The Biological Mechanisms Associated with Depo-Provera and HIV-1 Risk Acquisition in Women

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A research thesis submitted to the school of Health sciences, University of KwaZulu-Natal, Westville Campus, in fulfilment for the degree of Masters in Medical Sciences (Pharmaceutical Chemistry)

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This is a thesis in which the chapters are written as a set of discrete research publications, with an overall introduction and final summary.

This is to certify that the contents of this thesis are the original research work of Miss Funanani Takalani.

As the candidate's supervisor, I have approved this thesis for submission.

Supervisor: Prof Mahmoud Soliman

Signed: ..... Date: .....

ABSTRACT

Thirty-six years after the first identification of AIDS, the spread of its aetiological agents continues unabated, human immunodeficiency virus 1 (HIV-1) in particular. About 40% of HIV-1 infections have been reported to initiate in the female reproductive tract (FRT). However, the biological mechanisms through which these infections are spread are poorly understood hence there is now a major concern in women who use injectable hormonal contraceptives, particularly medroxyprogesterone acetate (MPA) administered as depot medroxyprogesterone acetate (DMPA) or Depo-Provera and an increase of HIV-1 risk acquisition.

As per literature, the glucocorticoid receptor (GR) and progesterone receptor (PR) are the main targets for Depo-Provera in the FRT. Therefore, in this study we performed molecular dynamic (MD) simulation on both the GR and the PR systems in relation to DMPA as a way of validating their docking poses and binding energy trends. We also investigated the nature of their overall interaction themes using post-dynamic analysis and most importantly, we postulated possible biological mechanisms in which DMPA may act *via* the GR and the PR in association with increased risk of HIV-1 infection in women.

Our findings revealed that, the effect of DMPA binding to both the GR and the PR could have a great impact on increased risk of HIV-1 infection in women. The reason being that when these receptors are activated by an agonist DMPA, they interact with a few residues in the ligand binding domain (LBD) which could affect the stability state of these receptors. They also interact with NFκB transcription factor as well as the p300 coactivator in the nucleus to cause transactivation in the ectocervical (Ect1/E6E7) epithelial cell line of the FRT, in turn, increasing mRNA and protein secretion levels of proinflammatory cytokines.

DECLARATION I - PLAGIARISM

I, Funanani Takalani, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original work.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

    a. Their words have been re-written, but the general information attributed to them has been referenced.
    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

5. This thesis does not contain text, graphics or tables copied from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references section.

A detail contribution to publications that form part and/or include research presented in this thesis is stated (include published and submitted publications).

Signed: **F. Takalani**

DECLARATION II - LIST OF PUBLICATIONS

1. Funanani Takalani, Ndumiso N. Mhlongo, Suri Moonsamy and Mahmoud E.S. Soliman (2017) Review on the biological mechanisms associated with Depo-Provera and HIV-1 risk acquisition in women. *Journal of Cell Biochemistry & Biophysics*. (*Published*)

Contribution:

Funanani Takalani: Author- Contributed to the review by performing all literature reviews, summing the information, preparation and writing of the manuscript.
Dr Suri Moonsamy: Came up with the idea of the manuscript
Dr Ndumiso Mhlongo: Assisted with editing of the manuscript
Prof Mahmoud Soliman: Supervisor

2. Funanani Takalani, Ndumiso N. Mhlongo, Suri Moonsamy and Mahmoud E.S. Soliman (2017) Computational modelling of DMPA interactions with GR and PR in association with increased risk of HIV-1 infection in women. *Journal of Systems Biology in Reproductive Medicine*. (*Under review*)

Contribution:

Funanani Takalani: Author- Contributed to the research article by performing all computational work and data analysis, interpretation of results, preparation and writing of the manuscript.
Dr Suri Moonsamy: Came up with the idea of the manuscript
Dr Ndumiso Mhlongo: Aided with technical aspects and editing of the manuscript.
Prof Mahmoud Soliman: Supervisor

RESEARCH OUTPUT

LIST OF PUBLICATIONS

1. Funanani Takalani, Ndumiso N. Mhlongo, Suri Moonsamy and Mahmoud E.S. Soliman (2017) Review on the biological mechanisms associated with Depo-Provera and HIV-1 risk acquisition in women. *Journal of Cell Biochemistry & Biophysics*, 1-10.

2. Funanani Takalani, Ndumiso N. Mhlongo, Suri Moonsamy and Mahmoud E.S. Soliman (2017) Computational modelling of DMPA interactions with GR and PR in association with increased risk of HIV-1 infection in women. *Journal of Systems Biology in Reproductive Medicine*. *(Submitted and under review).*

DEDICATION

- This thesis is dedicated to God who gave me the strength, knowledge and wisdom to complete all aspects of my work successfully; to Him I humble myself.

- It is also dedicated to the Takalani family: My mom, dad and siblings. I am grateful for all they have done for me; I want them to know that I have recognised each of their efforts towards this degree. It would not have been complete if it hadn't been for their support, thank you so much to all.

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LIST OF ABBREVIATIONS

ADCC	Antibody Dependent Cell Mediated Cytotoxicity
AF-1	Activation Function-1
AF-2	Activation Function-2
AIDS	Acquired Immune Deficiency Syndrome
AMPs	Anti-Microbial Peptides
ART	Antiretroviral Therapy
CBP	Creb Binding Protein
CCR5	C-C Chemokine Receptor 5
CD4	Cluster of Differentiation 4
CXCR4	C-X-C Chemokine Receptor type 4
DCs	Dendritic Cells
DMPA	Depot Medroxyprogesterone Acetate
DNA	Deoxyribonucleic Acid
EC_{50}	Half Maximal Effective Concentration
FRT	Female Reproductive Tract
GAFF	General Amber Force Field
GEC	Genital Epithelial Cell
GR	Glucocorticoid receptor
GPU	Graphics Processing Unit
HATs	Histone Acetyltransferases
HDACs	Histone Deacetylases
HIV	Human Immunodeficiency Virus
HREs	Hormone Response Elements
HSPs	Heat Shock Proteins
IFNs	Interferons
IκB	Inhibitor of Kappa B

IKK	Inhibitor of KappaB Kinase
LBD	Ligand Binding Domain
MD	Molecular Dynamics
MMFF	Merc Molecular Force Fields
MM/PBSA	Molecular Mechanics Poisson Boltzman Surface Area
MMV	Molegro Molecular Viewer
MPA	Medroxyprogesterone acetate
mRNA	Messenger Ribonucleic Acid
NFκB	Nuclear Factor Kappa B
NR	Nuclear receptor
ns	nanosecond
PME	Particle Mesh Ewald
PR	Progesterone receptor
R^2	Correlation Coefficient
Rg	Radius of gyration
RHD	Rel homology domain
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
RNA	Ribonucleic Acid
RTIs	Reproductive Tract Infections
SA	South Africa
SASA	Solvent Accessible Surface Area
SIVcpz	Simian Immunodeficiency Viruses from chimpanzees
SIVgor	Simian Immunodeficiency Viruses from gorillas
SRC-1	Steroid Receptor Coactivator-1
SSA	Sub-Saharan Africa
TAD	Transactivation Domain
TIF2	Transcriptional Intermediary Factor 2

UNAIDS	Joint United Nations Programme on HIV/AIDS
USA	United States of America
Vpr	Viral protein R
WHO	World Health Organisation

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CHAPTER 1

1. Introduction

1.1 Background & Rational

Acquired immune deficiency syndrome (AIDS) was first recognised as a new disease in 1981, but it became a more serious condition over the past years as its global statistics was revealed to increase every year (Sharp and Hahn,2011). The causative agent for this disease is Human Immunodeficiency Virus (HIV) which can further be classified into HIV-1 and HIV-2, with HIV-1 reported to be the major source of the AIDS pandemic (Dey et al., 2014; Plantier et al., 2009). Both HIV-1 and HIV-2 belong to the genus *Lentivirus* family of *Retroviridae* (Sharp and Hahn, 2010; Wain et al., 2007). Globally, HIV/AIDS is widely spread in sub-Saharan Africa (SSA) and Asia with the morbidity & mortality rate highest in young adults, women revealed to be the most vulnerable (Dey et al., 2014; Gouws et al., 2008; Sharp and Hahn, 2011). The method through which humans acquired ape precursors of HIV is poorly understood. However, it is believed that they could have come into contact with infected blood or other body fluid of these apes perhaps during hunting (Sharp and Hahn,2011).

An estimation of over 50 million women worldwide use Depo-Provera as a way of preventing pregnancy hence this has become one of the major reasons associated with their susceptibility to HIV infection (Hel et al., 2010; Morrison et al., 2012). Depo-Provera acts by binding to and regulating the activity of steroid receptors. Herein, we focussed our attention on the Glucocorticoid receptor (GR) and the Progesterone receptor (PR) as they are the main targets for Depo-Provera in the FRT (Schindler et al., 2003; Tomasicchio et al., 2013). Both GR and PR consist of various conserved domains at the centre but in this study, the ligand binding domain (LBD) was of great interest. Therefore, we performed molecular docking and molecular dynamic (MD) simulations on both the GR and PR protein systems in relation to Depo-Provera.

We also investigated their overall interaction themes using post dynamic analysis whereby 50 ns multiple MD simulations were employed to gain insight into the effect of Depo-Provera on the GR and PR. This was followed by binding free energy calculations, root mean square deviation (RMSD), root mean square fluctuation (RMSF) and radius of gyration (Rg) analyses.

1.2 Aim & objectives

The major aim of this work was to investigate possible biological mechanisms in which Depo-Provera may act *via* the GR & the PR signalling pathways in association with increased risk of HIV-1 infection in women. To accomplish this, the following objectives were outlined:

1. To estimate different binding affinities and orientations using molecular docking for Depo-Provera bound to the GR and the PR, respectively.

2. To validate docking poses and binding energy trends for Depo-Provera bound to the GR and the PR systems using half maximal effective concentration (EC_{50}) values and docking scores of the related approved drugs.

3. To perform 50ns multiple MD simulations for further verification on the resulted-docked systems.

4. To quantify individual amino acid interactions towards the total binding free energy and interaction forces (i.e. intramolecular and intermolecular forces computed per-residue interactions using MM/PBSA approach).

5. To investigate the nature of the overall interaction themes between Depo-Provera bound to the GR & PR target proteins and specific amino acids involved in ligand binding using post-dynamic analyses.

1.3 Novelty and significance of study

Antiretroviral therapy (ART) controls viral replication, restores the immune function and reduces viral load in HIV infected individuals (Katlama et al., 2013; UNAIDS, 2013). This treatment has been the medical solution against HIV/AIDS and it has been effective in reducing the number of death rates caused by the disease (Katlama et al., 2013; Sharp and Hahn,2011). However, it is incapable of destroying the virus completely and reliability on it for future curative abilities is uncertain (Sharp and Hahn,2011). Depo-Provera on the other hand, has been identified as one of the major risk factors associated with increased risk of HIV-1 in women (Ralph et al., 2015; Tomasicchio et al., 2013). However, the mechanisms through which it places women at risk are poorly understood. Likely, Schindler et al (2003) reported both the GR and PR as the main targets for Depo-Provera in the FRT since their biological activities are essential (Schindler et al., 2003).

This information is worth noting as it could mean that the GR and PR have effects on HIV-1 pathogenesis upon binding of Depo-Provera, particularly in the immune function. Therefore, we found it necessary to investigate HIV-1 pathogenesis in the FRT *via* the GR and PR signalling pathways in response to the effect of Depo-Provera. By conducting this research, we aim to provide new insights that will be useful in the development of new drugs against HIV/AIDS. A minimum of two articles will be published from this work and a thesis for an MSc in Medical Science degree will be submitted to the University of KwaZulu-Natal.

1.4 Overview of thesis

This thesis is divided into **six chapters**, chapter 1 included. Brief explanation of chapter 2, 3, 4, 5 & 6 is explained below:

Chapter 2

Explains the spread of HIV/AIDS across different countries.

Chapter 3

Discusses the background information of Molecular Modelling and provides brief explanation of the computational techniques performed in this study.

Chapter 4 (Accepted manuscript- this chapter is presented in the required format of the journal and is the final accepted version)

This is a review paper from the study titled "Review on the biological mechanisms associated with Depo-Provera and HIV-1 risk acquisition in women". It provides relevant literature that could be linked with the relationship between Depo-Provera and increased risk of HIV-1 acquisition in women.

Chapter 5 (Submitted manuscript- this chapter is presented in the required format of the journal and is the final submitted version)

This is a research article from the study titled "Computational modelling of DMPA interactions with GR and PR in association with increased risk of HIV-1 infection in women". It addresses objectives 1-5 as explained above.

Chapter 6

This chapter provides general conclusion of the overall thesis and future recommendations for future studies.

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CHAPTER 2

2. Introduction

This chapter explains the spread of HIV/AIDS across different countries. A detailed literature of this study is explained in chapter 4 (Review Paper).

2.1 The spread of HIV/AIDS

HIV/AIDS was first reported in the United States of America (USA) where it infected about 75 million individuals (Faria et al., 2014). It then spread to other countries such as South Africa (SA) where it infected approximately 6 million individuals (Kleinschmidt et al., 2007). In 1991, the AIDS global programme at World Health Organisation (WHO) predicted that by 2000 the cumulative global total of HIV infections in men, women and children would be 40 million. However, the cumulative global total of HIV infections appeared to be 56 million, proving a serious underestimate in the prediction (Piot et al., 2001). At the end of 2000, the region reported to have the worst epidemic was SSA with an estimation of 25.3 million people living with HIV (Piot et al., 2001).

In 2002, WHO reported 42 million people worldwide infected with HIV/AIDS while on the other hand, Simon et al (2006) reported about 46 million individuals worldwide infected with HIV-1 and approximately 25 million individuals already dead (Alimonti et al., 2003; Simon et al., 2006). Recent update (2016) provided by the TenoRes study group revealed more than 35 million people worldwide living with HIV-1 (Gregson et al., 2016). Up to date, the spread of HIV/AIDS is still estimated to increase across different countries yet the cure for this disease remains an ongoing challenge (Piot et al., 2001). The high vulnerability of women to HIV infection consequently places them at risk of transmitting the virus which in most cases can either be through direct transmission (mother to child) or horizontal transmission (heterosexual contact) (Anderson et al., 1991; Morison et al., 2001).

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CHAPTER 3

3. Methods

This chapter introduces the concept of Molecular Modelling and provides brief explanation of some computational techniques performed in this study such as molecular docking, molecular dynamic simulation and thermodynamic calculations. Further explanation of these techniques continues in chapter 5 (Research Article Paper).

3.1 Introduction to Molecular Modelling

Molecular modelling, term used synonymously with computational chemistry, provides tools for doing and teaching chemistry better, allowing scientists to investigate, interpret, explain and discover new phenomena (Nadendla, 2004). It uses computers to construct molecules and predict their chemical characteristics and behaviour by performing variety of calculations (Mukesh and Rakesh, 2011). Molecular modelling mimics the behaviour of molecules using computational techniques, data from experiments and all theoretical chemistry methods to predict molecular and biological properties (Nadendla, 2004). The level of description of atoms in molecular systems is a common feature of the molecular modelling technique (Rodrigues et al., 2008). Thus, molecular modelling technique allows consideration of atoms during simulation by reducing the system's complexity (Mukesh and Rakesh, 2011).

3.2 Molecular docking

Molecular docking is a computer based approach used to discover new compounds of therapeutically relevance using known target protein structures (Mukesh and Rakesh, 2011). The interest of molecular docking is to reproduce chemical potentials to estimate the binding affinity of a ligand within the receptor binding site and to predict bound conformations (Guedes et al., 2014; Trott and Olson, 2010). During the process of molecular docking, there are some conformational adjustments (referred to as "induced fit") that occur between the ligand and the protein to achieve what is referred to as the overall "best fit" (Mukesh and Rakesh, 2011).

These adjustments include optimal conformation of the ligand and the protein as well as their relative orientation (Figure 3.1) (Mukesh and Rakesh, 2011). Docking methodologies are useful in planning and designing of new drugs in addition to predicting the strength and type of signal produced (Guedes et al., 2014; Mukesh and Rakesh, 2011). Docking may also be applied to bioremediation, hit identification and lead optimization (Mukesh and Rakesh, 2011).

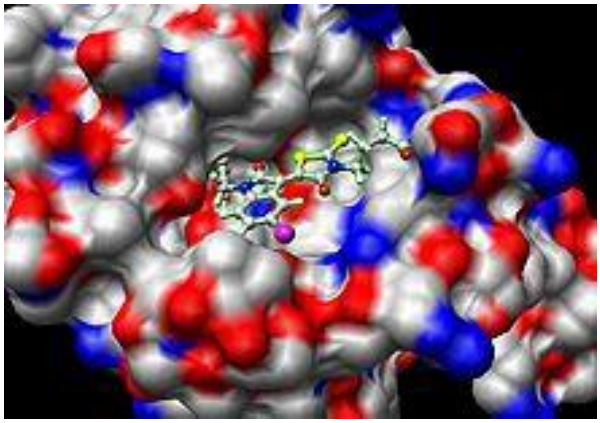

Figure 3.1 Optimized conformation of the ligand & the protein receptor. Figure adapted from Mukesh *et al* 2011 (Mukesh and Rakesh, 2011).

