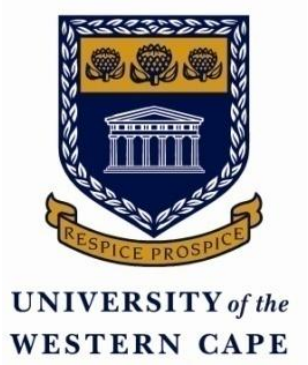
Molecular validation of putative antimicrobial peptides for improved Human Immunodeficiency Virus diagnostics via HIV protein p24

Mr. Monray Edward Williams



HIV/AIDS

- HIV/AIDS is a disease of the immune system caused by HIV

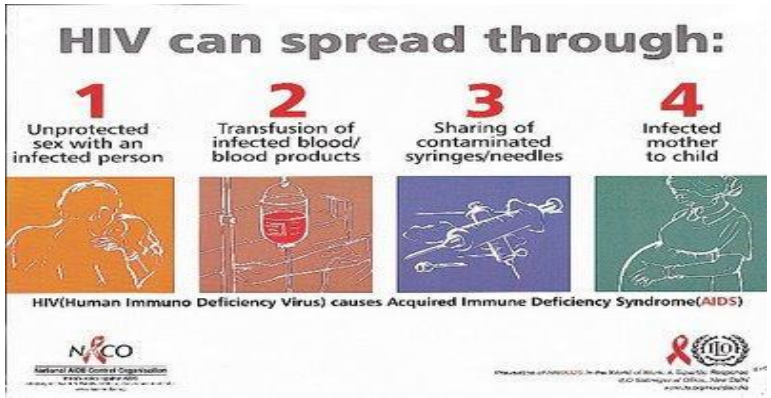


Figure 1: Common methods of HIV spread

- HIV functions by attacking the T-helper cells

- AIDS causes a weakened immune system

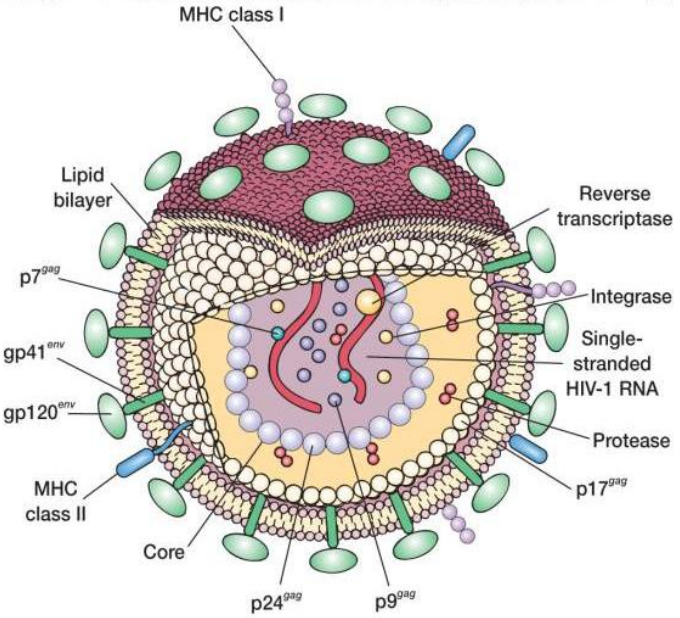
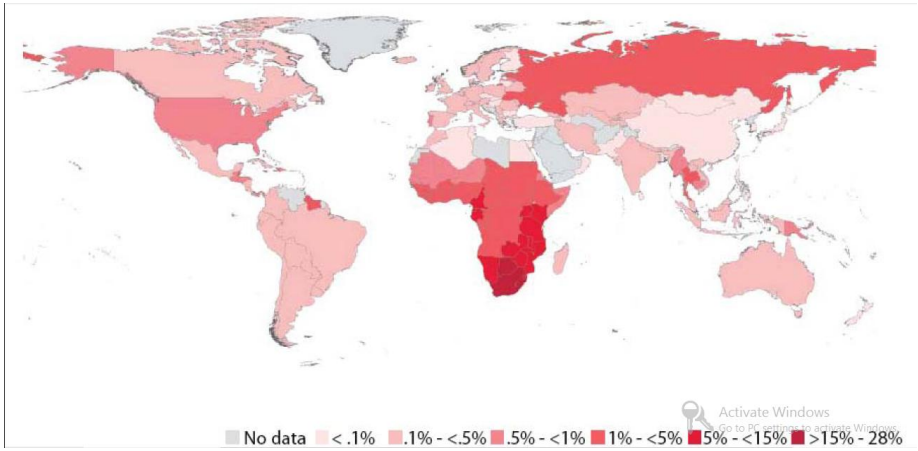


