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- Tincho, M.B., Gabere, M. N. and Pretorius, A (2016). *In-silico* identification and molecular validation of putative antimicrobial peptides for HIV therapy. *J. AIDS Clin. Res.*, **7**: 606. DOI: 10.4172/2155-6113.1000606.
- Tolton, J. C. and Doms, R. W. (2010). Entry inhibitors in the treatment of HIV-1 infection. *Antiviral research*, **85**: 91-100.
- Torrent, M., Di Tommaso, P., Pulido, D., Nogués, M. V., Notredame, C., Boix, E. and Andreu, D. (2012). AMPA: an automated web server for prediction of protein antimicrobial regions. *Bioinformatics*, **28** (1): 130-131.
- Tovchigrechko, A. and Vakser, I. L. (2006). GRAMM-X public web server for protein-protein docking. *Nucleic Acids Res.*, **34**: 310-314.
- Trkola, A. (2004). HIV-host interactions: vital to the virus and key to its inhibition. *Curr. Opin. Microbiol.*, **7** (4): 407-411.
- Trkola, A., Dragic, T., Arthos, J., Binley, J. M., Olson, W. C., Allaway, G. P., Cheng-Mayer, C., Robinson, J., Maddon, P. J. and Moore, J. P. (1996). CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5. *Nature*, **384**: 184-187.
- Turner, B.G. and Summers, M. F. (1999). Structural biology of HIV. *J. Mol. Biol.*, **285** (1): 1-32.
- U.S. Food and Drug Administration (2006-06-15). FDA Approves First Drug Treatment for Late-Stage Cervical Cancer. Retrieved 2007-12-02.
- UN. (1999). World urbanization prospects: the 1999 revision. New York, United Nations Population Division.
- UNAIDS, (2010). Report on the global AIDS epidemic.

UNAIDS, (2014). Global statistics report, Fact sheet 2014.

Uniprot, C. (2009). The Universal Protein Resource (UniProt) in 2010. *Nucleic Acids Research* **38** (Database issue): 142-148.

VanCompernelle, S. E., Taylor, R. J., Oswald-Richter, K., Jiang, J., Youree, B. E., Bowie, J. H., Tyler, M. J., Conlon, J. M., Wade, D., Aiken, C., Dermody, T. S., KewalRamani, V. N. Rollins-Smith L. A. and Unutmaz, D. (2005). Antimicrobial Peptides from Amphibian Skin Potently Inhibit Human Immunodeficiency Virus Infection and Transfer of Virus from Dendritic Cells to T cells. *J. Virol.*, **79** (18): 11598-11606.

Volberding, P. A. and Deeks, S. G. (2010). Antiretroviral therapy and management of HIV infection. *Lancet*, **376**: 49-62.

Wachinger, M., Kleinschmidt, A., Winder, D., von Pechmann, N., Ludvigsen, A., Neumann, M., Holle, R., Salmons, B., Erfle, V. and Brack-Werner, R. (1998). Antimicrobial peptides melittin and cecropin inhibit replication of human immunodeficiency virus 1 by suppressing viral gene expression. *Journal of General Virology*, **79**: 731-740.

Wachinger, M., Saermark, T. and Erfle, V. (1992). Influence of amphipathic peptides on the HIV-1 production in persistently infected T lymphoma cells. *FEBS Lett.*, **309**: 235-241.

Waggoner, S. E. (2003). Cervical Cancer. *The Lancet*. **361** (9376): 2217-2225

Wang, C., Li, H. B., Li, S., Tian, L. L. and Shang, D. J. (2012). Antitumor effects and cell selectivity of temporin-1CEa, an antimicrobial peptide from the skin secretions of the Chinese brown frog (*Rana chensinensis*). *Biochimie*, **94**: 434-441.

Wang, G., Buckheit, K. W., Mishra, B., Lushnikova, T. and Buckheit, R. (2011) De Novo Design of Antiviral and Antibacterial Peptides with Varying Loop Structures. *J. AIDS Clinic Res.*, **52**: 36-41.