Whether the docked conformation is good or bad can be determined based on Dock score, scoring functions or sum of steric and electrostatic Merc Molecular Force Fields (MMFF) (Mukesh and Rakesh, 2011). For a docking programme to be successful, two main components should be considered: (i) the search algorithm and (ii) the scoring function (Mukesh and Rakesh, 2011). These are the basic tools used to generate and evaluate ligand conformation (Guedes et al., 2014). Docking softwares include Auto Dock, Dock, Gold, V Life MDS, CDOCKER and Flex X (Erickson et al., 2004; Mukesh and Rakesh, 2011; Wandzik, 2006).

In this study, we used Auto Dock Vina which is an improved version of Auto Dock. This software uses a method known as sophisticated gradient optimization which allows better accuracy to predict the binding mode and has been shown to improve the speed by approximately two orders of magnitude compared to Auto Dock (Trott and Olson, 2010).

3.3 Molecular Dynamic (MD) simulations

Molecular dynamics is one of the major computational methods used to calculate properties of molecules (Nadendla, 2004). The aim of MD simulation is to understand properties of a group of molecules by looking at their structures and the microscopic interaction between them (Allen, 2004). These interactions comprise non-bonded interactions, bonding potentials and force calculations (Allen, 2004). Simulation softwares are designed in packages such as AMBER, OPLS, CHARMM and many others (Crowley et al., 2009; Van Gunsteren and Mark, 1998). In this study, we used AMBER software to carry out MD simulations. More than 40 scientific researchers have collaborated to get AMBER software running to solve biochemistry problems. To date, it is installed in more than 1000 sites worldwide (Salomon-Ferrer et al., 2012). This is a software which refers to simulation of biomolecules by a series of classical molecular mechanic's force fields (Salomon-Ferrer et al., 2012).

It includes general organic molecules (GAFF), amino acid and nucleic acid parameter sets (ff94, ff99SB & ff12SB), carbohydrates (Glycam) and phospholipids (Lipid11) (Salomon-Ferrer et al., 2012). The AMBER package also contains three main MD engines which are *sander*, *pmend* and *pmend.cuda* (Crowley et al., 2009; Salomon-Ferrer et al., 2012). Herein, the *pmend.cuda* engine which is a graphics processing unit (GPU)-accelerated version of *pmend* was utilised (Case et al., 2015; Salomon-Ferrer et al., 2012). In addition to this, AmberTools including LEaP, Antechamber, chamber, paramfit and Metal Center Parameter Builder (MCPB) were used to prepare for simulation while on the other hand, ptraj, cpptraj, pbsa and Mmpbsa tools were used to analyse MD simulations (Salomon-Ferrer et al., 2012).

3.4 Thermodynamic calculations

Two approaches are widely used to calculate binding free energies of ligands to proteins or proteins to proteins complexes: (i) Molecular Mechanics General Born-Surface Area (MM/GBSA) and (ii) Molecular Mechanics Poisson Boltzman Surface Area (MM/PBSA) (Fu et al., 2013; Sun et al., 2014). Both MM/PBSA and MM/GBSA are classified under free energy end-point methods and are implemented in software programmes such as Delphi, Schrödinger and Amber (Gilson and Zhou, 2007; Hayes and Archontis, 2012). Therefore, to carry out energy calculations, these approaches use MD simulations of a free ligand, free protein as well as their complex (Gilson and Zhou, 2007).

When calculating absolute binding free energies based on MD simulations, the MM/PBSA approach has been proven to give better results as compared to the MM/GBSA approach. However, this is not necessarily the case when calculating relative binding free energies (Hayes and Archontis, 2012). Several factors including charge models, force fields and selection of atomic radius parameters may affect the ability of accurate binding free energy estimates (Fu et al., 2013). Nevertheless, we employed the MM/PBSA approach implemented in AMBER 14 to evaluate binding free energies of DMPA in association with the GR & the PR. Equations to calculate such binding free energies are discussed in chapter 5.

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CHAPTER 4

Review on the biological mechanisms associated with Depo-Provera and HIV-1 risk acquisition in women

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Abstract

Women constitute more than 50% out of millions of individuals infected with HIV-1, the major causative agent of AIDS. About 40% of HIV-1 infections have been reported to initiate in the female reproductive tract (FRT). However, the mechanisms through which these infections are spread are poorly understood hence there is now a major concern in women who use long acting injectable hormonal contraceptives, particularly Depo-Provera and an increase of HIV-1 risk acquisition. Based on literature, Depo-Provera has an affinity for both the glucocorticoid receptor (GR) and the progesterone receptor (PR) in the FRT. Therefore, investigating HIV-1 pathogenesis in the FRT *via* the GR and the PR mechanisms in response to the effect of Depo-Provera is of great importance.

Key words: HIV-1, Depo-Provera, Glucocorticoid receptor, Progesterone receptor, Female reproductive tract.

4.1 Introduction

Human Immunodeficiency Virus (HIV) is the causative agent for the disease that has been recognised as a "global killer" since 1980s, the acquired immune deficiency syndrome (AIDS) (Moonsamy et al., 2014; Sharp and Hahn, 2011). Concerning its subdivisions (HIV-1 and HIV-2), HIV-1 has been reported to be the most studied as well as the most widespread. Moreover, this type of virus is divided into various groups which are M, N, O & P. Amongst these four groups, M has been revealed to be the most pandemic (Sharp and Hahn, 2011). All HIV-1 groups can lead to the depletion of $CD4^+$ T-cells in humans which in turn causes AIDS but the difference is how they are distributed within the population (Sharp and Hahn, 2011).

Statistics revealed that more than 20 million people have died of HIV infection while about 35 million people are infected (Herrera-Carrillo and Berkhout, 2015). It also showed that, women are more vulnerable to HIV-1 infections than men accounting for about 59% in sub-Saharan Africa (SSA) (Tomasicchio et al., 2013). Three major ways in which HIV-1 can be transmitted is through sexual intercourse, percutaneous and perinatal routes (Palmisano and Vella, 2011; Sharp and Hahn, 2011). The pathogenicity of HIV-1 is unique hence the mechanisms through which it places women at risk are poorly understood.

Previous studies suggested that one of the major risk factors could be that women use long acting injectable hormonal contraceptives as a way of preventing pregnancy, particularly medroxyprogesterone acetate (MPA) administered as depot medroxyprogesterone acetate (DMPA) or Depo-Provera (Polis et al., 2014; Ralph et al., 2015; Tomasicchio et al., 2013). Interestingly, Schindler et al (2003) reported that the biological activities of both the glucocorticoid receptor (GR) and the progesterone receptor (PR) are essential hence, they are the main targets for Depo-Provera in the FRT (Schindler et al., 2003).

From this information, it can be deduced that the GR and the PR could have effects on HIV-1 pathogenesis upon binding of DMPA, particularly in the immune function. However, further investigations regarding how much DMPA dose is responsible for causing an increase of HIV-1 infection in women still needs to be conducted. Herein, we review possible biological mechanisms occurring in the FRT via the GR and the PR in the presence of DMPA which could lead to an increase of HIV-1 infection in women.

4.2 HIV-1 viral life cycle

The life cycle of HIV is divided into various stages as is explained below and presented in Figure 4.1. (1) Binding: This is also known as an attachment stage because the infectious HIV uses its glycoproteins to attach itself to the CD4 receptors located on the surface of the cell membrane (NIAID, 2005; Palmisano and Vella, 2011). It does so in conjunction with chemokine co-receptors located at the CD4 T lymphocyte surface such as CCR5 or CXCR4 to make the process of invading the cell easier (Engelman and Cherepanov, 2013; NIAID, 2005). (2) Fusion/entry: The HIV envelope fuses with the CD4 cell membrane to continue the process of invading the cell (Berger et al., 2007).

(3) Reverse transcription & (4) Nuclear import: HIV uses one of its enzymes known as reverse transcriptase to convert its single stranded HIV-RNA to double stranded HIV-DNA in the cytoplasm of a CD4 cell to penetrate the nucleus (Berger et al., 2007; NIAID, 2005). (5) Integration: Located in the nucleus is the CD4 cell DNA (human DNA). Here, the HIV enzyme integrase is released to allow HIV-DNA (also referred to as the provirus) to insert itself or form part of the human DNA (Engelman and Cherepanov, 2013). (6) Transcription: Upon activation of the host cell by a signal in response to the presence of a provirus in the human DNA, small chains of virus proteins are formed during cell division (Herrera-Carrillo and Berkhout, 2015; NIAID, 2005).

This involves strands of HIV genomic material created by the provirus using a host enzyme called RNA polymerase and shorter RNA strands known as messenger RNA (mRNA). (7) Translation: Shorter mRNA strands give rise to long chains of HIV proteins which are chopped down into smaller proteins by HIV protease (NIAID, 2005). (8) Assembly: These smaller HIV proteins migrate to the surface of the CD4 cell and together with the newly formed HIV-RNA assemble into harmless immature HIV (Herrera-Carrillo and Berkhout, 2015; NIAID, 2005). (9) Maturation: the immature HIV finds its way outside the host cell but since it is an enveloped RNA virus, it steals part of host cell's envelope to cover itself (Berger et al., 2007).

In this way, the immature HIV becomes harmful because the host cell's envelope contains HIV glycoproteins which make it possible for the virus to attach itself again to the CD4 receptors and co-receptors, producing more new copies of HIV and spreading the infection to other cells (Berger et al., 2007).

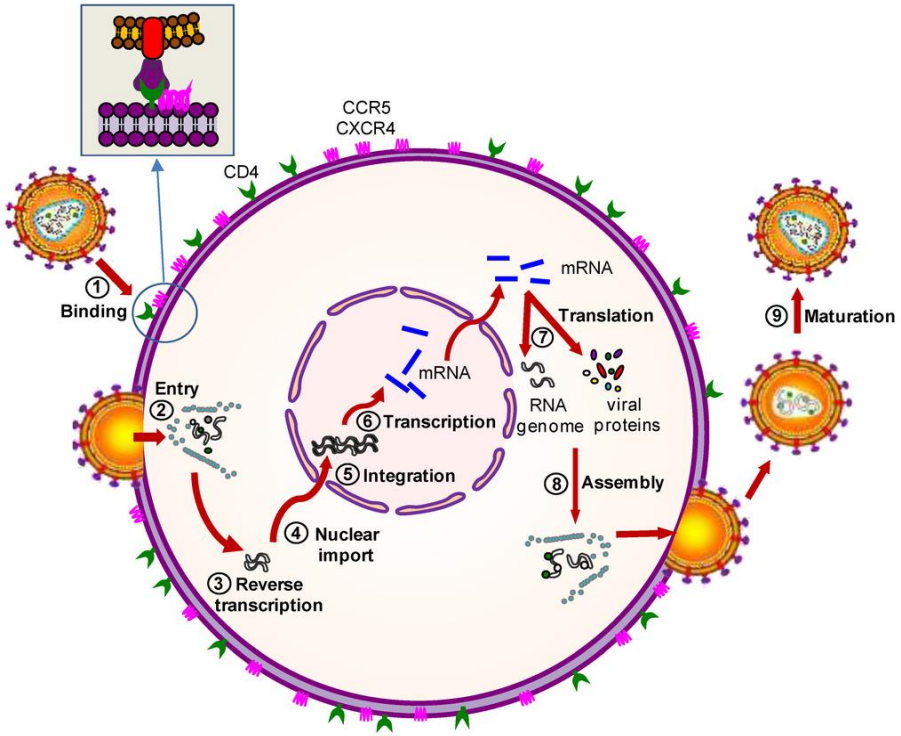

Figure 4.1. Steps of the HIV-1 viral life cycle. (1) infectious HIV binds to the surface of the cell, (2) infectious HIV enters the cell membrane, (3) & (4) single stranded HIV-RNA is converted to double stranded HIV-DNA which enters the nucleus, (5) HIV-DNA forms part of human DNA, (6) small chains of virus proteins are formed during cell division including mRNA strands, (7) mRNA strands give rise to HIV proteins, (8) HIV proteins migrate to the surface of the cell where it forms harmless immature HIV, (9) immature HIV comes into contact with the host's cell envelope which contains glycoproteins and becomes mature harmful HIV. Figure adapted from Herrera-Carrillo and Berkhout (2015).

4.3 Risk factors of HIV-1

The most common mode of infection in adults is through heterosexual transmission whereas in children is through mother-to-child transmission which occurs especially during the process of giving birth (Mostad et al., 1997). It has been reported that uncircumcised men have an increased risk of HIV infection of about 60% (Mmbaga, 2013). However, the joint United Nations programme on HIV/AIDS (UNAIDS) revealed that 52% of infected adults are women (Mmbaga, 2013). The cervix and vagina could be significant determinants of transmitting the virus sexually and vertically since 51% of HIV-1 infected cells are detected in the endocervix and 14% are detected in the vagina (Mostad et al., 1997). In addition to this, deficiency of vitamin A has been shown to result in shedding of HIV-1 infected cells in the vagina. When DMPA is administered, CD4 count is lowered and thickening of the cervical mucus in the genital tract is also affected resulting in an increase of the spread of HIV-1 infected cells.

Therefore, replication of the virus may either affect immune modulation or physiology of the local genital tract due to effects of hormonal contraceptives or vitamin A deficiency (Mostad et al., 1997). Every year in SSA, reproductive tract infections (RTIs) are estimated to give rise to new infections such as vaginitis, gonorrhoea and chlamydia because of lack of diagnostic services and poor health-seeking behaviours (Mmbaga, 2013).

4.4 Components of the immune system in the female reproductive tract (FRT) associated with HIV-1 infection

Although the pathogenesis is poorly understood, HIV infection is associated with immune dysfunction which can further lead to complications in various organs of the body (Palmisano and Vella, 2011). This dysfunction usually results from disturbances in components of the immune system. Therefore, understanding how the immune system works in conjunction with its components is vital (Berger et al., 2007). The immune system is divided into innate and adaptive immunity. Innate immunity is the response to acute infection which describes the first phase of HIV infection (Bruland, 2003). It consists of: (i) Interferons (IFNs) which have a wide variety of immunomodulatory and antiviral effects. (ii) Anti-Microbial Peptides (AMPs) which includes human alpha-and beta defensins that have been shown to have anti-viral activity including HIV-1 (Nguyen et al., 2014).

(iii) Natural Killer (NK) cells that are essential in anti-viral or anti-tumor responses hence, when activated, they function by either killing the infected virus or tumor cells. (iv) Genital Epithelial Cell (GEC) responses whereby cells in the epithelium recognize and tailor a response to a wide variety of viral pathogens in the lumen of the FRT. (v) Dendritic Cells (DCs) and Macrophages which process viral information from the external environment to adaptive immunity of the host system (Nguyen et al., 2014). Adaptive immunity is the response to secondary infection (e.g. AIDS, which describes the chronic stage of HIV infection). It involves two types of responses: (a) humoral response, mediated by B-cells and (b) cellular response, mediated by T-cells. Both humoral and cellular responses are activated during virus infection (Bruland, 2003).

Cellular T cells activate B cells to divide forming plasma cells that can produce antibodies to fight against antigens. They also produce memory B cells to protect the immune system against re-exposure to viral pathogens in future (Bruland, 2003). Antibodies produced prevent infection by targeting viruses for destruction by phagocyte cells, a process known as opsonisation, and by antibody dependent cell mediated cytotoxicity (ADCC). This is the process whereby lymphocyte cells lyse the target cell marked by an antibody (Bruland, 2003). Lymphocytes include Natural killer (NK) cells, Cytotoxic T cell ($CD8^+$), Helper T cell ($CD4^+$) and Memory B cell (Berger et al., 2007; Bruland, 2003). Changes in the B and T cells may increase HIV-1 infection.

As for humoral response, weak IgG responses may increase HIV-1 susceptibility (Nguyen et al., 2014). It is worth noting that HIV is capable of infecting monocytes, macrophages, DCs and $CD4^+$ T cells (Herrera-Carrillo and Berkhout, 2015). However, DCs and $CD4^+$ T cells are the main target cells of HIV and they are most abundant in the transformation zone of squamous ectocervix to columnar endocervix epithelium (Herrera-Carrillo and Berkhout, 2015; Nguyen et al., 2014).

4.5 Interaction of DMPA & HIV-1

Depot medroxyprogesterone acetate (DMPA) is a synthetic progestin derived from 17α-hydroxyprogesterone which makes it a progesterone-based contraceptive (Nguyen et al., 2014). It is the second most related to the original progesterone after retroprogesterone. However, this progestin has a half-life longer than that of progesterone (Schindler et al., 2003). This method of contraception is widely used in SSA as well as in some areas with high prevalence of HIV-1. Clinical studies provided evidence that DMPA increases the risk of HIV-1 acquisition in women which in turn leads to AIDS progression (Tomasicchio et al., 2013). Such evidence has been proven in workers who engage themselves in commercial sex and those involved in serodiscordant partnership (Ralph et al., 2015).

About 41 million women worldwide use Depo-provera as a way of preventing pregnancy (Hills-Nieminen et al., 2015; Ralph et al., 2015). Thus, a normal dose of 150 mg is injected every three months (Tomasicchio et al., 2013). Depo-Provera acts by binding to and regulating the activity of steroid receptors (Tomasicchio et al., 2013). In this review, we discuss the effect of its binding specifically to the GR and the PR. However, it has been reviewed previously that DMPA affinity to bind to PR is higher as compared to its affinity of binding to GR (Schindler et al., 2003). Investigating different mechanisms *via* the GR and PR in response to the effect of DMPA on HIV-1 pathogenesis is of greater importance.