Figure 2: Structure of HIV

Markowitz, 2007; Coutsoudis *et al.*, 2010

Epidemiology

- In 2014, 35 million infection since the discovery of HIV
- Sub-Saharan Africa (SSA) as the worlds most affected region, with an estimate of 25.8 million
- Swaziland has the world's largest prevalence rate (26.5%)
- South Africa is known to have the world largest HIV infected population (5.6 million)



Kalling, 2008; UNAIDS, 2011

Figure 3: Global Epidemiology of HIV

Current HIV diagnostics

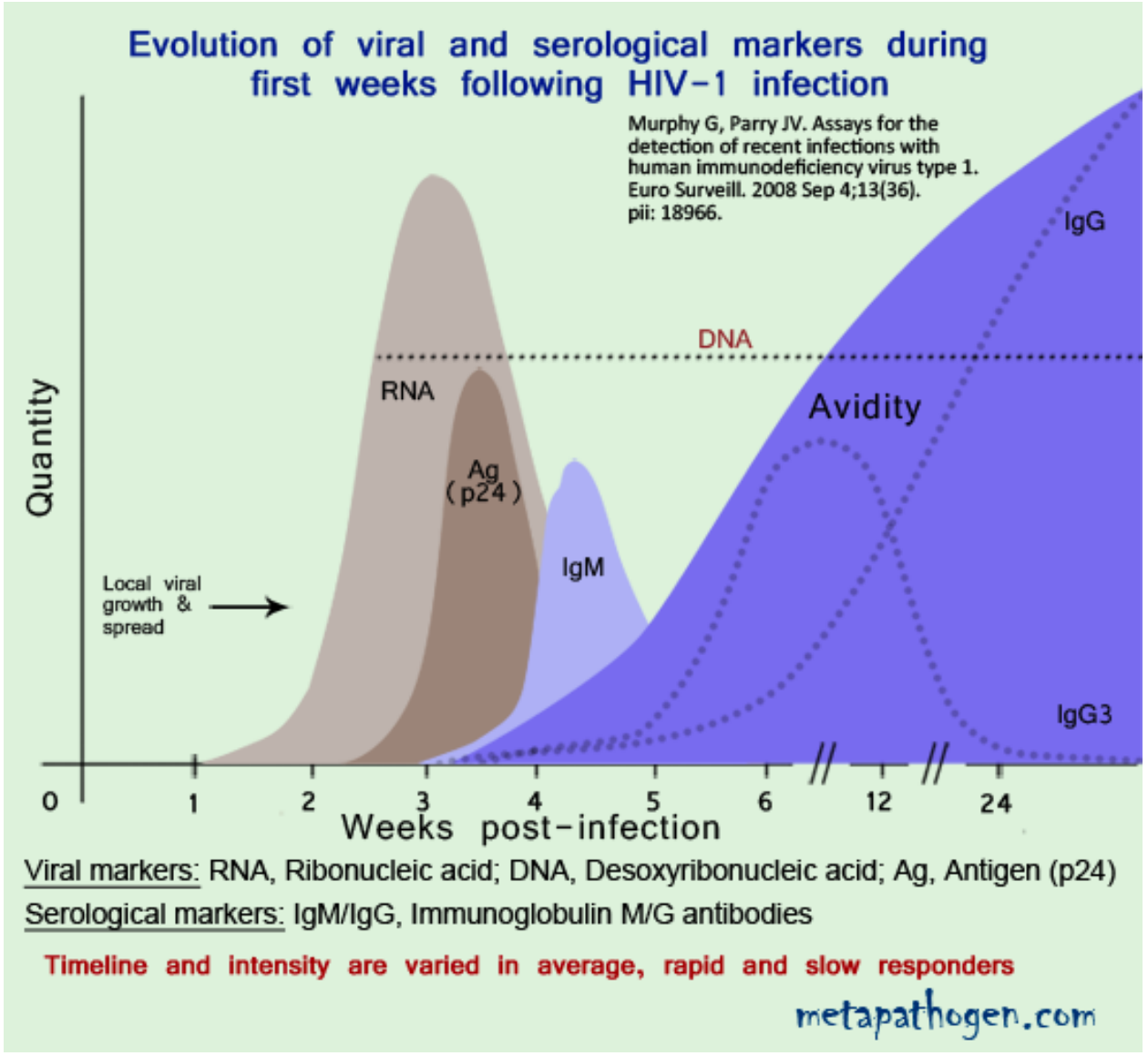


Figure 4: Evolution of serological markers during HIV infection

p24 antigen assay

- Considered as insensitive
- Displays false negatives in 50% of asymptomatic patients
- Insensitivity due to the binding of the host p24 antibody