Wang, G., Li, X. and Wang, Z. (2009). APD2: the updated antimicrobial peptide database and its application in peptide design. *Nucleic Acids Res.*, **37**: 933-937.

Wang, G., Watson, K. M., Peterkofsky, A. and Buckheit, Jr. R. W. (2010). Identification of Novel Human Immunodeficiency Virus Type 1-Inhibitory Peptides Based on the Antimicrobial Peptide Database, *Antimicrob. Agents Chemother.*, **54** (3): 1343-1346.

Wang, H. and Ng, T. B. (2002). Ascalin, a new anti-fungal peptide with human immunodeficiency virus type 1 reverse transcriptase-inhibiting activity from shallot bulbs. *Peptides*, **23**: 1025-1029.

Wang, H. X. and Ng, T. B. (2005). An antifungal peptide from the coconut. *Peptides.*, **26**: 2392-2396.

Wang, J. M., Ueda, H., Howard, O. M., Grimm, M. C., Chertov, O., Gong, X., Gong, W., Resau, J. H., Broder, C. C., Evans, G., Arthur, L. O., Ruscetti, F. W. and Oppenheim, J. J. (1998). HIV-1 envelope gp120 inhibits the monocyte response to chemokines through CD4 signal-dependent chemokine receptor down-regulation. *J. Immunol.*, **161** (8): 4309-4317.

Wang, S., Xu, F. and Demirci, U. (2010). Advances in developing HIV-1 viral load assays for resource-limited settings. *Biotechnol Advances.*, **28** (6): 770-781.

Wang, W., Owen, S. M., Rudolph, D. L., Cole, A. M., Hong, T., Waring, A. J., Lal, R. B. and Lehrer, R. I. (2004). Activity of alpha- and theta-defensins against primary isolates of HIV-1. *J. Immunol.*, **173** (1): 515-520.

Wang, W., Tao, R., Tong, Z., Ding, Y., Kuang, R., Zhai, S., Liu, J. and Ni, L. (2012). Effect of a novel antimicrobial peptide chrysopsin-1 on oral pathogens and *Streptococcus mutans* biofilms. *Peptides*, **33** (2): 1058-1068.

Wang, Z. and Wang, G. (2004). APD: the Antimicrobial Peptide Database. *Nucleic Acids Research*, **32**: 590-592.

Wei, L., Huang, E. S. and Altman, R. B. (1999). Are predicted structures good enough to preserve functional sites? *Structure*, **7**: 643-650.

Wei, X., Decker, J. M., Wang, S., Hui, H., Kappes, J. C., Wu, X., Salazar-Gonzalez, J. F., Salazar, M. G., Kilby, J. M., Saag, M. S., Komarova, N. L., Nowak, M. A.,

Hahn, B. H., Kwong, P. D. and Shaw, G. M. (2003). Antibody neutralization and escape by HIV-1. *Nature*, **422** (6929): 307-312.

Welch, B. D., VanDemark, A. P., Heroux, A., Hill, C. P. and Kay, M. S. (2007). Potent D-peptide inhibitors of HIV-1 entry. *Proc. Natl. Acad. Sci. U.S.A.*, **104**: 16828-16833.

WHO (1998). Obesity: preventing and managing the global epidemic. Working Group on Obesity. Geneva, World Health Organization.

WHO (2011). Progress report 2011: Global HIV/AIDS response.

Wild, C., Greenwell, T. and Matthews, T. (1993). A synthetic peptide from HIV-1 gp41 is a potent inhibitor of virus-mediated cell-cell fusion. *AIDS Res. Hum. Retroviruses*, **9**: 1051-1053.

Wild, C., Oas, T., McDanal, C., Bolognesi, D. and Matthews, T. (1992). A synthetic peptide inhibitor of human immunodeficiency virus replication: correlation between solution structure and viral inhibition. *Proc. Natl. Acad. Sci. U.S.A.*, **89**: 10537-10541.

Wilkinson, D. (1996). HIV-1: cofactors provide the entry keys. *Curr. Biol.*, **6**: 1051-1053.

Wiley, R. L., Maldarelli, F., Martin, M. A. and Strebel, K. (1992a). Human immunodeficiency virus type 1 Vpu protein regulates the formation of intracellular gp160-CD4 complexes. *J. Virol.*, **66**: 226-234.