These effects could include alterations in the mucosal microflora and thinning of the cervical or vaginal epithelium (Tomasicchio et al., 2013). Nguyen et al (2014) reported that: in the mucosal immune system of the FRT, DMPA inhibits the activity of $CD8^+$ T cells and blocks the expression of perforins in T cells, it reduces antibody production, interferon β (IFN- β) production and antibody dependent cytotoxicity (ADCC).

4.6 Antiretroviral (ARV) treatment

Antiretroviral (ARV) has been the drug of choice against HIV/AIDS and it has been effective in reducing the number of death rates caused by the disease (Sharp and Hahn, 2011).

This treatment controls viral replication and reduces viral load in HIV infected individuals (UNAIDS, 2013). Medical history revealed that people staying in well-developed countries are at an advantage of getting full access to the treatment. However, it's unfortunate for those located in geographical areas like Africa as they are the most affected and yet treatment is limited (Palmisano and Vella, 2011). Per literature, North Africa has the poorest access in providing ARV therapy as it only caters for 11% of its population while SSA has been reported to take the lead in bettering access of providing ARV therapy because it caters for up to 7.5 million people (UNAIDS, 2013). Taken together, treatment is not prevailing everywhere and reliability on it for future curative abilities is uncertain (Sharp and Hahn, 2011). It is also incapable of destroying the virus completely hence therapy should be made to be lifelong and health care systems should also be of reasonable cost (Palmisano and Vella, 2011).

Up to date, there is still no cure for HIV/AIDS hence this disease is still predicted to increase soon due to untreated or undiagnosed HIV infected individuals (Lundgren et al., 2015; Palmisano and Vella, 2011; Sharp and Hahn, 2011). In fact, previous studies performed on model simulation revealed that any 20% increase of HIV, particularly by injectable contraceptives would lead to 27,000 additional infections per year which could in turn lead to an increase of unintended pregnancies and birth, unsafe abortion as well as maternal death (Polis et al., 2014). Per Lundgren et al (2015), the risk of AIDS has never been zero among patients using antiretroviral therapy or even among those who had viral suppression in the process of receiving antiretroviral therapy.

This calls for further research on treatments to use along with antiretroviral therapy to reduce disease among HIV positive patients (Lundgren et al., 2015). However, there are some inhibitors that have been reported to lower the viral load by interacting with the HIV viral life cycle. These include enfuvirtide, zidofudine, ritonavir, raltegravir, saquinavir and many more (Hicks and Gulick, 2009; Palmisano and Vella, 2011). Thus, new HIV infections and illnesses are prevented and many lives are saved by using ARV drug.

4.7 Interaction of DMPA and ARV treatment

Almost half of HIV infected adults are women of reproductive age who choose to prevent falling pregnant by making use of contraceptives such as DMPA (Watts et al., 2008). This contraceptive is recommended safe to be used by HIV infected women under ARV treatment because it doesn't have effects on the levels of HIV RNA and $CD4^+$ cell count (Hills-Nieminen et al., 2015; Watts et al., 2008). However, during the period of seroconversion to HIV-1; the use of DMPA has been reported to increase levels of HIV RNA as compared to non-use of DMPA during seroconversion (Watts et al., 2008). In addition to this, using DMPA for longer periods results in weight gain; the condition which may be worsened by using ARV treatment in HIV infected women (Bonny et al., 2006).

4.8 Background of the Progesterone receptor (PR) & the Glucocorticoid receptor (GR)

These receptors belong to a superfamily of type 1 nuclear receptor (NR) (Mckay et al., 1999). Per phylogenetic analysis, they are classified under a subfamily of oxosteroids receptors (Bledsoe et al., 2002). They both consist of various conserved domains at the centre but in this review, we focus more on the ligand binding domain (LBD) as is illustrated in a graphical representation of DMPA bound to both the PR and the GR (Figure 4.2). The PR and GR LBDs share a homology sequence of 50-57% (Africander et al., 2011). These LBDs are involved not only in binding of hormones but also in dimerization, chaperone association and binding coactivators (Bledsoe et al., 2002).

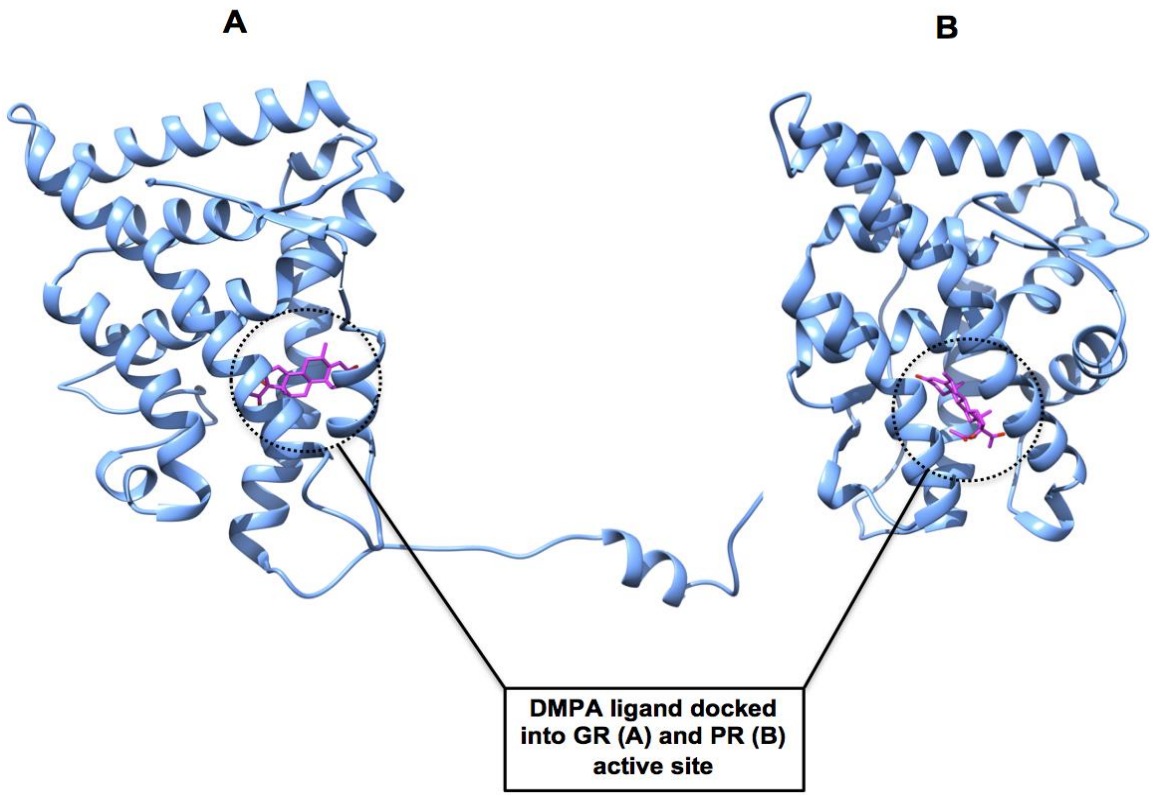

Figure 4.2. Graphical representation of DMPA ligand docked into the GR (A) and the PR (B) LBDs.

Over the years, it has been shown that binding of a ligand to nuclear receptors causes the LBD to fold leading to formation of a hydrophobic pocket (Bledsoe et al., 2002). The activation function-2 (AF-2) domain, located within the LBD has been proven to be crucial in ligand regulation for transcriptional activity of the receptor because unlike activation function-1 (AF-1), the transcriptional activity of AF-2 acts in the presence of a ligand (Africander et al., 2011; Aranda and Pacual, 2001; Bledsoe et al., 2002). This AF-2 domain is also known to be a site where cofactors can be docked within the LBD (Africander et al., 2011). Present in the AF-2 domain is the LxxLL motif which interacts with some cofactors in the LBD (Africander et al., 2011; Bledsoe et al., 2002).

Upon activation of the receptor by the ligand, AF-2 is stabilised in its active conformation preventing interaction between LBD and corepressors but associating with coactivator proteins such as steroid receptor coactivator-1 (SRC-1) and transcriptional intermediary factor 2 (TIF2) (Africander et al., 2011; Bledsoe et al., 2002).

4.9 General mechanism of type 1 nuclear receptors (GR & PR)

In the absence of a ligand, the GR is in the cytoplasm where it's complexed with heat shock proteins (HSPs) (De Bosscher et al., 2008). This prevents the GR from being activated as HSPs act as molecular chaperones to prevent activation (Africander et al., 2011). In contrast to the GR, location of the PR when not bound to the ligand is predominantly in the nucleus but part of the PR that is in the cytoplasm is also complexed with HSPs (Africander et al., 2011). Upon binding of a ligand, these receptors are highly phosphorylated leading to dissociation of HSPs and formation of NR dimer (Aranda and Pacual, 2001). The NR dimer translocate from the cytoplasm to the nucleus where it binds to hormone response elements (HREs), followed by remodelling of the chromatin, components of the transcription machinery and cofactors recruitment to bring about positive transcription regulation (Africander et al., 2011). This process is referred to as transactivation (Sedwick, 2014).

However, when transcription is regulated negatively, the process is termed transrepression. This can either be through a direct mechanism where receptors bind straight to DNA or via indirect mechanism where receptors interact with transcription factors bound to DNA like nuclear factor kappa B (NFκB) and CCAAT-enhancer-binding protein (C/EBP) (Africander et al., 2011). The GR has been reported to be the most studied under transrepression processes; it follows indirect mechanism since it uses its GR monomer to bind NFκB, a process known as tethering (Africander et al., 2011; Sedwick, 2014). Although the mechanism of tethering is less studied, it has been reported to activate transcription regulation (Africander et al., 2011).

It is worth noting that whether regulation of transcription leads to activation or repression process depends mostly on whether the ligand is an agonist or antagonist. Generally, binding of an agonist to the receptor leads to transactivation whereas binding of an antagonist to the receptor results in transrepression (Africander et al., 2011).

4.10 General mechanism of NFκB transactivation

It has been reported that expression of genes involved in the immune response is positively regulated by NFκB. Such genes (including cytokines, immunoreceptors, viral proteins and many more) serve as targets during the process of NFκB transactivation (Mckay et al., 1999; Zhong et al., 1998).

In the absence of a stimulus in the cell, the NFκB dimer is in the cytoplasm where it is complexed with the inhibitor of NFκB (IκB), particularly IκBα which is the most studied in a family of IκB (Mckay et al., 1999; Oeckinghaus and Ghosh, 2009). However, in the presence of a stimulus, the IκBα is phosphorylated by a complex of IκB kinase (IKK), ser/thr kinases (IKK-1 & IKK-2). This in turn leads to dissociation of IκBα which later undergoes proteosomal degradation from its exposure to ubiquitination, leaving the NFκB dimer to translocate to the nucleus where it up-regulates the expression of genes to cause transcriptional activity (Mckay et al., 1999; Zhong et al., 1998). The general signalling pathway of NFκB is brought about by the Rel p65/p50 heterodimer, hence it has been reported to be the most studied and found in almost all types of cells among the Rel NFκB dimers (Mckay et al., 1999; Oeckinghaus and Ghosh, 2009).

The Rel family is a term given to several related proteins that act as subunits of NFκB (Mckay et al., 1999). Members of the Rel family can form a homo or heterodimeric complex when they associate with each other as most of them form part in the Rel homology domain (RHD) made up of 300 amino acids (Mckay et al., 1999; Oeckinghaus and Ghosh, 2009). This RHD consist of DNA binding, dimerization and nuclear localization functions of NFκB. In the p65/p50 heterodimer, the p50 subunit has been shown to lack the ability to cause transactivation since it doesn't have the transactivation domain (TAD) like the p65 subunit (Oeckinghaus and Ghosh, 2009; Zhong et al., 1998). However, it is said that at least one Rel of the NFκB dimer is enough to cause transactivation.

4.11 Mediated response of the GR & the PR upon DMPA binding associated with increased risk of HIV-1 in women

The GR and NFκB signalling pathways have been recognized as important in regulation of immunity and inflammation because the immune response contains cytokines and cytokine induced genes regulated and activated through these pathways (Barnes et al., 2005; Mckay et al., 1999). The figures below summarise three possible biological mechanisms which could occur in the FRT *via* the GR/PR and NFκB in the presence of DMPA, leading to an increase of HIV-1 infection in women. These mechanisms are explained as follows:

(i) In the ectocervical (Ect1/E6E7) epithelial cell line, interleukin 12 (IL-12) p40 has been reported to be the cytokine induced gene regulated by the GR in the female genital tract and mediated by NFκB to bring about transactivation (Louw-du Toit et al., 2014; Mckay et al., 1999). However, this IL-12p40 contains none of the functional GR responsive elements hence transactivation of this mechanism is through binding of the ligand-activated GR to NFκB which tethers to C/EBPβ that can attach itself in the IL-12p40 binding site (Figure 4.3). As for the PR mediated response, although the mechanisms involved are poorly understood, there is a high possibility that they could be similar to those of the GR

which are well studied (Africander et al., 2011). Like Bledsoe et al (2002) reported, the GR is homologous to the PR hence the functioning of their signalling pathways is closely related. This involves binding of the ligand, recruitment of coactivators as well as dimerization. Therefore, we postulate that DMPA or other agonist ligands could have a tendency of binding to the PR following the same GR mechanism as is explained above. However, the mechanism of DMPA-PR could be stronger and more effective as compared to that of DMPA-GR because of the difference in their binding affinities to DMPA (Schindler et al., 2003).

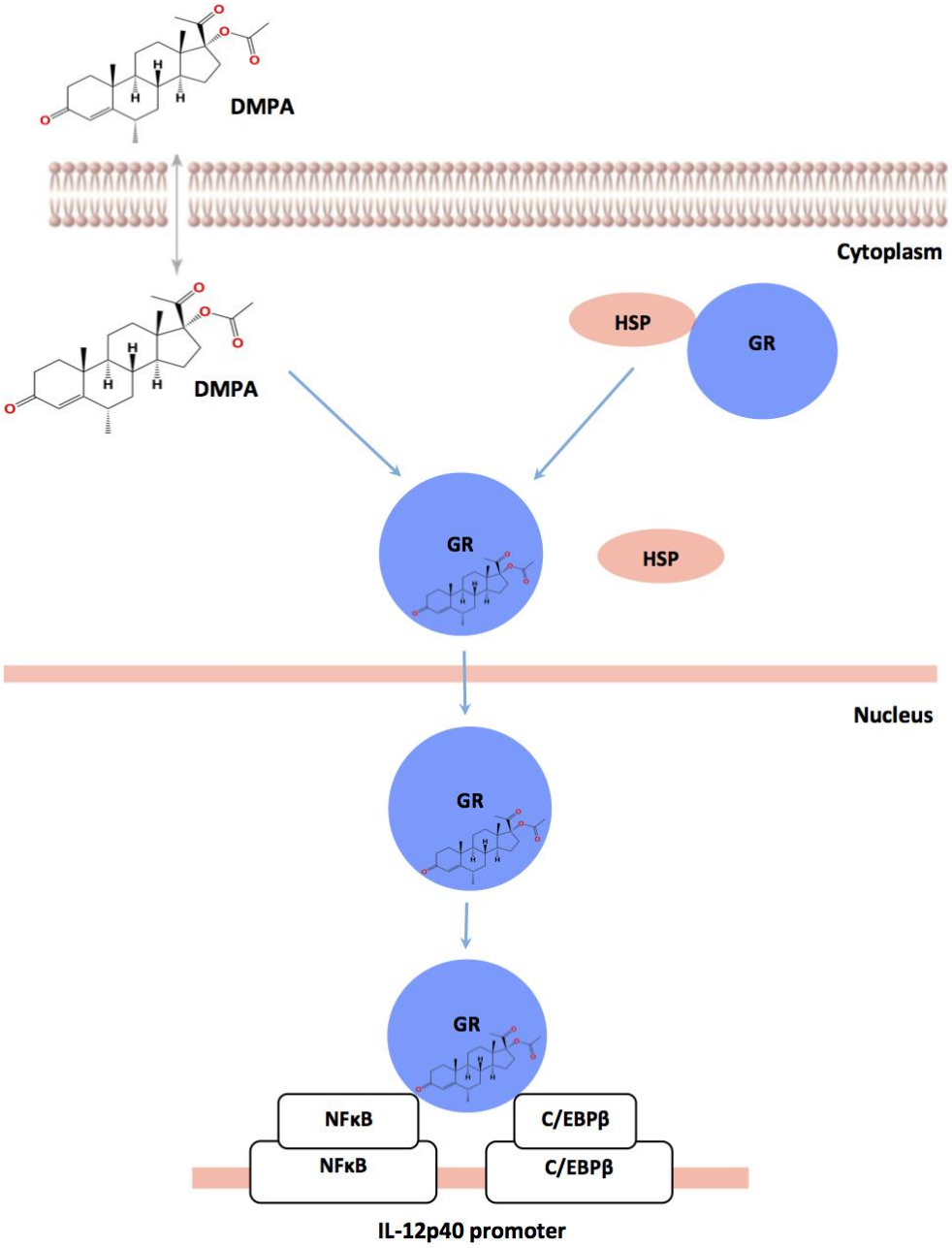

Figure 4.3. Upon binding of DMPA to the GR, the HSP in the cytoplasm dissociates from the GR allowing the DMPA-activated GR to translocate to the nucleus. In the nucleus, the DMPA-activated GR binds to NFκB which tethers to C/EBPβ that can bind IL-12p40 promoter to cause transactivation. Figure adapted from Africander et al (2011) & Louw et al (2014).