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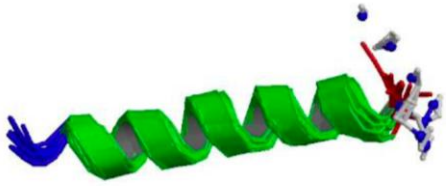
Mutual Conformational Adaptations in Antigen and Antibody upon Complex Formation between an Fab and HIV-1 Capsid Protein p24

Figure 5: Binding of the p24 antibody at C-terminal domain of p24 antigen

Antimicrobial peptides (AMPs)

- Important components of the innate immune system of many species
- Found in eukaryotes and prokaryotes



Magainin-2

- They are small, positively charged, amphipathic molecules
- Antimicrobial peptides have activity against gram-positive and gram-negative bacteria, protozoa, fungi as well as viruses.
- It is highly unlikely that pathogens can develop resistance against AMPs due to their diversity

Zasloff, 1987; Zasloff, 2002

Peptides Vs. Antibodies

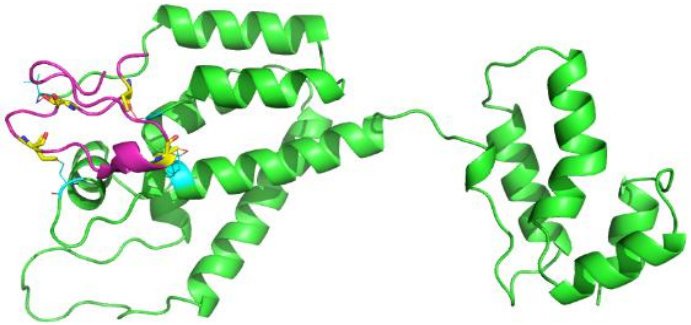
Peptides	Antibodies
1. Small size	1- Labour and machine intensive
2- Rapid and reproducible synthesis	2- Limited assay utility
3- Simple and controllable modification	3- Non-specific binding to non-target molecules
4- High stability	4- Time consuming
5- Non-toxic	5- Poor linearity of dilution
6- Lack of immunogenicity	

Methodology: Previous research

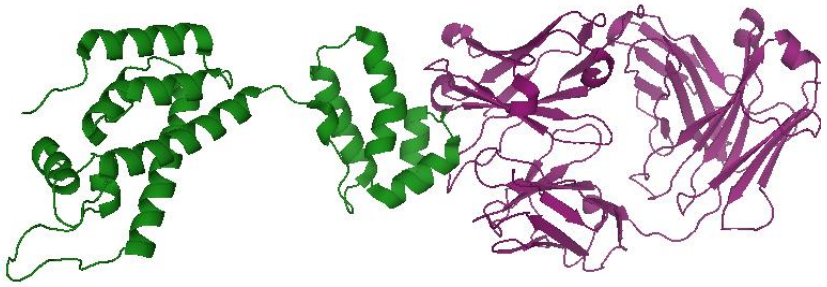
(Tincho, 2013)

Identified novel AMPs by using a Mathematical algorithm HMMER → Used top 10 novel AMPs with lowest e-value considered → *In silico* Study: 3-D structure prediction of AMPs → Protein–protein interaction studies between HIV protein p24 and AMPs

Identification of AMPs which bind N-terminal domain of the p24 protein



AMP binding N-terminal domain of p24 protein



p24 antibody binding C-terminal domain of p24 protein

Aims of study

- Identification of derivative AMPs, which bind the p24 N-terminal domain with greater affinity
- Molecularly validation of binding between AMPs and the p24 protein
- Prototype development with specific AMPs conjugated to AuNPs to accurately detect HIV within patient samples