Wiley, R. L., Maldarelli, F., Martin, M. A. and Strebel, K. (1992b). Human immunodeficiency virus type 1 Vpu protein induces rapid degradation of CD4. *J. Virol.*, **66**: 7193-7200.

Williams, M. E., Tincho, M. B., Gabere, M.N., Uys A, Meyer, M., [SEP]Pretorius, A. (2016). Molecular validation of putative antimicrobial peptides for improved human immunodeficiency virus diagnostics via HIV protein p24. *J. AIDS Clin. Res.* **7**: 571. DOI:10.4172/2155- 6113.1000571.

Wong, J. H. and Ng, T. B. (2003). Gymnin, a potent defensin-like antifungal peptide from the Yunnan bean (*Gymnocladus chinensis* Baill). *Peptides*, **24** (7): 963-968.

Wong, J. H. and Ng, T. B. (2005a). Sesquin, a potent defensin-like antimicrobial peptide from ground beans with inhibitory activities toward tumor cells and HIV-1 reverse transcriptase. *Peptides*, **26** (7): 1120-1126.

Wong, J. H. and Ng, T. B. (2005b). Lunatusin, a trypsin-stable antimicrobial peptide from lima beans (*Phaseolus lunatus* L.). *Peptides*, **26** (11): 2086-2092.

World Health Organization global status report on non-communicable diseases (2014). Attaining the nine global non-communicable diseases targets; a shared responsibility.

Wrin, T., Crawford, L., Sawyer, L., Weber, P., Sheppard, H. W. and Hanson, C. V. (1994). Neutralizing antibody responses to autologous and heterologous isolates of human immunodeficiency virus. *J. Acquir. Immune Defic. Syndr.*, **7**: 211-219.

Wu, S., Skolnick, J. and Zhang, Y. (2007). *Ab initio* modeling of small proteins by iterative TASSER simulations. *BMG Biol.*, **5**: 17.

Xiong, W. (2010). Clinical efficacy of treating infant cytomegalovirus hepatitis with ganciclovir and impact on cytokines. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* (in Chinese). **26** (11): 1130-1132.

Yeaman, M. and Yount, N. (2003) Mechanism of antimicrobial peptide action and resistance. *Pharmacological reviews*, **55**: 27-55.

Zhang, L., Benz, R. and Hancock, R. E. W. (1999). Influence of proline residues on the antibacterial and synergistic activities of alpha-helical peptides. *Biochemistry*, **38** (25): 8102-8111.

Zhang, L., Rozek, A. and Hancock, R. E. W. (2001). Interaction of cationic antimicrobial peptides with model membranes. *J. Biol Chem.*, **276**: 35714-35722.

Zhang, Y. (2008). I-TASSER server for protein 3D structure prediction. *BMC Bioinformatics*, **9**: 40 doi: 10.1186/1471-2105-9-40.

Zhou, T., Xu, L., Dey, B., Hessel, A. J., Van Ryk, D., Xiang, S. H., Yang, X., Zhang, M. Y., Zwick, M. B., Arthos, J., Burton, D. R., Dimitrov, D. S., Sodroski, J., Wyatt, R., Nabel, G. J. and Kwong, P. D. (2007). Structural definition of a conserved

neutralization epitope on HIV-1 gp120. *Nature*, **445** (7129): 732-737.

Zhu, X. and Mitchell, J. C. (2011). KFC2: A knowledge-based hot spot prediction method based on interface solvation, atomic density and plasticity features. *Proteins*, 79 (9): 2671-2683.

Zolla-Pazner, S. (2004). Identifying epitopes of HIV-1 that induce protective antibodies. *Nature Reviews Immunol.*, **4**: 199-210.