(ii) The chromatin structure in the nucleosome is made up of core histones around which DNA is wound. Modification of these histones is useful in determining which genes are repressed or activated during the process of transcription (Barnes et al., 2005). The histone acetylation process, controlled by histone acetyltransferases (HATs) has been reported to cause a major modification. This is a process whereby DNA unwinds due to change in charge of the core histone (Barnes et al., 2005). This change is caused by the presence of lysine residues in the core histone which are likely to be acetylated. Histone acetylation causes the chromatin structure to change from being in a resting closed conformation to being in an activated open form (Barnes et al., 2005). Coactivators such as p300 and the Creb Binding Protein (CBP) have been reported to contain intrinsic HAT activity (Barnes et al., 2005).

Interestingly, HIV transcriptional activation by viral protein R (Vpr) which is an accessory protein of HIV-1 is also mediated by the P300 NFκB co-activator (Felzien et al., 1998). Both Vpr and NFκB are localised and regulated in the nucleus where Vpr co-operates through indirect mechanism with p300 to further regulate transcription of NFκB (Felzien et al., 1998). Vpr is made up of 96 amino acids with a molecular weight of 11.38kDa (Guenzel et al., 2014; Wu et al., 2016; Zahoor et al., 2014). As a pleiotropic protein, it interacts with cellular factors inside the host through multiple mechanisms to bring about viral infection (Zahoor et al., 2014). Aranda and Pascual (2001) revealed that the CBP/P300 can either bind directly or indirectly to nuclear receptors. Direct binding occurs through the NH_2-terminal domain while indirect binding occurs through interaction with p160 coactivators.

In the event of indirect binding, a complex of corepressors such as SMRT/NcoR: mSin3 can bind to the GR heterodimer in the absence of a ligand by allowing recruitment of histone deacetylases (HDACs), a process known as deacetylation (Aranda and Pascual, 2001). Histone deacetylation causes chromatin compaction which leads to repression (Figure 4.4A). In contrast to this, the p160 coactivators such as SRC-1 have been reported to contain HAT activity in addition to its ability to interact with nuclear receptors. Moreover, binding of an agonist to the receptor has been reported to trigger hyperacetylation which is AF-2 dependent (Aranda and Pascual, 2001). Therefore, upon binding of DMPA to the GR, the CBP/P300: SRC-1 coactivator complex binds to the GR heterodimer through HAT to cause transactivation (Aranda and Pascual, 2001; De Bosscher et al., 2008) (Figure 4.4B).

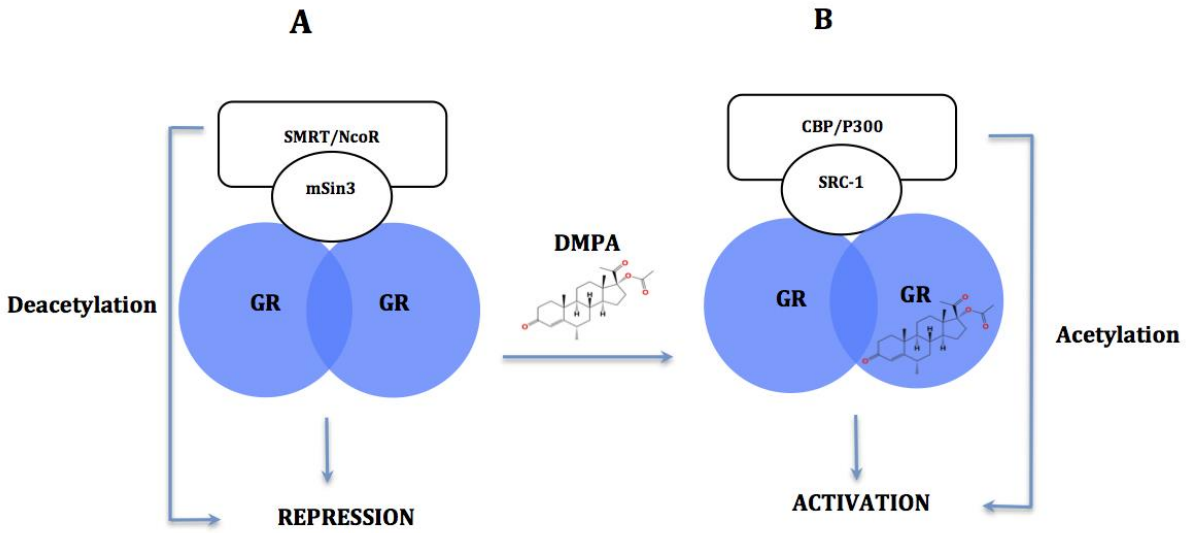

Figure 4.4. A, in the absence of a ligand, the nuclear receptor (GR or PR) is bound to a corepressor complex such as SMRT/NCoR: mSin3 via the deacetylation process, this in turn causes repression. **B**, upon binding of a ligand, the GR heterodimer recruit histone acetyltransferases to bind the CBP/P300: SRC-1 coactivator complex, process known as acetylation. This coactivator complex then binds to the AF-2 domain to cause transactivation. Figure adapted from Aranda and Pascual, (2001).

(iii) As part of the NFκB general signalling pathway, protein kinase A catalytic subunit (PKAc) has been shown to be present in the cytosolic NFκB: IκBα complex held in its inactive state by the IκBα (Zhong et al., 1998). Phosphorylation of IκBα by IKK leads to dissociation of IκBα as well as activation and dissociation of PKAc. The IκBα gets degraded via ubiquitin pathway while the activated PKAc phosphorylates the p65 subunit of the NFκB heterodimer allowing it to translocate to the nucleus for transcriptional activity (Mckay et al., 1999; Zhong et al., 1998) (Figure 4.5A). Interestingly, just like NFκB, Vpr is localised and regulated in the nucleus where it co-operates with p300 to further regulate transcription of NFκB (Felzien et al., 1998; Guenzel et al., 2014).

In association with NFκB, p300 has been reported to regulate transcriptional activity through phosphorylation of p65 by PKAc (Zhong et al., 1998). On the other hand, it has been shown to interact with NFκB p65 Rel protein by acting as a coactivator in the p65 mediated transactivation. Moreover, the CBP/P300 has been identified as the GR transcriptional coactivator which enables transactivation upon binding of a ligand (Mckay et al., 1999). In summary, p300 has been reported to contain the receptor interacting domain (RID). Within this RID is the LxxLL motif which is also present in the AF-2 domain located at the C-terminus within the LBD in nuclear receptors. This LxxLL motif causes transactivation by p300 to be AF-2 dependent (Aranda and Pascual, 2001). Interestingly, Unlike AF-1, the AF-2 domain plays a role in ligand regulation for transcriptional activity because it acts in the presence of a ligand and it's also a site where cofactors can be docked within the LBD (Africander et

al., 2011; Aranda and Pascual, 2001). Thus, the AF-2 domain associate with coactivator proteins upon activation of the receptor by the ligand (Africander et al., 2011; Bledsoe et al., 2002). Therefore, upon binding of an agonist ligand such as DMPA to nuclear receptors (GR/PR); the p300 binds to the LBD through the LxxLL motif to cause transactivation. Based on this explanation, one can postulate that since p300 interacts with NFκB transcription factor by acting as a coactivator in NFκB p65 mediated transactivation, it is probable that the phosphorylated NFκBp65/p50: p300 complex in the nucleus could bind to nuclear receptors through the p300 to further regulate transcriptional activity (Figure 4.5B).

Sedwick (2014) recently reported that, just like the GR dimer would interact with NFαB p65 to cause transactivation, the GR monomer is also capable of exerting the same quality. Taken together, all these three mechanisms increase mRNA and protein secretion levels of proinflammatory cytokines in the ectocervical (Ect1/E6E7) epithelial cell line, region which is flooded by HIV-1 target cells (DCs and $CD4^+$ T) in the FRT (Louw-du Toit et al., 2014; Nguyen et al., 2014).

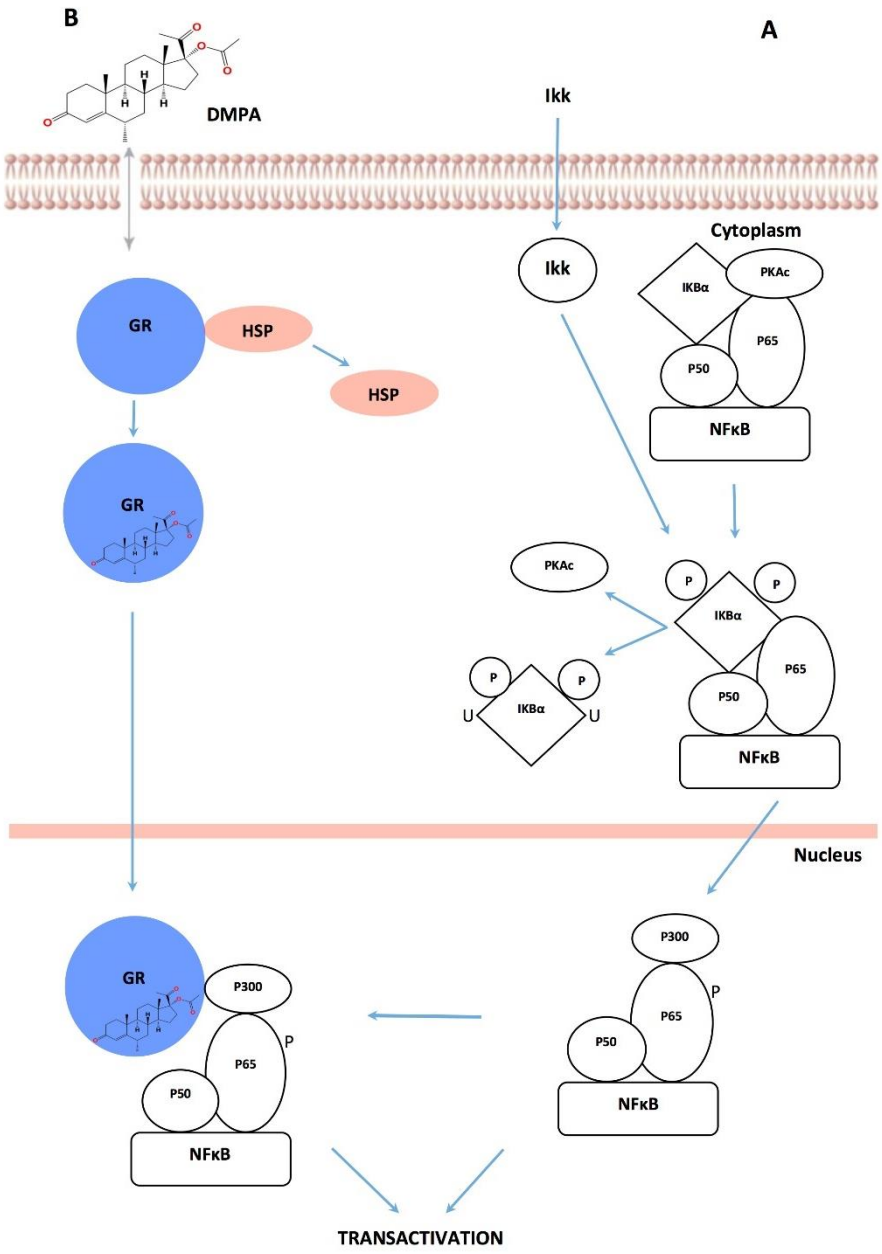

Figure 4.5. A, The NFκB p65/p50 heterodimer is in complex with IκBα and PKAc in the cytoplasm. But in the presence of IKK, IκBα is phosphorylated and exposed to ubiquitin pathway where it gets degraded. On the other hand, IKK causes PKAc to be activated which in turn phosphorylates the NFκB p65/p50. In the nucleus p300 interacts with NFκB transcription factor *via* the phosphorylated p65 subunit to cause transactivation. **B**, Binding of DMPA to the GR causes the HSP in the cytoplasm to dissociate allowing the DMPA-activated GR to translocate to the nucleus where it could interact with the phosphorylated NFκB p65/p50 *via* the p300 to cause further transcriptional activity. Figures adapted from Mckay et al (1999) and Louw-du Toit et al (2014).

4.12 Summary and conclusion

The effect of Depo-Provera when bound to both the GR and the PR is likely to be a major cause of an elevated risk of HIV-1 infection in the female reproductive tract. This is because nuclear receptors can interact with NFκB transcription factor in the nucleus as well as p300 which contains the RID. Within this RID is the LxxLL motif which is also present in the AF-2 domain located at the C-terminus within the LBD in nuclear receptors. This LxxLL motif causes transactivation by p300 to be AF-2 dependent which is interesting information because the AF-2 domain plays a crucial role in ligand regulation for transcriptional activity as it acts in the presence of a ligand and it's also a site where cofactors can be docked within the LBD.

Thus, the AF-2 domain associate with coactivator proteins upon activation of the receptor by the ligand. In addition to this, HIV transcriptional activation by Vpr which is the accessory protein of HIV-1 is known to be mediated by the p300 NFκB coactivator. Therefore, various mechanisms regulated and mediated by the NFκB and p300 through the GR and the PR are likely to cause transactivation upon binding of an agonist ligand as explained above. This increases mRNA and protein secretion levels of proinflammatory cytokines in the Ect1/E6E7 epithelial cell line, in turn, increasing the risk of HIV-1 infection. Further investigations can still be carried out regarding how much DMPA dose is responsible for causing an increase of HIV-1 infection in women.

4.13 Brief summary of docking approach

Crystal structures of the GR in complex with dexamethasone [PDB code: 1M2Z (Bledsoe et al., 2002)] & that of the PR in complex with progesterone [PDB code: 1A28 (Williams & Sigler, 1998)] were downloaded from the protein data bank (PDB) for subsequent simulation whereas on the other hand, the DMPA ligand was downloaded separately from the Zinc database (Irwin & Shoichet, 2005). Docking preparation was carried out in AutoDock Vina (Trott & Olson, 2010) whereby a grid box with the spacing and exhaustiveness of 1Å and 8, respectively was designed for each of the GR and PR systems based on residues involved in their LBDs. Then the centre & dimension values were recorded to carry out molecular docking.

4.14 Acknowledgements

The authors would like to acknowledge the University of KwaZulu-Natal, College of Health Sciences for funding and the Centre for High Performance Computing for computational resources.

4.15 Conflict of Interest

None declared.

4.16 References

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CHAPTER 5

Computational modelling of DMPA interactions with GR and PR in association with increased risk of HIV-1 infection in women

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Abstract

Over the 30 years since it was first identified, the HIV/AIDS epidemic has continued to increase across different countries. To date, women have become victims of HIV-1 infection as they use injectable hormonal contraceptives frequently, particularly depot medroxyprogesterone acetate (DMPA). This triggered a search as to which DMPA target is likely to contribute to the progression of HIV-1 pathogenesis. As per literature, DMPA targets both the Glucocorticoid receptor (GR) and the progesterone receptor (PR) in the female reproductive tract (FRT). However, fewer computational work has been performed regarding the interaction of DMPA and these targets as well as their association with HIV-1 infection in women.

Therefore, in this study we perform Molecular Dynamic (MD) simulation on both the GR and the PR systems in relation to DMPA as a way of validating their docking poses and binding energy trends. We also investigate the nature of their overall interaction themes using post-dynamic analysis. Our findings revealed that, the effect of DMPA binding to both the GR and PR could elevate the risk of HIV-1 infection in women. The reason being that when DMPA is bound to GR and PR, it interacts with a few residues in the ligand binding domain (LBD) which could affect the stability state of these receptors. Furthermore, the results showed that DMPA is more comfortable binding to the PR as compared to the GR.

Key words: HIV-1, DMPA, Glucocorticoid receptor, Progesterone receptor, Molecular docking, Molecular dynamics

5.1 Introduction

Since it was first noted in the 1980s, acquired immune deficiency syndrome (AIDS) constitute one of the most serious crises currently facing human development (Piot et al., 2001). The causative agent for this disease is Human Immunodeficiency Virus (HIV) which can further be divided into HIV-1 and HIV-2 (Sharp & Hahn, 2011). However, HIV-1 is the most prevalent in countries such as sub-Saharan Africa (SSA) where infected women account for about 59% (Takalani et al.,2017). The major common mode of transmission is by heterosexual sex and mother-to-child transmission (Palmisano & Vella, 2011; Piot et al., 2001). Within an estimation of 1.1 million children living with HIV in SSA, more than 90% were reported to have acquired the infection from their mother (Piot et al., 2001).

The emergence of HIV is not properly understood. However, it represents a major challenge to global public health (Piot et al., 2001). Given that the glucocorticoid receptor (GR) and the progesterone receptor (PR) are the main targets for DMPA (Figure 5.1) in the female reproductive tract (FRT) (Schindler et al., 2003), it is essential to perform *in silico* study of such interactions in association with increased risk of HIV-1 infection in women. Hence, we perform molecular docking and molecular dynamic (MD) simulations on the GR and PR protein systems in relation to DMPA to investigate their overall interaction themes using post dynamic analysis. Conducting MD simulations is advantageous in presenting conformational changes of a single molecule that has been examined over time and providing new insights to protein dynamics (Mhlongo & Soliman, 2015).