Methodology: *In silico* approach Identification of derivative AMPs

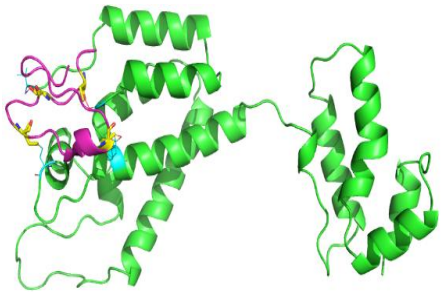
Knowledge base FADE and contacts server: Identify "hotspot residues" between parent AMPs and p24 protein

In silico site directed mutagenesis

I-TASSER software: 3-D structure prediction of derivative AMPs

PatchDock server: Protein-protein interaction studies

PyMol visualization: and results interpretation



Methodology: Molecular approach

Peptide synthesis and recombinant p24 protein expression

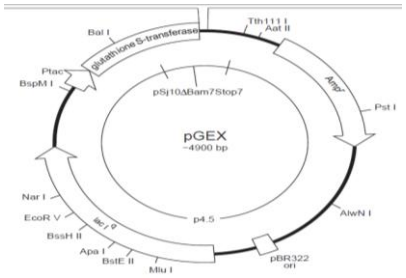


Figure 6: Map of fusion vector pGEX-6P-2

Protein interaction study: Lateral flow binding assay between selected AMPs and protein p24