## APPENDIX A

### Supplementary materials for Chapter TWO

>Molecule 1:

CLRYKKPECQSDWQCPGKKRCCPDTCGIKCLDPVDTPNPTRRKP GKCPVTYG  
QCLMLNPPNFCEMDGQCKRDLKCCMGM

>Molecule 3:

RWKLFKKIEKVGRNV RDGLIKAGPAIAVIGQAKSLGK

>Molecule 7:

RWKIFKKIEKMGRNIRDGIVKAGPAIEVLGSAKAIGK

>Molecule 8:

CLKSGAICHVPFCPRRYKQIGTCGLPGTKCCKKP

>Molecule 10:

WNPFKLEKAGQRV RDAIISAKPAVDVVGQATAIIK

**Table A.1:** The parental sequences of the anti-HIV AMPs sequence obtained after the initial docking of the HIV gp120 protein and the putative AMPs, published in Tincho *et al.*, 2016.

>Mutated Molecule 1 or Molecule 1.1: F62W

CLRYKKPECQSDWQCPGKKRCCPDTCGIKCLDPVDTPNPTRRKPGKCPVTYG  
QCLMLNPPNWCMDGQCKRDLKCCMGM

>Mutated Molecule 3 or Molecule 3.1: V28L

RWKLFKKIEKVGRNVRDGLIKAGPAIALIGQAQSLGK

>Mutated Molecule 7 or Molecule 7.1: W2H

RHKIFKKIEKMGRNIRDGIVKAGPAIEVLGSAKAIGK

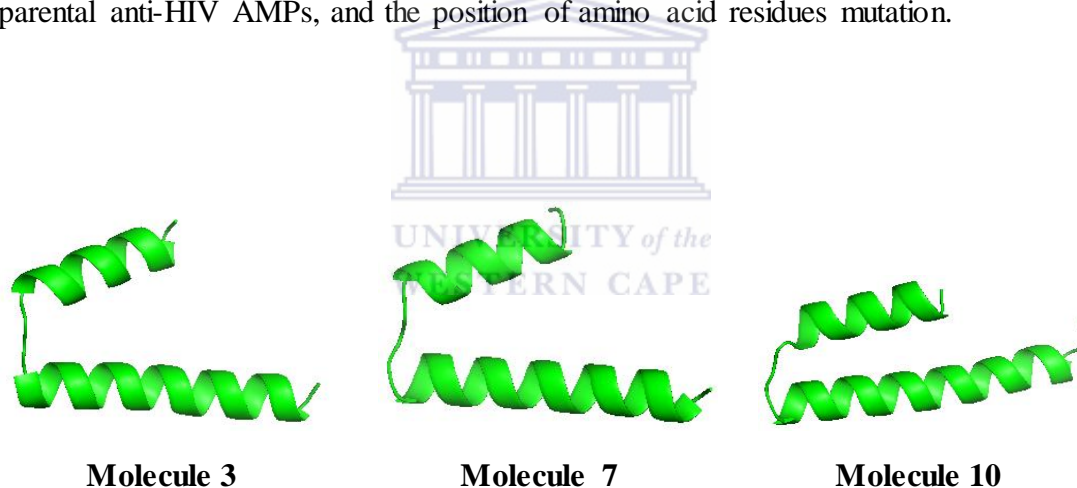
>Mutated AMP 8 or Molecule 8.1: F12H

CLKSGAICHVHCPRRYKQIGTCGLPGTKCCKKP

>Mutated Molecule 10 or Molecule 10.1: V25L

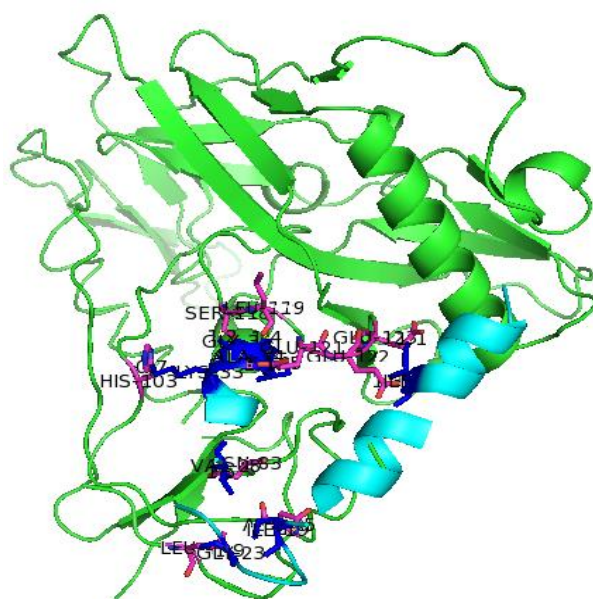
WNPFKELEKAGQRVRDAIISAKPALDVVGQATAIK

**Table A.2:** Mutated AMPs sequence obtained after site-directed mutagenesis of parental anti-HIV AMPs, and the position of amino acid residues mutation.