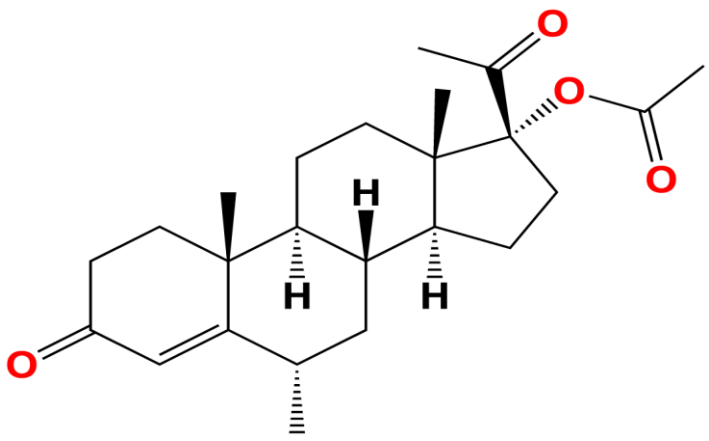

Figure 5.1. Two-dimensional structural representation of DMPA ligand (Irwin & Shoichet, 2005)

These insights could be gained by applying multiple-trajectory MD simulations as this enables conformational space sampling by reducing the impact of entrapment in local minima (Mhlongo & Soliman, 2015).

Herein, 50 ns multiple-trajectory MD simulations were employed to gain insight into the effect of DMPA on GR and PR. This was followed by binding free energy calculations, root mean square deviation (RMSD), root mean square fluctuation (RMSF) and radius of gyration (Rg) analyses. By conducting this research, we aim to provide new insights that will be useful in the development of new drugs against HIV/AIDS.

5.2 Computational methods

5.2.1 Preparation of protein structures

The 3D agonist crystal structures of the GR in complex with dexamethasone [PDB code: 1M2Z (Bledsoe et al., 2002)] & that of the PR in complex with progesterone [PDB code: 1A28 (Williams & Sigler, 1998)] were retrieved from the protein data bank (PDB) for subsequent simulation. The DMPA ligand was downloaded separately from the Zinc database (Irwin & Shoichet, 2005).

5.2.2 DMPA-GR and DMPA-PR complex preparations

Crystal structures obtained from the PDB contained multiple chains but we only made use of chain A which was bound to dexamethasone in the GR and to progesterone in the PR. Unwanted water molecules, ligands and other protein chains except chain A, were removed from both structures and chain A was kept to generate the binding pockets for DMPA-GR and DMPA-PR complexes. The DMPA ligand was prepared in Molegro Molecular Viewer (MMV) and in Chimera (Yang et al., 2012) software. Taken together, the binding pockets of DMPA-GR and DMPA-PR were designed based on dexamethasone-GR and progesterone-PR ligand binding domains (LBDs), respectively (Bledsoe et al., 2002; Williams & Sigler, 1998) (shown in Figure 5.2 and Table 5.1).

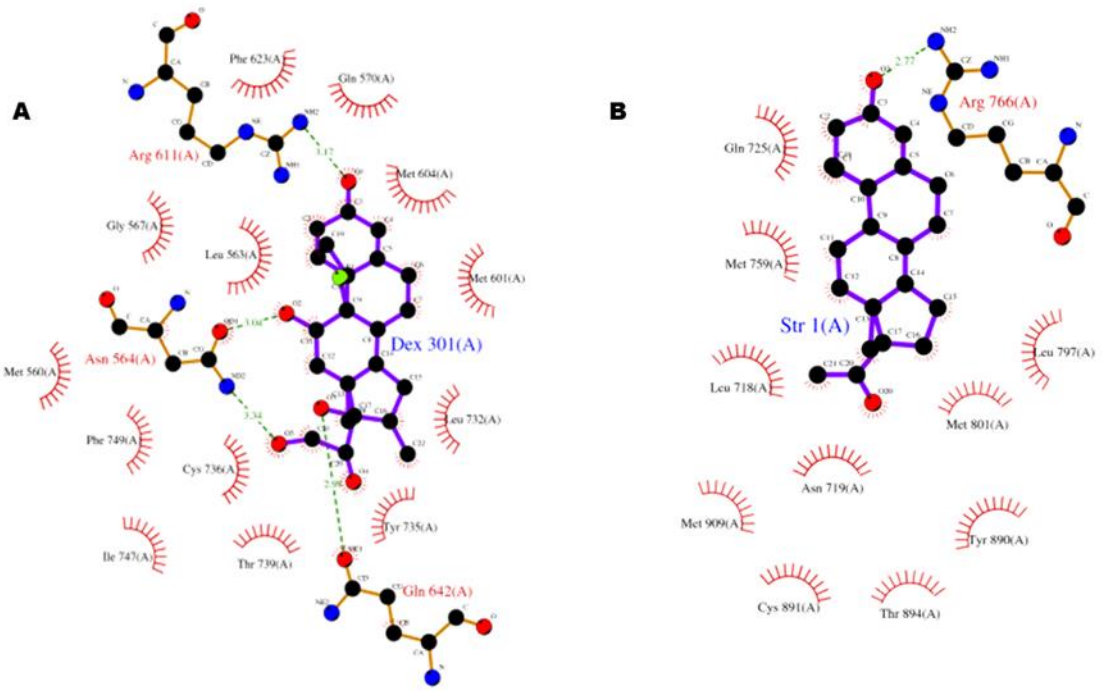

Figure 5.2. Ligplot diagrams showing A: Dexamethasone-GR (1M2Z) and B: Progesterone-PR interactions (1A28). Figures adapted from Wallace et al (1996).

5.2.3 Molecular Docking

To the best of our knowledge, there are no available x-ray structures of the GR and PR complexed to DMPA. Neither the "bound forms of agonistic PR and GR LBD structures" complexed to similar compounds with well-defined active sites. However, there are well-studied crystal structures (1M2Z and 1A28, as explained above) in the PDB bound to their respective agonist ligands (dexamethasone and progesterone, respectively). Hence, we made use of these structures to design the binding pockets of DMPA-GR and DMPA-PR, respectively since DMPA is also an agonist ligand. The docking procedure was conducted in AutoDock Vina software (Trott & Olson, 2010). For each of the GR and PR systems, a grid box (with the spacing and exhaustiveness of 1Å and 8, respectively) was designed to encompass the binding site residues shown in Figure 5.2A & B, respectively. Then the acquired grid box coordinates (shown in Table 5.1) were used to dock DMPA into GR and PR active site. Complexes of highest stability were analysed and saved (Figure 5.3) using Chimera software (Yang et al., 2012).

Table 5.1. Amino acid residues (Wallace et al.,1996) used to design the grid box for DMPA-GR and DMPA-PR protein systems, and the grid box coordinates for the respective systems.

Protein structures	LBD pocket residues	Grid box centre & dimensions	
GR (1M2Z)	Asn 564, Gln 570, Arg 611, Phe 749,	Centre	Dimension
	Gly 567, Gln 642, Thr 739, Ile 747,	X= -48.194	X= 24
	Leu 563, Met 560, Met 601, Tyr 735,	Y= 12.78	Y= 20
	Met 604, Leu 732, Cys 736, Phe 623.	Z= -43.095	Z= 26
PR (1A28)	Arg 766, Gln 725, Met 759,	Centre	Dimension
	Leu 718, Leu 797, Met 801,	X= 22.737	X= 18
	Asn 719, Met 909, Cys 891	Y= 9.318	Y= 26
	Thr 894, Tyr 890.	Z= 60.861	Z= 24

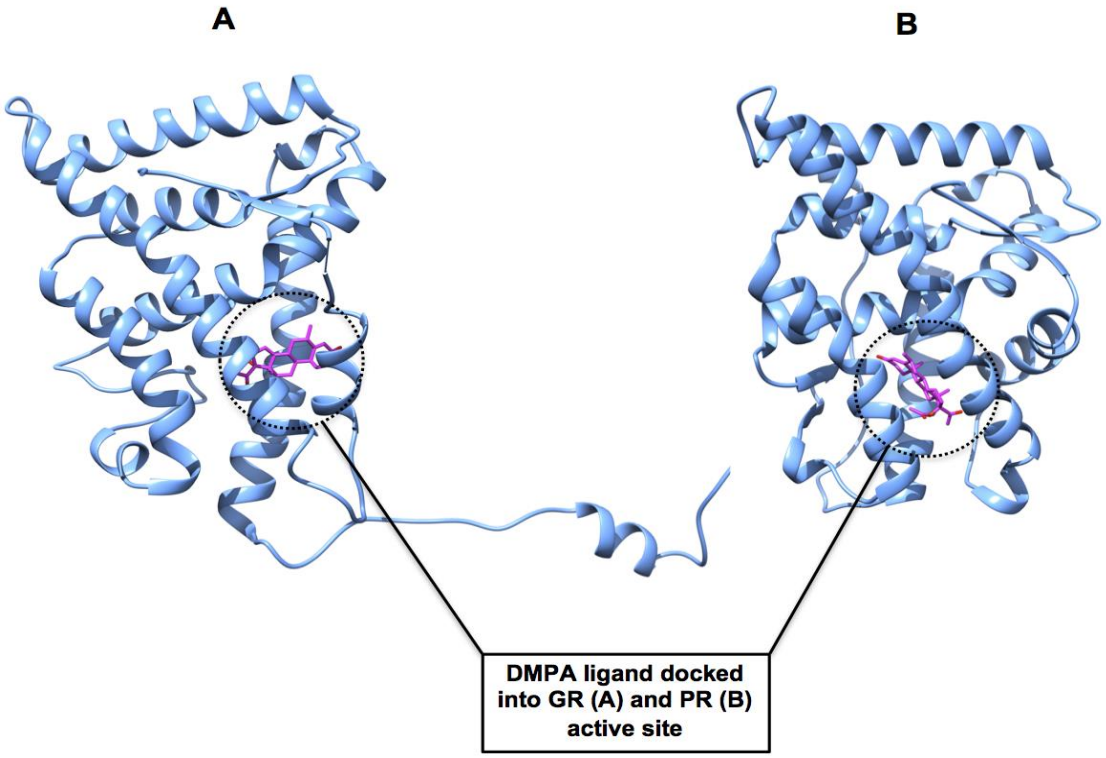

Figure 5.3. Graphical representation of DMPA ligand docked into the GR (A) and the PR (B) active site. Diagrams constructed using Chimera software (Yang et al., 2012).

5.2.4 Molecular dynamic (MD) simulation

Molecular dynamic simulations were performed using Amber 14-intergrated GPU version of PMEMD engine (Andreas et al., 2012; Case et al., 2015). The coordinate and topology files were created using LEaP and antechamber modules integrated in amber 14 (Case et al., 2015). Thus, Tleap programme (Case et al., 2015) and general amber force field (GAFF) (Wang et al., 2004) were employed. Hydrogen atoms and counter-ions were added to ensure the systems are neutralised. In addition to this, the FF14SB force field of Amber 14 suite (Lindorff-Larsen et al., 2010) was used to further define parameters in the protein systems. These systems were submerged in a TIP3P (Jorgensen et al., 1983) water box such that no protein atoms were within 10 Å of box dimensions during simulations.

Long-range electrostatic interactions with a direct space and van der Waals cut from 12 Å were treated using the particle mesh Ewald (PME) method (Harvey & De Fabritiis, 2009). Afterwards, a restraint potential of 500 kcal/mol $Å^2$ was applied to the solute to carry out partial minimization of 2000 steps (1000 steps using steepest descend method and another 1000 steps using conjugate gradients). This was followed by 1000 steps of full minimization with no restrain carried out by conjugate gradients algorithm. With a harmonic restrain potential of 5 kcal/mol $Å^2$ and a Langevin thermostat of 1/ps collision frequency, the systems were gradually heated from 0 to 300 K prior to minimization using a

canonical (NVT) ensemble MD simulation. Then isothermal isobaric (NPT) ensemble was used to equilibrate the systems at 300 K for 500 ps without restraint using Berendsen temperature coupling (Berendsen et al., 1984) to maintain pressure of the systems at 1 bar. The bonds of all hydrogen atoms at a time of 2 fs were constrained using SHAKE algorithm (Ryckaert et al., 1977) while all MD runs were performed using the SPFP precision model (Grand et al., 2013). Multiple 50 ns production MD runs were performed with a pressure coupling constant of 2 ps and a target pressure of 1 bar in an NPT ensemble at a temperature of 300 K.

5.2.5 Post dynamic analysis

Integrated with Amber 14 are the PTRAJ and CPPTRAJ programs (Case et al., 2015); these were used to analyse MD trajectories namely: root mean square deviation, Root of mean square fluctuation and Radius of gyration. Origin data software (http://www.originlab.com/) was used to plot results for analysis.

5.2.6 Thermodynamic calculations

The binding free energies of the GR and PR systems were calculated using the MM/PBSA method (Sun et al., 2014). From a 50 ns production run, these binding free energies were calculated considering 1000 snapshots. Binding free energy calculations tell us about the ligand-receptor interactions and equations to describe such interactions are given by:

$$\Delta G_{bind} = G_{complex} - G_{receptor} - G_{ligand} \quad (1)$$

$$\Delta G_{bind} = E_{gas} + G_{sol} - T\Delta S \quad (2)$$

$$E_{gas} = E_{int} + E_{vdw} + E_{ele} \quad (3)$$

$$G_{sol} = G_{PB} + G_{SA} \quad (4)$$

$$G_{SA} = \gamma_{SASA} \quad (5)$$

Where E_{gas} is equivalent to energy gas phase evaluated from the FF14SB force field directly; E_{int} is equivalent to bond, angle & dihedral internal energies; and E_{vdw} and E_{ele} are equivalent to van der Waals and electrostatic energies, respectively. The solvation free energy is denoted by G_{sol} which can further be divided into polar electrostatic solvation energy (G_{PB}) and non- polar electrostatic solvation energy components (G_{SA}). A water probe radius of 1.4 Å is used to determine G_{SA} estimated from the solvent accessible surface area (SASA). The $T\Delta S$ is equivalent to the conformational temperature and total solute entropy upon binding.

5.3 Results and discussion

5.3.1 Validation of molecular docking

Apart from DMPA, there is experimental evidence (Hudson et al.,2007; Schindler et al., 2003; Zhang et al.,2007) of other agonist ligands which have been reported to interact with both GR and PR. This includes dexamethasone and prednisolone in the GR system as well as progesterone and norethindrone in the PR system. Hence, we retrieved half maximal effective concentration (EC_{50}) values (shown in table 5.2) from the Binding Database (Chen et al., 2001; Chen et al., 2002) for these ligands complexed to their respective receptors and correlated them with their docking scores to further validate their docking poses. Docking was calculated as explained in section 5.2.3. The docked complexes and docking scores are shown in figure 5.4 and table 5.2, respectively.

It is worth noting that less information provided in table 5.2 is due to a limited number of agonist ligands that interact with the GR and PR. To the best of our knowledge, these are the most studied agonist ligands for GR and PR with available EC_{50} values and that have also been approved by the food and drug administration (FDA). The GR system revealed an excellent correlation coefficients (R^2) of 0.83219 which can be attributed to the fact it is well studied, unlike the PR system which revealed a poor correlation of 0.5021. The results for these correlations are presented graphically in Figure 5.5 and 5.6, respectively.

The binding poses of GR and PR to ligands presented herein are consistent with previous experimental work carried out by Zhang et al (2007) and Hudson et al (2007). Per Neubig et al (2003), the EC_{50} value measures the potency of the drug hence it depends on both the affinity of a drug for its receptor as well as the efficacy which explains the drug-receptor interaction due to maximum biological effect (Neubig et al., 2003). The smaller the EC_{50} value, the more potent is the drug. Therefore, it can be deduced from our docking results and the EC_{50} values that DMPA has a higher binding affinity for the PR as compared to its affinity for the GR hence it is more potent when complexed to the PR.

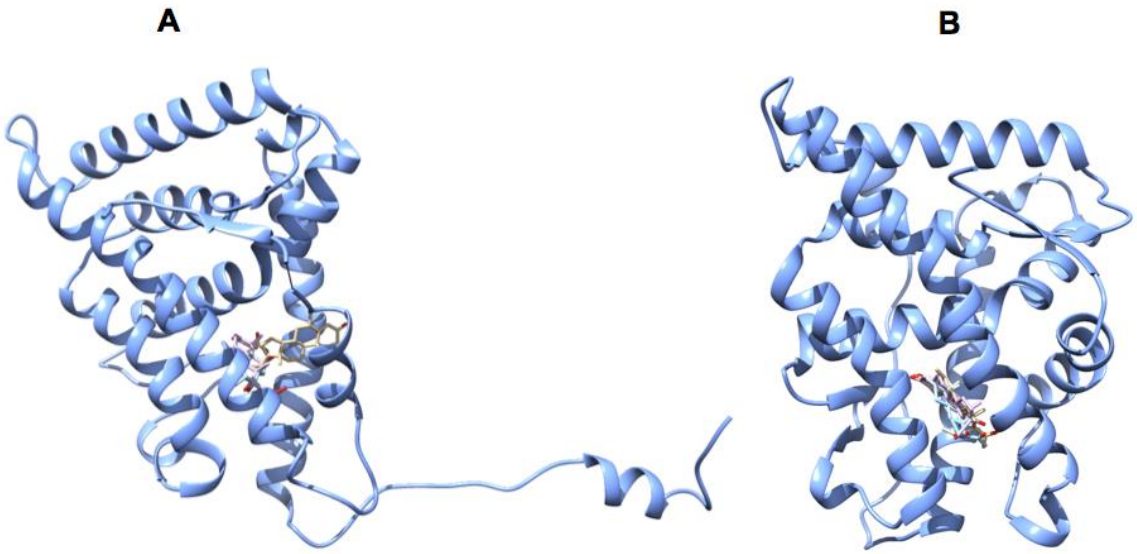

Figure 5.4. A, the GR protein structure docked with dexamethasone (light purple), prednisolone (light blue) and DMPA (brown). B, the PR protein structure docked with norethindrone (light blue), progesterone (light purple) and DMPA (brown). Diagrams constructed using Chimera software (Yang et al., 2012).