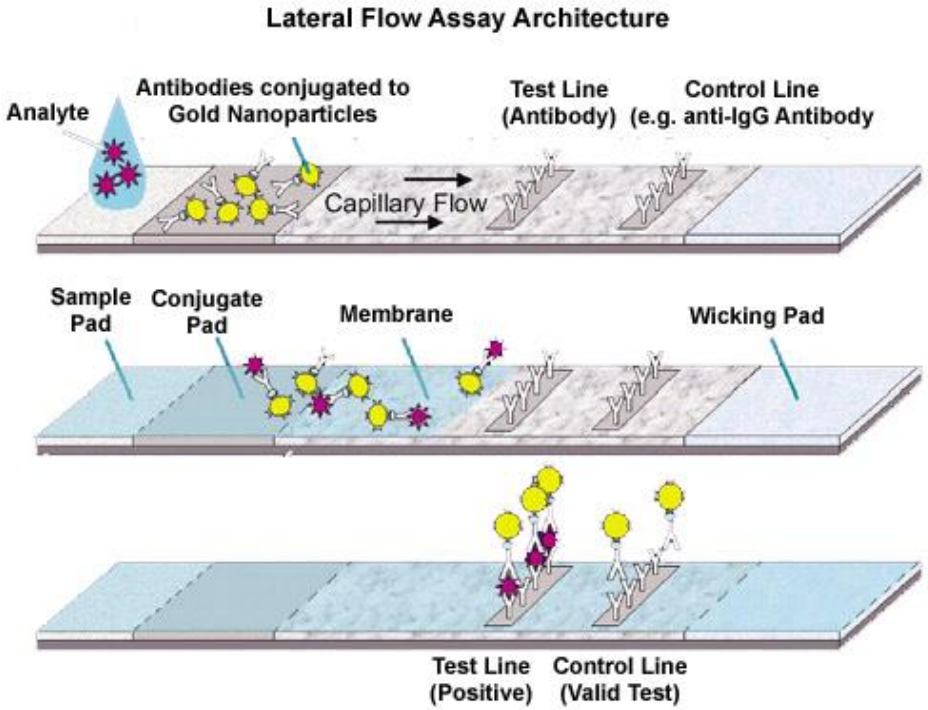


Figure 7: Lateral Flow Assay Architecture

Lateral flow device: Prototype development for HIV detection

Results: *In silico* validation of AMPs

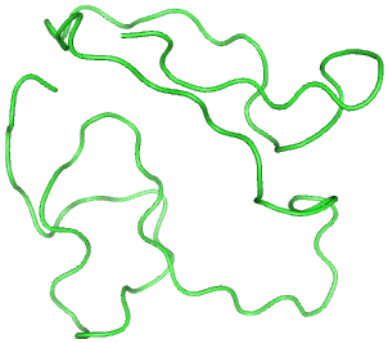
In silico site-directed mutagenesis

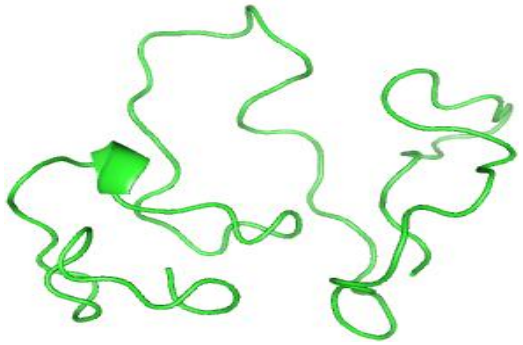
Putative HIV AMPs	Mutation
Amp 1	$F_{62}W$
Amp 2	$W_{2}H$
Amp 3	$K_{7}R$
Amp 4	$V_{28}L$
Amp5	$W_{2}H$
Amp6	$A_{34}V$
Amp 7	$K_{3}R$
AMP 8	$F_{12}H$
Amp 9	$D_{23}N$
Amp 10	$W_{1}H$

Physicochemical profiles

AMP	Arg %	Lys %	Cys %	Hydroph-obicity	Molecular weight	Total Net charge	Size	pI	Boman index	Instability index	Half-life: mammal	Common Amino Acids
AMP 1.1	**6**	**11**	**16**	34	8942.752	**+6**	79	8.37	**2.18**	**44.30**	1.2 h	Cys
AMP 1.2	**5**	**18**	**0**	40	3979.759	**+8**	37	11.49	**1.45**	**14.38**	1.3 h	Lys
AMP 1.3	**10**	**16**	**0**	43	4068.907	**+8**	37	12.16	**1.62**	**38.90**	1 h	Lys
AMP 1.4	**5**	**21**	**0**	43	4102.953	**+7**	37	11.25	**1.24**	**22.15**	1.3 h	Lys
AMP 1.5	**8**	**18**	**0**	37	4028.834	**+9**	37	11.48	**1.57**	**23.94**	1 h	Lys
AMP 1.6	**5**	**18**	**0**	45	4059.937	**+6**	37	11.17	**0.97**	**1.40**	1.3 h	Lys
AMP 1.7	**10**	**16**	**0**	43	4101.958	**+7**	37	11.75	**1.7**	**64.17**	1 h	Lys & Ile
AMP 1.8	**5**	**14**	**17**	35	3660.516	**+9**	34	9.60	**1.29**	**48.28**	1.2h	Cys
AMP 1.9	**0**	**7**	**22**	51	2779.416	**+1**	27	7.73	**-0.19**	**44.23**	7.2 h	Cys
AMP 1.10	**5**	**11**	**0**	44	3859.492	**+3**	26	10.33	**1.52**	**11.04**	3.5 h	Ala