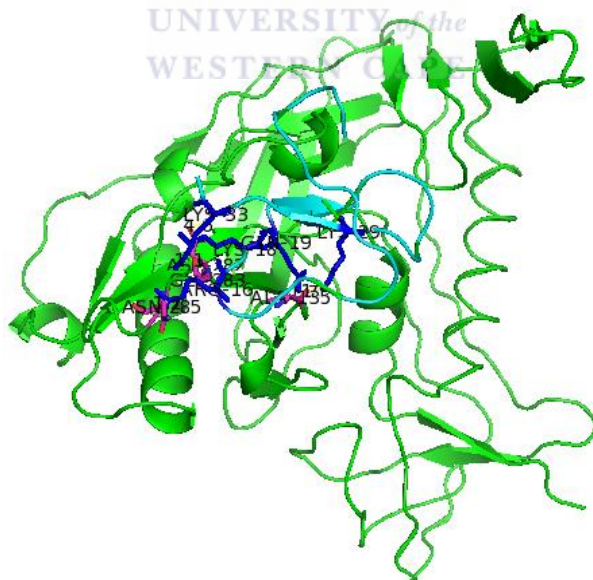


**Figure A.1:** The 3-D structures of the three alpha-helical parental anti-HIV AMPs Molecule 3, Molecule 7 and Molecule 10 predicted by I-TASSER server and represented in cartoon representation by PyMOL 1.3. Software

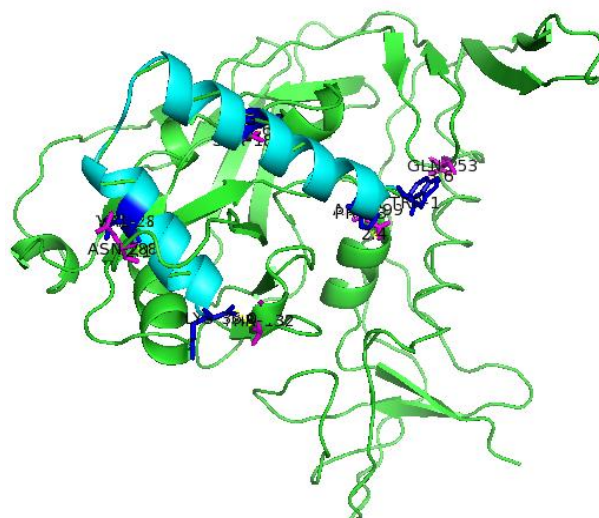




**Figure A.4:** gp120-Molecule 7 complex formation during anti-HIV-gp120 interaction. The cartoon representation in green colour is the HIV protein gp120 and the putative anti-HIV AMP (Molecule 7) is represented in light blue colour. The purple colour represents the stick representation of gp120 amino acids interacting with Molecule 7 amino acid stick representation in dark blue. Each amino acid is labelled with the position of their amino acid.



**Figure A.5:** gp120-Molecule 8 complex formation during anti-HIV-gp120 interaction. The cartoon representation in green colour is the HIV protein gp120 and the putative anti-HIV AMP (Molecule 8) is represented in light blue colour. The purple colour represents the stick representation of gp120 amino acids interacting with Molecule 8 amino acid stick representation in dark blue. Each amino acid is labelled with the position of their amino acid.



**Figure A.6:** gp120-Molecule 10 complex formation during anti-HIV-gp120 interaction. The cartoon representation in green colour is the HIV protein gp120 and the putative anti-HIV AMP (Molecule 10) is represented in light blue colour. The purple colour represents the stick representation of gp120 amino acids interacting with Molecule 10 amino acid stick representation in dark blue. Each amino acid is labelled with the position of their amino acid.