Table 5.2. Docking scores (DS) and EC_{50} values of the GR & PR systems with their respective ligands

Ligand	**DS (Kcal/mol)**	**EC_{50}(nM)**
Glucocorticoid receptor (GR)		
Dexamethasone	-8.8	0.2 nM
Prednisolone	-8.4	2.1 nM
DMPA	-8.1	10 nM
Progesterone receptor (PR)		
DMPA	-9.7	0.1 nM
Progesterone	-11.2	0.9 nM
Norethindrone	-11.0	2.2 nM

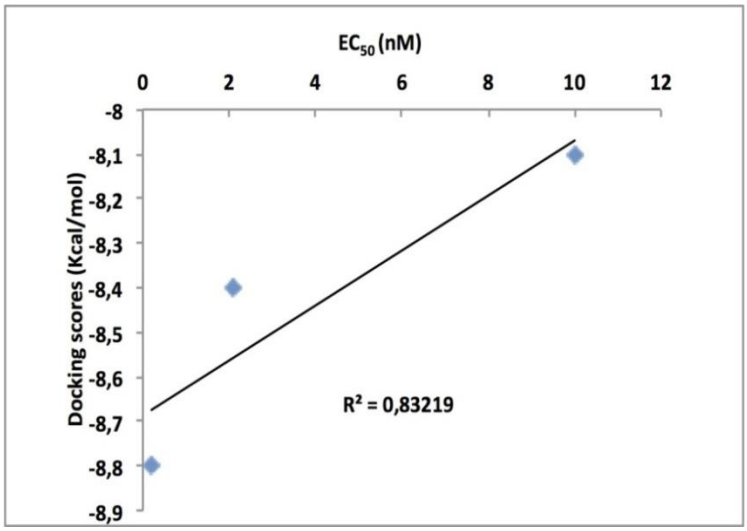

Figure 5.5. Correlation graph showing docking scores (Kcal/mol) versus EC_{50} (nM) values for the GR system.

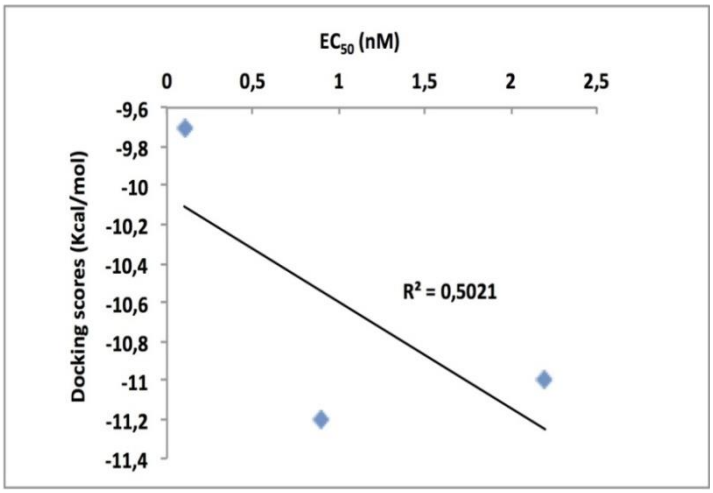

Figure 5.6. Correlation graph showing docking scores (Kcal/mol) versus EC_{50} (nM) values for the PR system.

5.3.2 Ligplot diagrams of DMPA interactions with GR and PR protein

Given that DMPA is an agonist and agonist molecules bind in the bottom half of the LBD (Bledsoe et al., 2002; Nicolaides et al., 2010); DMPA was found to interact with which corresponds to Met 227 & Ile 231 in the active site of the GR system (Figure 5.7A) corresponding to M752 & I756, respectively in the human glucocorticoid receptor (hGRa) LBD sequence alignment (Figure 5.8). In the PR system (Figure 5.7B), residues Cys 210, Phe 224 & Met 228 interact with the ligand at the active site, corresponding to C891, F905 & M909, respectively in the human progesterone receptor (hPRb) LBD sequence alignment (Figure 5.8).

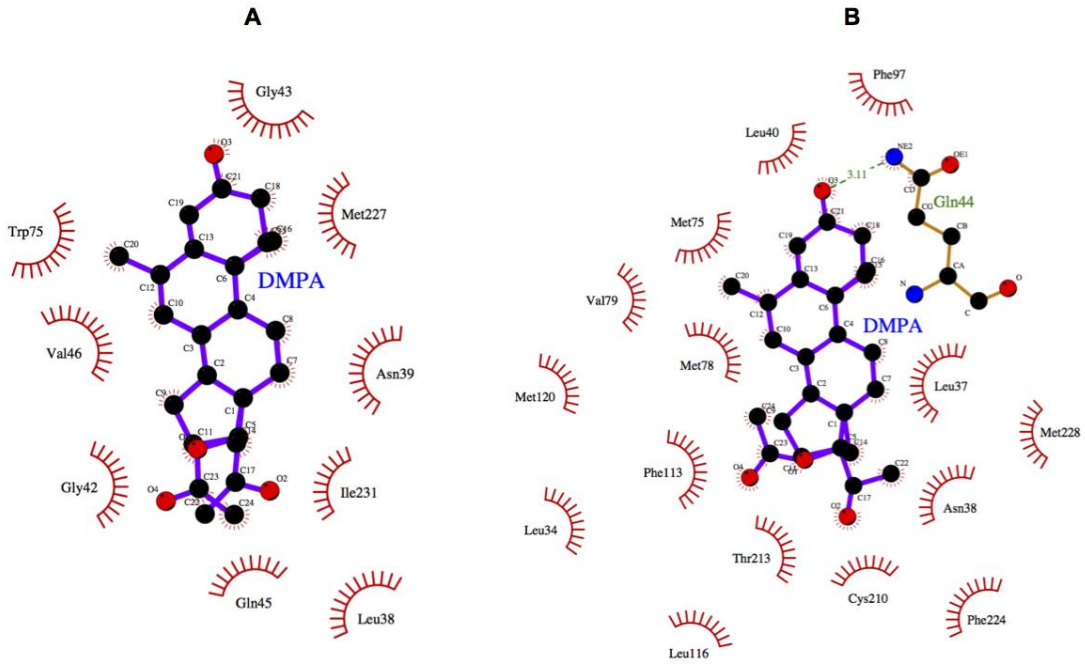

Figure 5.7. Ligplot diagram showing, A: DMPA-GR and B: DMPA-PR interactions.

Figure 5.8. Sequence alignment of LBDs from selected steroid and nuclear receptors. The secondary structure determined by crystallography is in green (α helix) and blue (β sheet) whereas residues are highlighted by function: magenta (hormone binding) & yellow (dimerization). Navy blue and red triangles show the position of the intermolecular disulphide bond and the C-terminus of the HERαLBD used for crystallization, respectively. The activation function 2 core and "signature sequence" are underlined in red and navy blue, respectively. Figure adapted from Tanenbaum et al (1998).

5.3.3 Post dynamic analysis of DMPA-GR and DMPA-PR complexes

5.3.3.1 Root mean square deviation (RMSD)

RMSD of the C-alpha backbone for DMPA with respect to GR and PR was monitored throughout the 50ns simulations to ensure stability within the systems. Apo (protein only) and bound (protein & ligand) average RMSD for the GR system was 6.83Å and 6.77Å, respectively. At the beginning of the simulation, apo conformation revealed a fluctuation of ~2.5 Å whereas bound conformation was stable from 4-30 ns and later on revealed a fluctuation of ~3Å within 30-40 ns. (Figure 5.9). These results demonstrated that DMPA is structurally related to GR. However, a great fluctuation within 30-40 ns towards the end of the simulation shows that the GR system could have undergone some conformational changes which could have resulted in the loss of stability of this complex.

This can be explained by the interaction of DMPA with residues Met 752 and Ile 756 in the GR system as revealed in section 5.3.2. Both these residues are located in helix 12 (H12) of the hGRa LBD sequence alignment which is crucial in the formation of ligand binding pocket and the activation function-2 (AF-2) surface that facilitates interaction with coactivators (Nicolaides et al., 2010). Thus, upon binding of an agonist the receptor undergoes large conformational changes leading to alterations of H11 and H12 positions and the formation of an interaction surface that allows coactivators to bind to the AF-2 through their LxxLL motifs (Nicolaides et al., 2010).

In contrast to the GR system, apo and bound average RMSD for the PR system was 1.13 Å and 1.40 Å, respectively (Figure 5.10). It is clear that average RMSD value for the apo form is lower than that of the bound form which can be attributed to DMPA interacting with Asn 38 as shown in the ligplot diagram (Figure 5.7B), corresponding to Asn 719 in the hPRb sequence alignment. Recent studies carried out by Zheng et al (2016) revealed that this is one of the key residues that has been found to be crucial for the dynamics of helix 12, helix 11 and the loop between them (895-908) resulting in stable apo-conformations of PR-LBD (Zheng et al., 2016). RMSD for this PR system was reasonably stable around 1 Å which confirms stable trajectories.

Unlike in the GR system where residues interacting with DMPA in H11/H12 positions revealed large conformational changes, residues C891 (located in H11), F905 and M909 (located in H12) interacting with DMPA in the PR system resulted in a higher binding affinity and a more stable complex. Therefore, the large difference observed between RMSD behaviour of DMPA-GR and DMPA-PR confirms that progestins (DMPA in this case) bind better to the progesterone receptor as compared to the glucocorticoid receptor (Schindler et al., 2003). In addition to this, the ligand binding pocket of PR has

been reported to be flexible enough to accommodate ligands with different sizes and chemical species (Zheng et al., 2016).

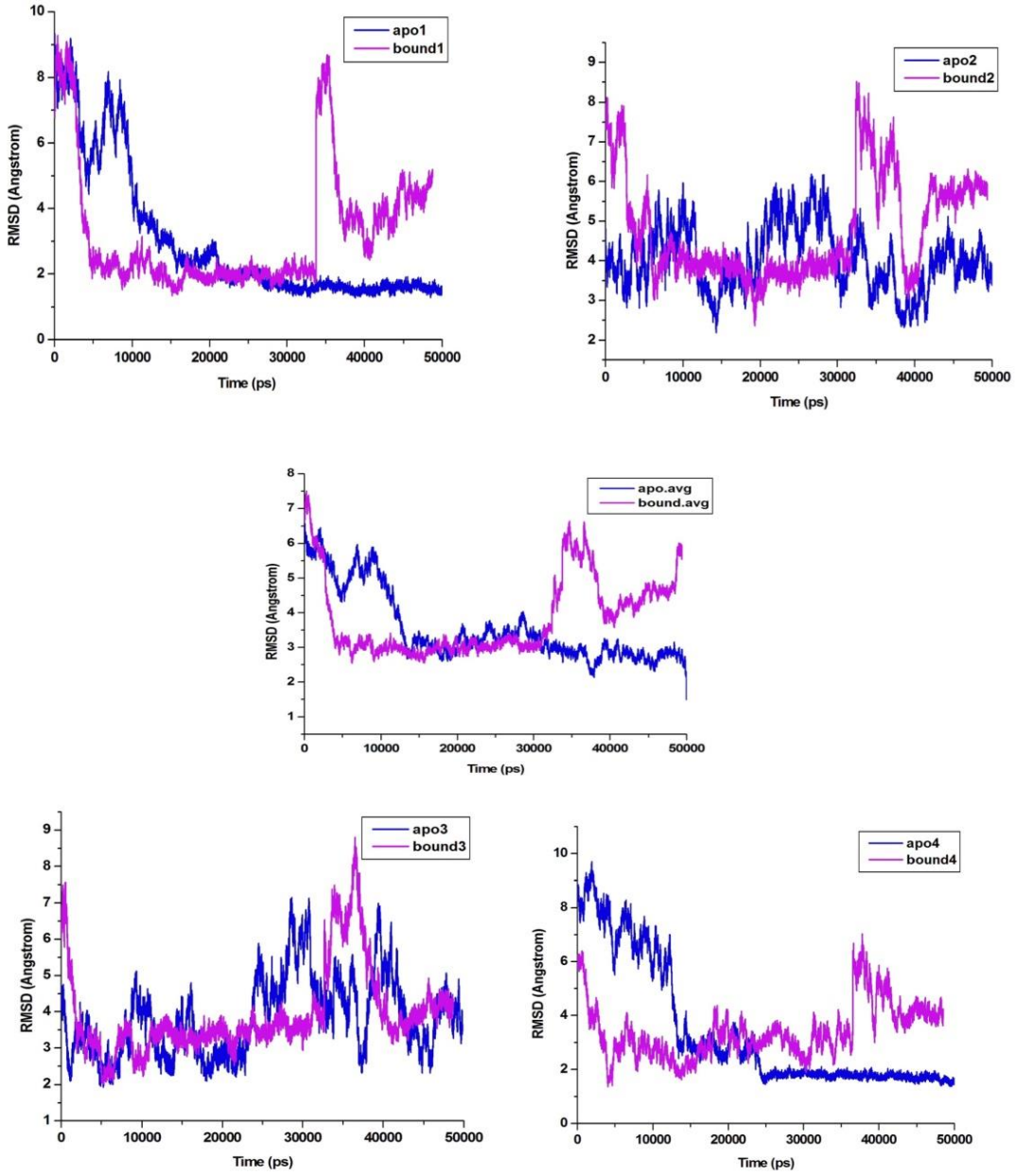

Figure 5.9. RMSD for DMPA-GR showing stability of apo and ligand bound conformations. The average for apo and bound were 6.83Å and 6.77Å, respectively.

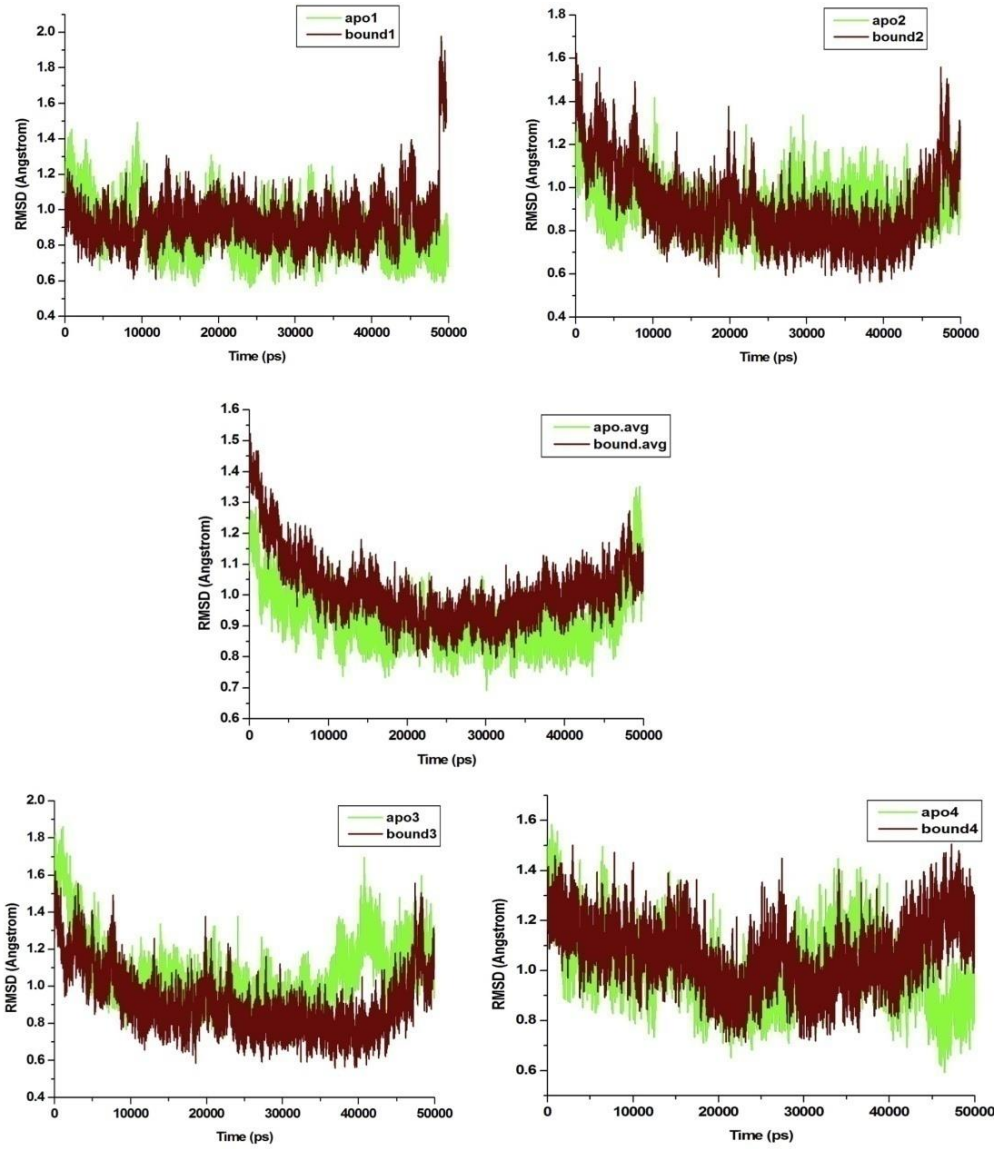

Figure 5.10. RMSD for DMPA-PR showing stability of apo and ligand bound conformations. The average for apo and bound were 1.13Å and 1.40Å, respectively.