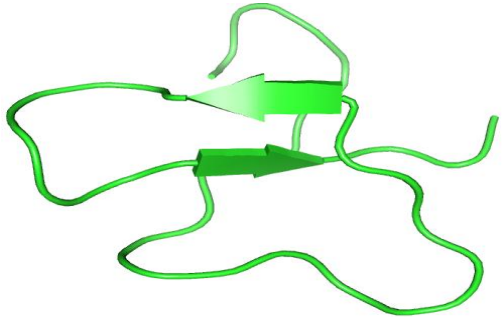
3-D Structure prediction





Parent AMP 1

Derivative AMP 1.1



Parent AMP 8

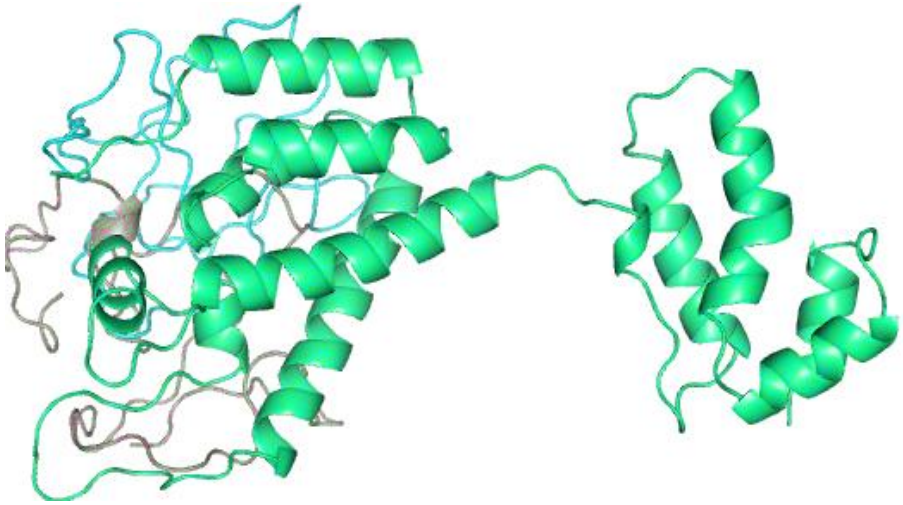
Derivative AMP 1.8

Figure 8: Structural variation between parental and derivative AMPs

In silico docking studies

Molecule	Binding affinity of parent AMPs	Binding pocket of parent AMPs	Binding affinity of derivative AMPs	Binding pocket of derivative AMPs	Difference in Binding affinity	% Increase
AMP1	**14708**	**N-terminal**	**15328**	**N-terminal**	**620**	**4.2%**
AMP2	12114	N-terminal	12620	N-terminal	506	4.2%
AMP3	12310	N-terminal	13584	N-terminal	1274	10%
AMP4	11188	N-terminal	12040	N-terminal	852	7.6%
AMP5	11974	N-terminal	13170	N-terminal	1196	9.9%
AMP6	12534	N-terminal	14100	N-terminal	1566	12.5%
AMP7	11930	N-terminal	13354	N-terminal	1424	11.9%
AMP8	**9418**	**Between N and C - terminal domain**	**10704**	**N-terminal**	**1286**	**13.6%**
AMP9	8618	N-terminal	9230	N-terminal	612	7.1%
AMP10	11560	N-terminal	12218	N-terminal	658	5.6%

Docking of HIV proteins with AMPs



Provisional patent filed for all AMP sequences

Figure 9: Binding of AMP 1 (turquoise) and AMP 1.1 (brown) to the N-terminal domain of HIV protein p24

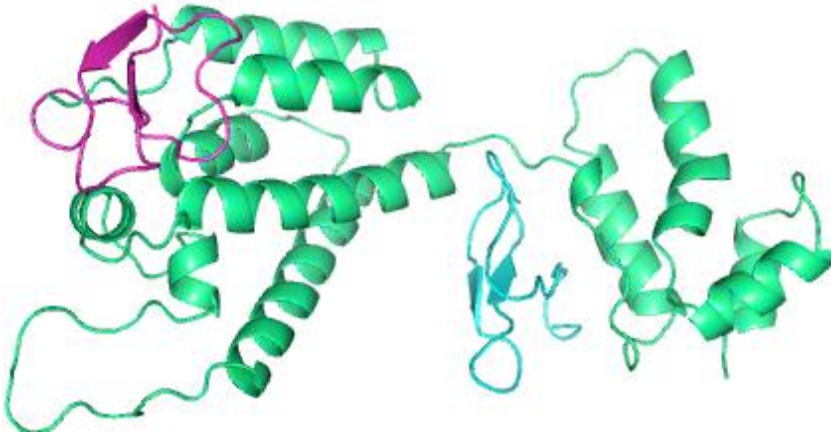


Figure 10: Binding shift of AMP 8 (turquoise) to the N-terminal domain AMP 1.8 (purple) of HIV protein p24