**Table A.2:** The area cover and the ACE's from the docking the gp120-putative-anti-HIV AMP using the PatchDock docking server. These interactions are the results generated from the previous work (Tincho *et al.*, 2016).

	gp120		
	Area (Å <sup>2</sup> )	ACE	Transformation coordinates
Molecule 1	2433.90	281.93	-0.94 0.61 -2.68 14.27 19.05 2.11
Molecule 3	1926.00	410.28	-1.31 0.66 1.01 -5.19 -9.03 -16.06
Molecule 7	1906.00	295.00	2.31 1.12 2.99 8.08 -27.26 11.81
Molecule 8	1564.90	71.02	-1.23 0.07 1.76 9.50 5.77 -10.61
Molecule 10	1916.20	-34.06	-2.33 0.66 -2.29 9.00 14.84 -0.33

## APPENDIX B

### Supplementary materials for Chapter THREE

**Table B.1:** Chemical/Reagents and suppliers

<u>Material</u>	<u>Supplier</u>
Acetic acid	Merck
40 % 37.5:1 Acrylamide:bis-acrylamide	Promega
Agarose	Promega
Ampicillin	Sigma
Ammonium Persulfate (APS)	Sigma
Bacteriological agar	Merck
Boric acid	Merck
Bromophenol blue	Sigma
Coomassie Brilliant Blue R-250	Sigma
Dithiothreitol (DTT) <sub>SEP</sub>	Roche
Disodium phosphate	Merck
Ethylene Diamine Tetra-acetic acid (EDTA)	Merck
Ethanol	Merck
Ethidium bromide (EtBr) <sub>SEP</sub>	Promega
L-reduced Glutathione-S-Transferase	Sigma
Glycerol	Merck
Glycine	Sigma
Isopropanol	Merck
Isopropyl $\beta$ -D-thiogalactopyranoside (IPTG)	Sigma
Lysosome	Sigma
Methanol	Merck



Monopotassium phosphate	Merck
cOomplete EDTA-free protease inhibitor	Roche
PageRuler™ Unstained Protein Ladder	Fermentas
Potassium chloride	Merck
Sodium acetate	Merck
Sodium Azide	Sigma
Sodium Chloride (NaCl)	Merck
Sodium Hydroxide	Merck
Sodium dodecyl sulphate (SDS)	Merck
Urea	Merck
<i>N, N, N', N'</i> -Tetramethylethylenediamine (TEMED)	Sigma
Tris [hydroxymethyl] aminoethane (Tris)	Merck
Triton X-100 (iso-octylphenoxypoly-ethoxyethanol)	Sigma
Tryptone	Merck
Tween-20 (Polyoxyethylene [20] sorbitan)	Merck
Yeast Extract	Merck

**Table B.2:** Buffers and Solutions

2 X SDS Sample buffer:

62.5 mM Tris-HCl (pH 6.8), 2 % SDS, 25 % glycerol, 0.01 % bromophenol blue, 5 % β-mercaptoethanol.

10 X TBE

0.9 M Tris, 0.89 M boric acid, 0.032 M EDTA stored at room temperature.

10 X Tris-EDTA (TE)

10 mM Tris-HCl, 1 mM EDTA, pH 7.5

10 X Phosphate-buffered saline (PBS)

150 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 2.0 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4.

#### DNA Loading buffer

0.25 % Bromo-phenol-blue, 0.25 % xylene cyanol and 30 % glycerol

#### Ampicillin

100 mg/ml ampicillin in distilled water; filter sterilized.

#### Sodium Chloride-Tris-EDTA/lysozyme (Lysis buffer)

10 mM Tris, pH 8, 150 mM NaCl, 1mM EDTA and 100 µg/ml lysozyme

#### Ammonium persulphate (APS)

A 10 % stock solution was prepared in deionised water.