5.3.3.2 Root of mean square fluctuation (RMSF)

RMSF of the C-alpha atoms was calculated to examine the dynamic movement of amino acid residues in the GR and PR systems. Figure 5.11 shows DMPA-GR average RMSF; smooth RMSF was observed within residues 20-80 which can be attributed to DMPA interacting with residues Asn 39 and Gln 45 as shown in figure 5.7A, corresponding to N564 and Q570 in the hGRa sequence alignment. These residues have been shown to contain hydrogen bonds which are important for holding the backbone of the steroid tightly in position (He et al., 2014). Thus, N564 interacts with the C-11 hydroxyl group of the steroid C ring whereas Q570 interacts with the C-3 keto group of the steroid A ring (He et al., 2014). This was followed by small fluctuations within residues 80-100 which per Bledsoe et al (2002) and He et al (2014) can be attributed to the presence of Phe 81, Phe 88 and Phe 98 within this region,

corresponding to F606, F613 and F623, respectively in the hGRa sequence alignment. Phenylalanine residues have been associated with solubility problems in the glucocorticoid receptor which may cause local instability that makes the protein prone to aggregation or misfolding (Bledsoe et al., 2002; He et al.,2014). Lastly in the RMSF-GR plot, we observed high fluctuations of ~2-2.5 Å at residues Glu180 and Met220, corresponding to E705 and M745 in the hGRa sequence alignment. Based on the explanation provided in section 5.3.3.1 concerning alterations that occur in the H12 position upon binding of an agonist, it is clear that residue M745 also contributed to the large conformational changes that resulted upon binding of DMPA as it is also located in the H12 position and it revealed a high fluctuation.

As for E705, long side chains of glutamic acids located in helix 9 of the GR have also been associated with solubility problems that may hinder crystallization. However, this residue is far away from the ligand binding pocket hence it doesn't affect ligand-mediated GR transactivation or transrepression functions (He et al., 2014). In contrast to the GR system, DMPA-PR average RMSF (Figure 5.12) revealed much flexibility at residues K26, K109, K180, K238 and K245 corresponding to K707, K790, K861, K919 and K926 in the hPRb LBD sequence alignment, respectively. From these results it can be deduced that Lysine residues seem to play a major role of high conformational flexibility in the DMPA-PR system. This can be attributed to change in loop dynamics of 703-712, 785-808 and 925-927 which may be correlated with ligand binding in agonistic conformation of the PR LBD (Williams and Sigler, 1998; Zheng et al., 2016).

Per Williams and Sigler (1998), the C-terminal extension (residues 922-933) which is crucial for hormone binding is tightly fixed in position by an antiparallel β-sheet interaction between amino acids 925-927. K919 on the other hand which is found in helix 12 has been reported to stabilize helix 12 in the apo-form of PR-LBD through its interaction with Glu 723 in helix 3 (Zheng et al., 2016) whereas K861 which is found in helix 11 gets altered upon binding of an agonist to form an interaction surface that allows coactivators to bind to the AF-2 through their LxxLL motifs (Nicolaides et al., 2010).

Therefore, a large difference observed between average RMSF values of DMPA-GR and DMPA-PR shows that even though residues in the GR system didn't seem to fluctuate much as compared to the PR system, those that fluctuated (F606, F613, F623, E705 and M745) caused large conformational changes leading to some loss of its stability. Whereas large fluctuations observed in the PR system which were due to lysine residues didn't disturb much of the stability state of this system, confirming that DMPA is indeed more comfortable binding to the PR as compared to the GR.

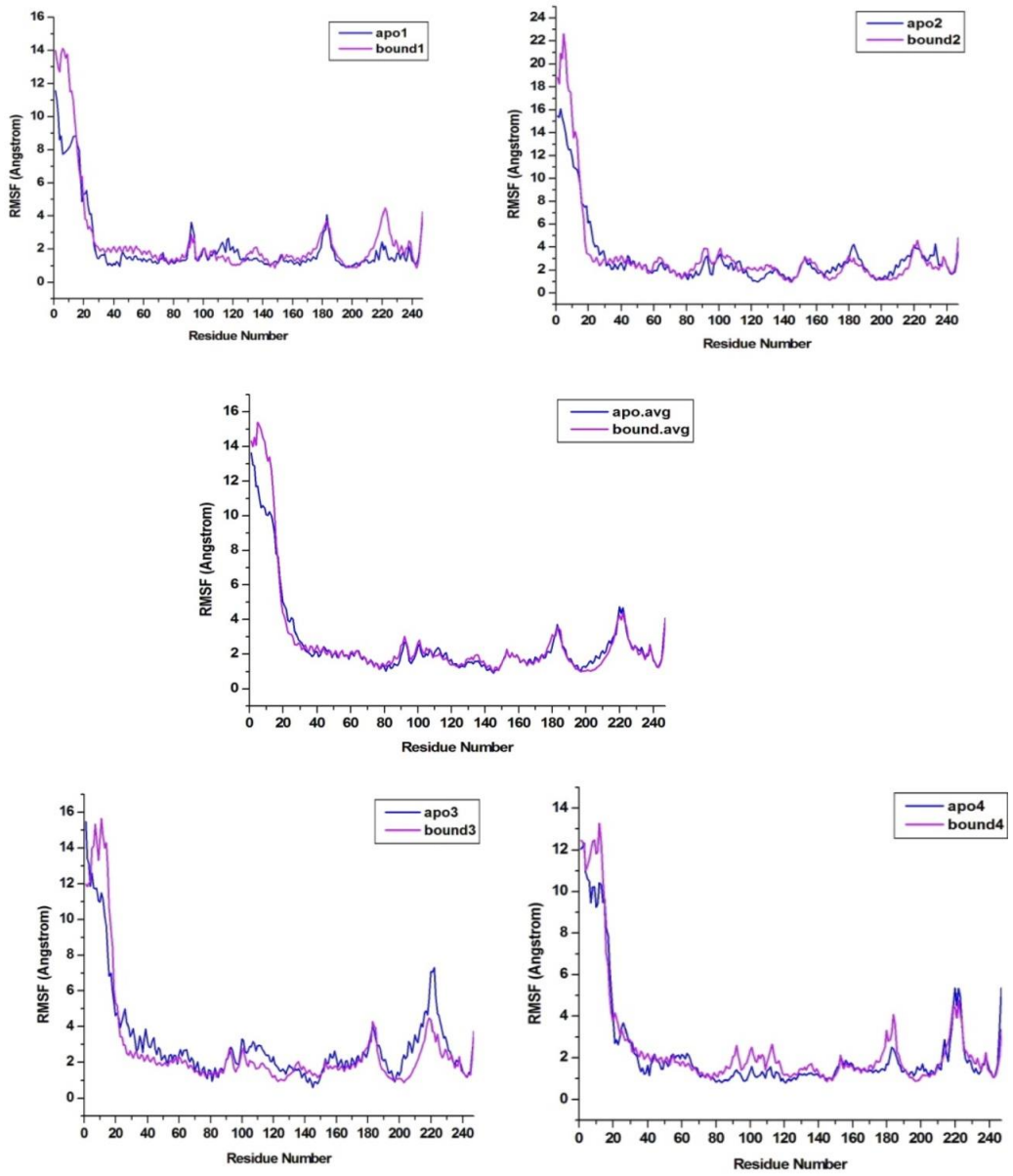

Figure 5.11. RMSF for DMPA-GR and apo conformations. The average for apo and bound were 13.63Å and 14.31Å, respectively.

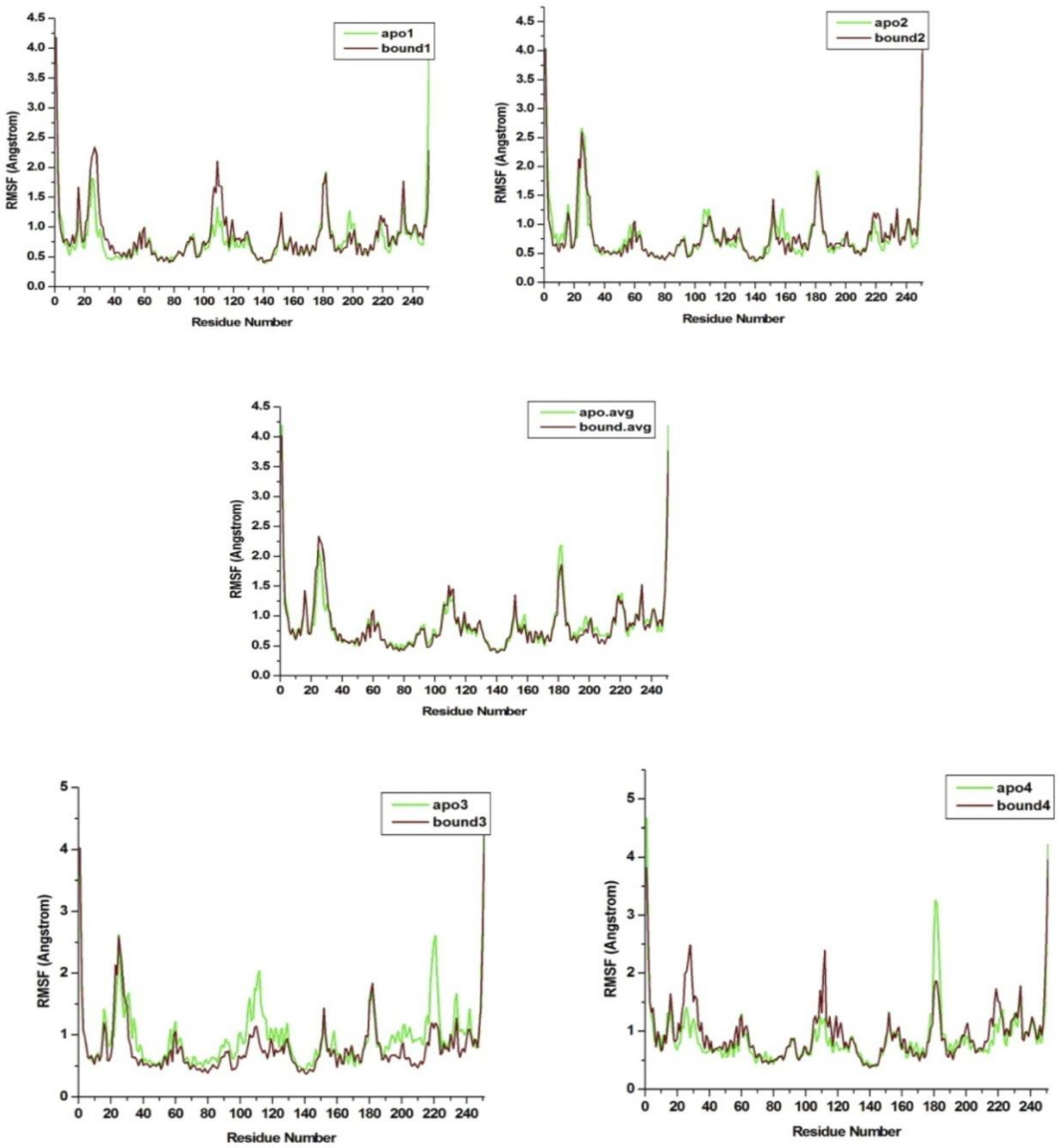

Figure 5.12. RMSF for DMPA-PR and apo conformations. The average for apo and bound were 4.19Å and 4.01Å, respectively.

5.3.3.3 Radius of gyration

The radius of gyration (Rg) provides insight into the stability state of the system. However, this is a parameter linked to tertiary structural volume of a protein. Herein, average Rg for DMPA-GR was stable from 7-31 ns followed by a great fluctuation of ~3 Å within 31-41 ns (Figure 5.13). On the other hand, average Rg for DMPA-PR revealed a more compact system from the beginning of the simulation until the end, which confirms very high stability (Figure 5.14). These results are consistent with both our RMSD and RMSF results explained in section 5.3.3.1 and 5.3.3.2, respectively. Thus, a great fluctuation observed within 31-41 ns in the GR system could be explained by the H12 alterations which

occurred upon binding of DMPA whereas high stability in the PR system could be explained by a high number of lysine residues interacting with DMPA. The overall Rg profile for both DMPA-GR and DMPA-PR (apo and bound) showed large variations during the simulation time which indicates that both proteins are much flexible. This is also in accordance with previous reports which revealed that during MD simulations, radical conformational changes occur upon ligand binding (Kumalo & Soliman, 2016).

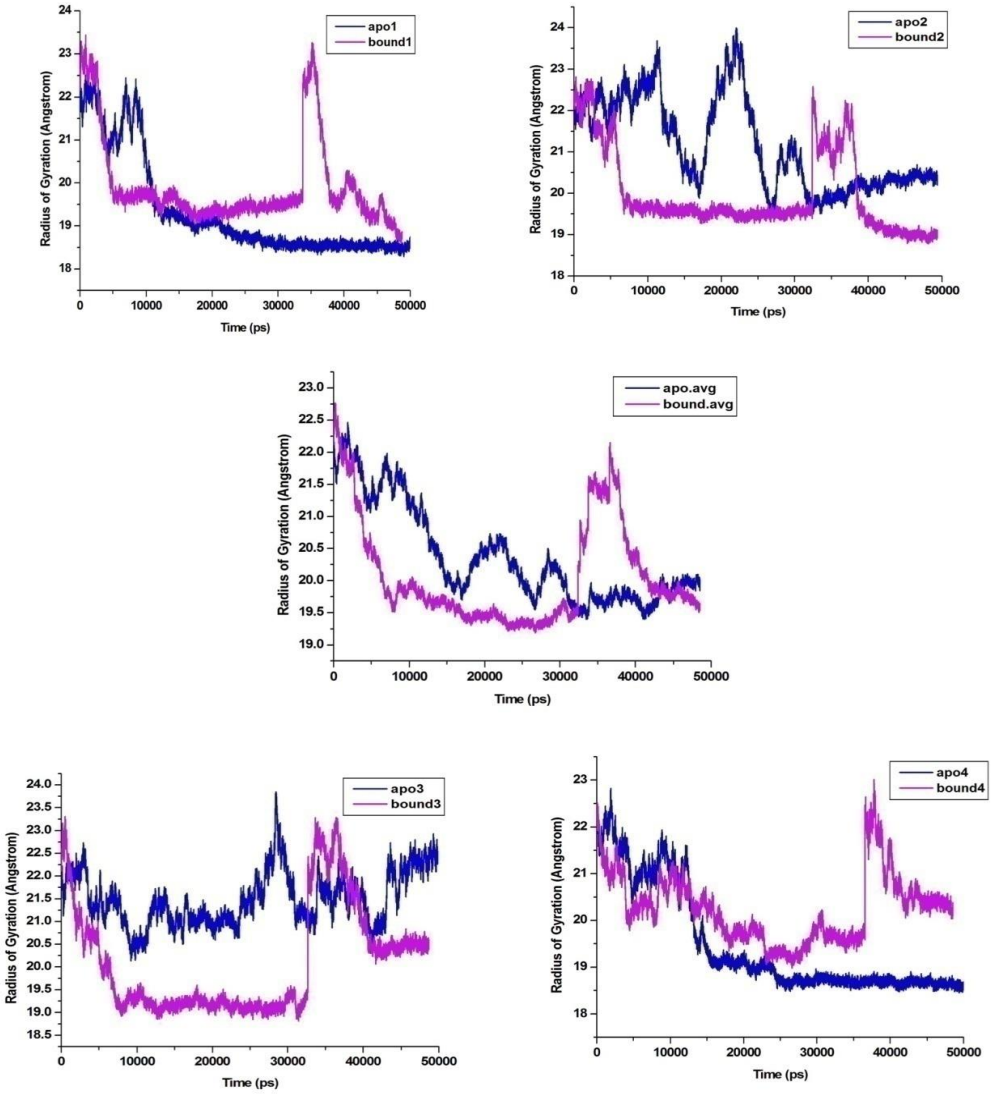

Figure 5.13. Rg of DMPA-GR and apo conformations. The average for apo and bound were 22.20Å and 22.25Å, respectively.