#### Coomassie Brilliant Blue R-250 Staining Solution

0.25 g Coomassie Brilliant Blue R 250, 50 % ethanol and 10 % acetic acid

#### Cleaning buffer 1:

0.5 M borate buffer, (pH 8.5): 0.5 M NaCl

#### Cleaning buffer 2:

0.1 M acetate buffer, (pH 4.5): 0.5 M NaCl

#### Destaining solution

16.5 % ethanol and 5 % acetic acid.

#### Dithiothreitol (DTT)

A 1 M stock solution was prepared in 0.01 M Sodium acetate, pH 5.2. This solution was sterilized by filtration.

#### Ethylene diamine tetra acetic acid (EDTA)

A stock solution was prepared at a concentration of 0.5 M in deionised water, pH 8.0.

10 % Sodium dodecyl sulphate (SDS) 10 % SDS in distilled water.

#### Elution buffer

5 mM reduced glutathione, 50 mM Tris- HCl pH 9.0.

#### Isopropyl $\beta$ -D-thiogalactopyranoside (IPTG)

A 1 M stock solution was prepared in deionised water. The solution was sterilised by filtration.

#### Luria Agar

14 g/l Bacteriological agar, 10 g/l Tryptone, 5 g/l Yeast Extract and 5 g/l NaCl

#### Luria Broth

10 g/l Bacto-tryptone, 5 g/l Bacto-Yeast Extract and 5 g/l NaCl

#### Lysozyme

A stock solution was prepared at a concentration of 50 mg/ml in deionised water.

#### PBS-T

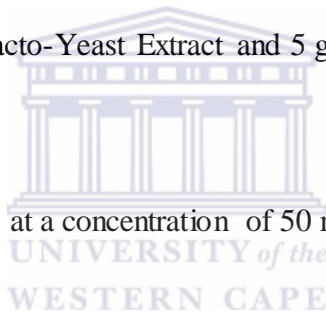
150 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 2 mM KH<sub>2</sub>PO<sub>4</sub> and 1 % Triton-X (100)

#### Storage buffer

2 M NaCl, 1 mM Sodium Azide

#### U-Buffer

8 M Urea, 50 mM Tris, 5 mM EDTA, 5 mM DTT and cOmplete EDTA-free protein inhibitor tablet



**Table B.3:** Equipment and suppliers

<u>Equipment</u>	<u>Suppliers</u>
211DS Shaking Incubator	Labnet
5415D Benchtop Microcentrifuge Centrifuge	Eppendorf Beckman Coulter
Tube Roller SRT9D <sup>[SEP]</sup>	Stuart
UVP BioSpectrum Imaging System	UVP LLC
Mini-PROTEAN Tetra Cell	BioRad
CanoScan LiDE 120 electronic scanner	Canon

EVVLVNVTEENFNMWKNDMVEQMHEDIISLWDQSLKPCVKLTPLCVGAGSC  
NTSVITQACPKVSFEPIPIHYCAPAGFAILKCNNKTFNGTGPCTNVSTVQCTHG  
IRPVVSSQLLLNGSLAEEEEVVIRSVNFTDNAKTIIIVQLNTSVEINCTGAGHCNIA  
RAKWNNTLKQIASKLREQFGNNKTIIFKQSSGGDPEIVTHWFNCGGEFFYCNS  
TQLFNSTWFNSTWSTEGSNNTSGSDTITLPCRKQIINMWQKV GKAMYAPPIS  
GQIRCSSNITGLLLTRDGGNSNNESEIFRPGGGDMRDNRSELYKYKVVKIE

**Figure B.1:** Protein sequence of HIV gp120, extracted from the complex of PDB ID 2NXZ (2NXZ: A|PDBID|CHAIN|SEQUENCE) deposited in the Protein Database Bank.

## APPENDIX C

### Supplementary materials for Chapter FOUR

**Table C.1:** Chemicals/Reagents and Suppliers

<u>Material</u>	<u>Suppliers</u>
Dimethyl Sulphoxide (DMSO)	Sigma
Roswell Park Memorial Institute medium (RPMI)	Lonza
Fetal Calf Serum (FCS)	Merck
Dulbecco's Phosphate Buffer Saline (DPBS)	Lonza
2.5 % Trypsin (10X)	Gibco
PEN-STREP	Lonza
2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-carboxanilide (XTT)	Sigma
3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS)	Sigma

**Table C.2:** Buffers and Solutions

#### RPMI Complete

50 ml Fetal Calf Serum and 5 ml PEN-STREP in 500 ml Roswell Park Memorial Institute Medium (RPMI), mix solution to obtain a homogeneous distribution.

#### XTT Solution

5 mg/ml XTT: Dissolve 5 mg of lyophilized XTT in 1 ml distilled water and filter sterilize.

#### MTS Solution

5 mg/ml MTS: Dissolve 5 mg of lyophilized MTS in 1 ml distilled water and filter sterilize.

## APPENDIX D

### Supplementary materials for Chapter FIVE

**Table D.1:** Chemicals/Reagents and Suppliers

<u>Material</u>	<u>Suppliers</u>
Tryptone Soya Agar CM0131 (TSA)	Oxoid
Tryptone Soya Broth CM0129 (TSB)	Oxoid
Iodonitrotetrazolium Chloride (INT)	Sigma
Ampicillin	Sigma

**Table D.2:** Buffers and Solutions

Tryptone Soya Agar (TSA)

40 g/l Tryptone Soya Agar CM0131 was prepared, and it contains a pancreatic digest of casein 15.0 g; enzymatic digest of soya bean 5.0 g; sodium chloride 5.0 g; agar 15.0 g.

Tryptone Soya Agar (TSB)

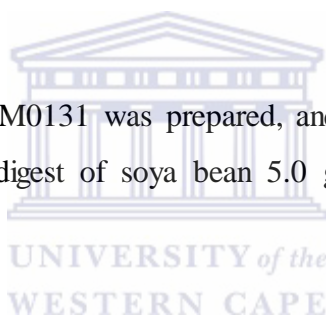
30 g/l Tryptone Soya Broth CM0129 was prepared, and it contains a pancreatic digest of casein 17.0 g; enzymatic digest of soya bean 3.0 g; sodium chloride 5.0 g; di-potassium hydrogen phosphate 2.5 g; glucose 2.5 g.

Iodonitrotetrazolium Chloride (INT)

A stock solution was prepared at a concentration of 4 mg/ml, by dissolving 4 mg of lyophilized INT with 1 ml distilled water and filter sterilized

Ampicillin

5 mg/ml lyophilized ampicillin in distilled water and filter sterilized.

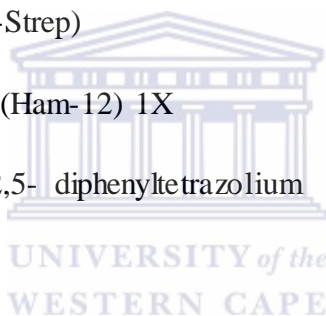


## APPENDIX E

### Supplementary materials for Chapter SIX

**Table E.1:** Chemicals/Reagents and Suppliers

<u>Material</u>	<u>Suppliers</u>
Dimethyl Sulphoxide (DMSO)	Sigma-Alrich
Dulbecco's Modified Eagle's medium (DMEM)	Lonza
Fetal Bovine Serum (FBS)	Merck Group
Dulbecco's Phosphate Buffer Saline (DPBS)	Lonza
2.5 % Trypsin (10X)	Gibco
Penicillin-Streptomycin (Pen-Strep)	Lonza
Hams F-12 Nutrient Mixture (Ham-12) 1X	Gibco
3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide (MTT)	Sigma-Alrich



**Table E.2:** Buffers and Solutions

#### DMEM Complete

50 ml Fetal Bovine Serum and 5 ml Penicillin-Streptomycin in 500 ml Dulbecco's Modified Eagle's Medium (DMEM), mix reagents to obtain a homogeneous solution.

#### Ham F-12 Complete

50 ml Fetal Bovine Serum and 5 ml Penicillin-Streptomycin in 500 ml Hams F-12, mix reagents to obtain a homogeneous solution.

#### MTT Solution

5 mg/ml MTT: Dissolve 5 mg of lyophilized MTT in 1 ml distilled water and filter sterilized.