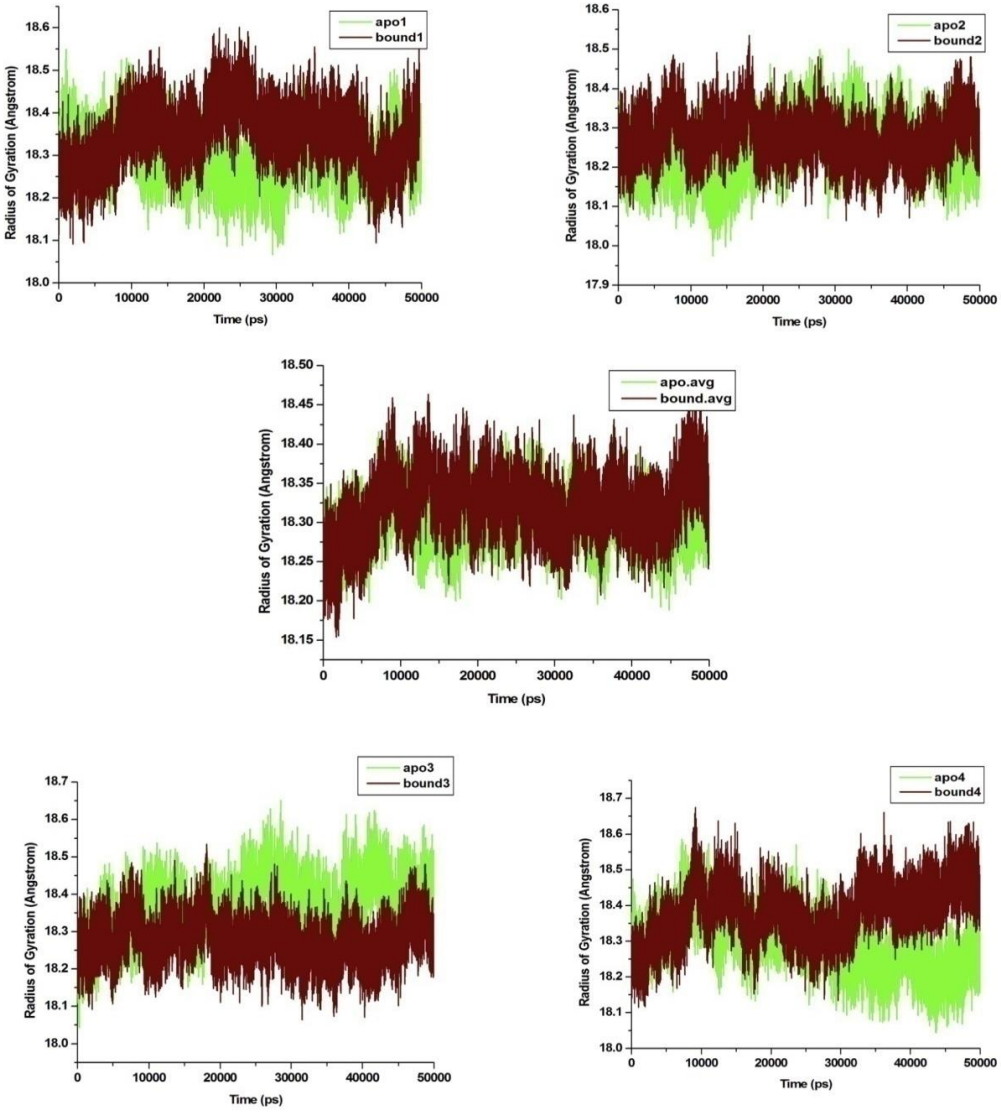

Figure 5.14. Rg for DMPA-PR and apo conformations. The average for apo and bound were 18.22Å and 18.27Å, respectively.

5.3.4 Thermodynamic calculations

Binding free energy calculations for complexed systems (DMPA-GR and DMPA-PR) were conducted using the MM/PBSA method as is presented in Table 5.3. Average binding free energy (ΔG_{bind}) of DMPA-GR was found to be -42.7928 kcal mol^{-1} whereas that of DMPA-PR was -56.6511 kcal mol^{-1}. Thus, the overall binding free energy of DMPA-PR was higher than that of DMPA-GR by ~ 14 kcal mol^{-1}. On the other hand, ΔE_{ele} (electrostatic) energy contributions to total binding free energy was revealed to be -3.9171 kcal mol^{-1} in the GR and -9.1289 kcal mol^{-1} in the PR, while ΔE_{vdw} (van der Waals) contributions were revealed to be -50.7022 kcal mol^{-1} and -59.1000 kcal mol^{-1} in the GR and PR, respectively. Moreover, ΔG_{gas} (bond, angle and dihedral energies) contributions were -54.6193 kcal

mol^{-1} in the GR and -68.2290 kcal mol^{-1} in the PR while ΔG_{sol} (polar and non-polar electrostatic solvation energies) contributions were 11.5766 kcal mol^{-1} and 11.5778 kcal mol^{-1} in the GR and PR, respectively. It is worth noting that contributions of all components to binding free energy were also higher in the DMPA-PR system than in the DMPA-GR system. Thus, contributions of all components to binding free energy were higher in the DMPA-PR system than in the DMPA-GR system with the overall binding free energy of DMPA-PR higher than that of DMPA-GR by ~ 14 kcal mol^{-1}. This is in great accordance with what has been reported by Govender et al (2014) that DMPA is a high affinity PR agonist and a full to partial GR agonist.

Table 5.3. Binding free energy calculations (MM/PBSA) of DMPA-GR and DMPA-PR complexes

Complexes	ΔG_{bind}	ΔE_{ele}	ΔE_{vdw}	ΔG_{gas}	ΔG_{sol}
GR bound1	-43.4264±0.2164	-4.6704±0.2418	-51.3190±0.2254	-55.9893±0.3203	12.5626±0.2285
bound2	-42.3400±0.2320	-4.4488±0.2424	-50.3101±0.2288	-54.7589±0.3679	12.4189±0.2343
bound3	-44.3741±0.2337	-2.4663±0.1888	-52.4331±0.2338	-54.8994±0.2694	10.5253±0.1829
bound4	-41.0307±0.1960	-4.0829±0.2284	-48.7467±0.1923	-52.8296±0.3157	10.7989±0.2254
bound average	**-42.7928**	**-3.9171**	**-50.7022**	**-54.6193**	**11.5766**
PR bound1	-56.5561±0.1645	-9.6677±0.1320	-58.8929±0.1491	-68.5606±0.2032	12.0045±0.1138
bound2	-56.9602±0.1686	-8.3772±0.1189	-59.4803±0.1584	-67.8575±0.1923	10.8973±0.0991
bound3	-56.9602±0.1686	-8.3772±0.1189	-59.4803±0.1584	-67.8575±0.1923	10.8973±0.0991
bound4	-56.1281±0.1784	-10.0937±0.1278	-58.5468±0.1679	-68.6405±0.1885	12.5124±0.0977
bound average	**-56.6511**	**-9.1289**	**-59.1000**	**-68.2290**	**11.5778**

5.4 Understanding the mechanisms of action of GR and PR upon binding of DMPA in association with increased risk of HIV-1 infection in women

The GR and PR are classified under steroid hormone receptors (SRs) which belong to the large nuclear receptor (NR) family (Fang et al., 2006). These NRs are transcription factors which get activated by hormones that bind through a ligand binding pocket located within the LBD. The LBD is made up of two transactivation domains, namely AF-1 and AF-2 (Nicolaides et al., 2010). The AF-1 domain is ligand-independent whereas the AF-2 domain acts in the presence of a ligand hence it plays a major role in ligand regulation for transcriptional activity. This AF-2 domain is located at the C-terminus within the LBD in nuclear receptors and it contains the LxxLL motif through which it interacts with some cofactors (Takalani et al., 2017).

Most often, unliganded receptors are located either in the cytoplasm or in the nucleus where they are complexed with heat shock proteins (HSPs). However, upon binding of a ligand these HSPs dissociate allowing receptors to form dimers competent to bind DNA at hormone response elements (HREs) (De Bosscher et al., 2008; Aranda and Pacual, 2001). During transcriptional control of gene expression by nuclear receptors, coactivators or corepressors are required to stimulate or repress, respectively, expression of the target gene (Sementchenko and Watson, 2000). However, it is worth noting that coactivators do not bind to DNA directly hence they associate with histone acetyltransferases (HATs).

Per Barnes et al (2005), HATs control histone acetylation which is a process whereby DNA unwinds due to change in charge of the core histones around which DNA is wound in the chromatin structure of nucleosomes. This change is associated with the presence of lysine residues in histones which loosen their association with DNA (Barnes et al., 2005). Thus the chromatin structure opens up, allowing transcription factors that couldn't bind DNA in a closed chromatin configuration and RNA polymerase II to bind, resulting in enhanced transcriptional activity. Conversely, corepressors together with histone deacetylases (HDACs) which control histone deacetylation reverse this acetylation process, resulting in gene silencing and decreased transcriptional activity (Barnes et al., 2005).

Aranda and Pascual (2001) reviewed more interesting information on coactivators and histone acetylation; they revealed that Creb Binding Protein/p300 (CBP/P300) and p160 complexes function as enzymes hence, they have the ability to modify chromatin structure by acetylation (Aranda and Pascual, 2001). The interaction of CBP/300 with nuclear receptors has been identified both in vivo and vitro and it was found that CBP/P300 binds directly and indirectly to nuclear receptors (Spencer et al., 1997). Direct binding is through interaction with the NH_2-terminal whereas indirect binding is achieved by associating with p160 coactivators through the COOH-terminal region (Aranda and Pascual, 2001). In the case of p160 coactivators, steroid receptor coactivator-1 (SRC-1) possesses histone acetyltransferase activity that maps the COOH-terminal region (Spencer et al., 1997). Moreover, SRC-1 interacts with various nuclear receptors in an agonist and AF-2 dependent manner stimulating transcriptional activity in mammalian cells and yeasts (Aranda and Pascual, 2001).

Given that HIV-1 provirus is packed into chromatin after integration in the host cell genome (Van Lint et al.,1996) and that HIV transcriptional activation by Viral Protein R (Vpr); an accessory protein of HIV-1 is mediated by the p300 transcription factor with HATs activity (Felzien et al., 1998;), one can postulate that conformational changes observed in RMSF plot of the DMPA-PR system (Figure 5.12) which were due to a high number of lysine residues resulted in histone acetylation and enhanced transcriptional activity. Thus, upon binding of an agonist DMPA to PR, the PR recruited CBP/P300:SRC-1 coactivator complex which associated with AF-2 at the C-terminal region. Because the CBP/P300:SRC-1 coactivator complex possesses HAT activity, this could have resulted in

chromatin decondensation and gene activation. It is worth noting that hyperacetylation is only triggered by agonists and is AF-2 dependent (Aranda and Pascual, 2001). The HAT activity is important for gene activation as mutation or removal of the HAT domain leads to loss of function for many transcription factors (Aranda and Pascual, 2001). Apart from SRC-1, P300/CBP-Associated Factor (p/CAF) which has also been reported to contain strong HAT activity, binds directly and indirectly to CBP/300 and nuclear receptors (Blanco et al., 1998; Korzus et al., 1998). Per Korzus et al (1998), the p/CAF HAT activity for nuclear receptor activation appears to be indispensable (Korzus et al., 1998).

On the other hand, p300 has been reported to contain the LxxLL motif within its receptor interacting domain (RID) through which it interacts with the AF-2 domain in nuclear receptors to regulate transcriptional activity (Aranda and Pascual, 2001). Interestingly, this AF-2 domain is located within helix 12 (H12) in the LBD which gets altered upon binding of an agonist in order to create an interface for NR coactivators to bind (Hellal-Levy et al., 2000; Nicolaides et al., 2010). Therefore, we can also postulate that large conformational changes observed in the GR system at residues Met 752 and I756 (Figure 5.9) and at residue M745 (Figure 5.11) found in H12 position of the hGRa sequence alignment resulted from helix 12 alteration as the GR associate with CBP/P300:SRC-1 coactivator complex upon binding of DMPA.

Conversely, a higher binding affinity observed in the PR system at residues F905 and M909 in Figure 10 and at residues K919 and K926 in Figure 5.12 found in H12 position of the hPRb sequence alignment can also be attributed to helix 12 alterations upon binding of DMPA as the PR associate with CBP/P300:SRC-1 coactivator complex. Taken together, mechanisms postulated herein could result in an enhanced ligand-dependent transcriptional activity which could in turn lead to increased risk of HIV-1 infection in women. However, based on our results we noticed that upon binding of DMPA to both GR and PR, the PR system would lead to a more enhanced transcriptional activity as compared to the GR system that undergoes large conformational changes resulting in some loss of its stability.

5.5 Conclusion

This work revealed from a computational perspective that binding of DMPA to residues M745, M752 & I756 within the GR LBD causes large conformational changes that could result in loss of stability of this complex whereas binding of DMPA to residues K707, K861, K790, F905, M909, K919 and K926 within the PR LBD results in a higher binding affinity that could lead to a more enhanced transcriptional activity. However, knowing that the GR and PR LBDs share a homology sequence of 50-57%, both GR and PR remain important targets of DMPA. Thus, alterations in the GR and PR actions upon binding of DMPA may have major implications on the regulation of transcriptional activity, leading to an

increase of mRNA and virus production which further leads to increased risk of HIV-1 infection in women.

5.6 Acknowledgements

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5.7 Conflict of Interest

None declared.

5.8 References

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CHAPTER 6

6. Conclusion and future recommendations

Outlined in this chapter is the general conclusion of the entire thesis based on our findings and recommendations for future studies.

6.1 General conclusion

From a computational perspective, this work revealed that DMPA is structurally related to GR. However, binding of DMPA to residues M745, M752 & I756 within the GR LBD causes large conformational changes that could result in loss of stability of this complex whereas binding of DMPA to residues K707, K861, K790, F905, M909, K919 and K926 within the PR LBD results in a higher binding affinity that could lead to a more enhanced transcriptional activity. Thus, alterations in the GR and PR actions upon binding of DMPA may have major implications (especially in the PR) on the regulation of transcriptional activity, leading to an increase of mRNA and virus production which can further lead to increased risk of HIV-1 infection in women.

Furthermore, and most importantly it revealed that the HAT activity possessed by the CBP/P300, p160 SRC-1 and pCAF coactivators is crucial in facilitating nuclear-receptor mediated hormone signalling hence there is indeed a strong link between histone acetylation, chromatin remodelling and gene regulation, supporting the notion that was previously reported by Kevin Struhl in 1998. Taken together, DMPA binds more tightly to the PR as compared to the GR; supporting the idea that although the PR mechanisms are poorly understood, there is a high possibility that upon binding of an agonist ligand such as DMPA they could follow mechanisms similar to those of the GR which are well studied to cause transactivation in the FRT (Louw et al., 2014; McKay and Cidlowski, 1999). This would increase mRNA and protein secretion levels of proinflammatory cytokines in the Ect1/E6E7 epithelial cell line, region which contains the main target cells ($CD4^+$ T cells) of HIV (Louw et al., 2014; Nguyen et al., 2014).

On the other hand, previous literature revealed that p300 interact with NFκB p65 Rel protein by acting as a coactivator in the p65 mediated transactivation and has been identified as the GR transcriptional coactivator which enables transactivation upon binding of a ligand (McKay and Cidlowski, 1999; Zhong et al., 1998). This p300 is found in the AF-2 domain at the C-terminus within the LBD and within the RID in nuclear receptors. Given that the LxxLL motif present in the AF-2 domain causes transactivation by p300 to be AF-2 dependent (Aranda and Pascual, 2001), it is probable that mechanisms regulated and mediated by NFκB and p300 *via* the GR and PR are likely to cause

transactivation upon binding of an agonist ligand due to the presence of the LxxLL motif, leading to an elevated risk of HIV-1 infection in women.

6.2 Recommendations and future studies

This study does not discourage women from using DMPA as this would lead to lack of available and affordable birth control methods and increased rate of mother-to-child transmission in areas with high prevalence of HIV-1 (Hel et al., 2010). However, it encourages women living in areas with high prevalence of HIV-1 and using DMPA as their contraceptive method to seek diagnosis for reproductive tract infections (RTIs) as this gives rise to infections that may expose the non-infected to HIV-1 (Hel et al., 2010; Mmbaga, 2013). It also motivates these women to opt for counselling regarding polygamous relationships and making use of condoms during sexual intercourse (Morrison et al., 2012). Moreover, this study advises doctors, nurses or anyone who is responsible for injecting DMPA to patients to be careful with the sharing and reusing of needles during the process of administering DMPA as this has been associated with HIV-1 infection (Hel et al., 2010). Lastly and most importantly, it is worth noting that multiple factors such as dose, method used to select subjects of the study and size of the population would affect interpretation of studies differently. Therefore, great care should be taken when analysing the results (Hel et al., 2010). Herein, further investigations regarding how much DMPA dose is responsible for causing an increase of HIV-1 infection in women still needs to be conducted.

6.3 References

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APPENDICES

Appendix 1. Input files for docking DMPA into the GR system

receptor = rec.pdpqt

exhaustiveness = 8

center_x = -48.194

center_y = 12.78

center_z = -43.095

size_x = 24

size_y = 20

size_z = 26

Appendix 2. Input files for docking DMPA into the PR system

receptor = rec.pdpqt

exhaustiveness = 8

center_x = 22.737

center_y = 9.318

center_z = 60.861

size_x = 18

size_y = 26

size_z = 24