Recombinant HIV p24 protein expression

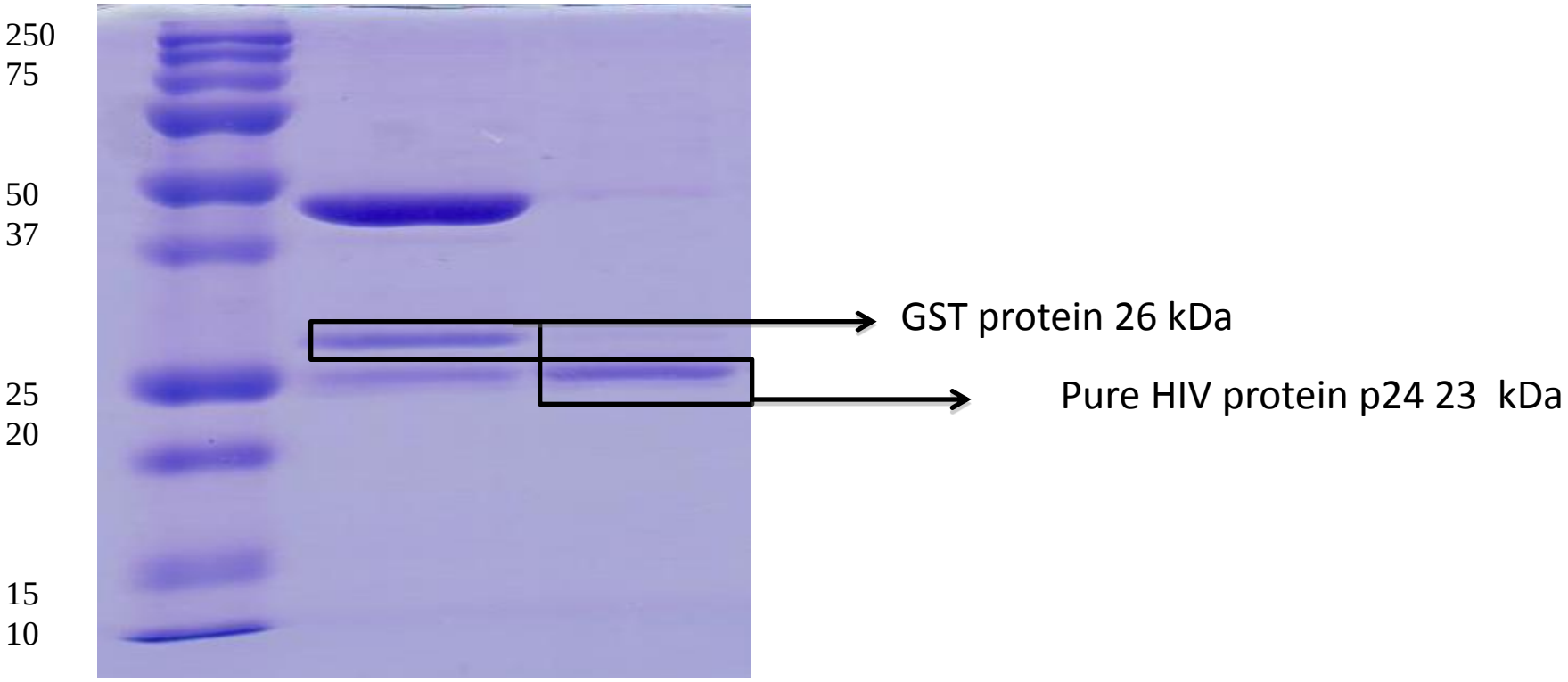
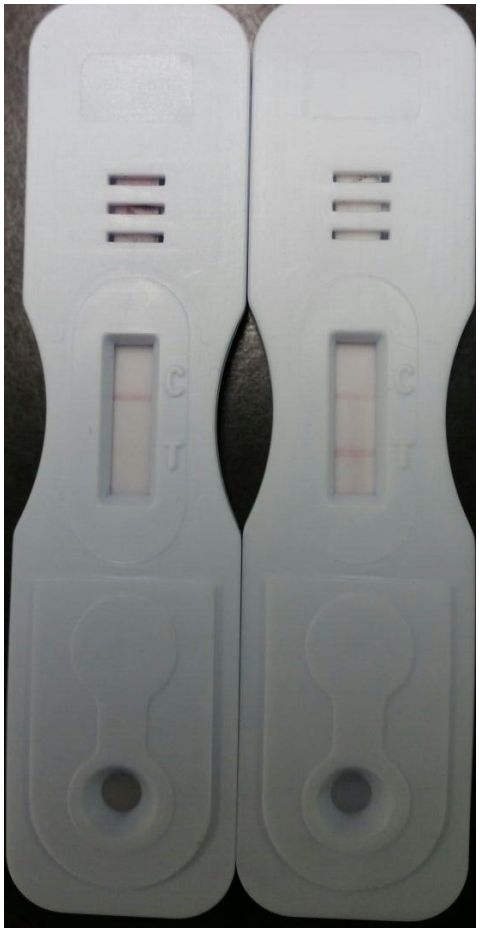


Figure 11: SDS PAGE analysis of pure purified HIV protein p24 after cleavage by protease HRV 3C

Protein-protein interaction study: LFD binding assay

AMP	Sample tested	G rating
AMP 1	p24	G8
AMP 3	p24	G1
AMP 5	p24	G1
AMP 6	p24	G1
AMP 7	p24	G1
AMP 8	p24	G4
AMP 1.1	p24	G10
AMP 1.8	p24	G6

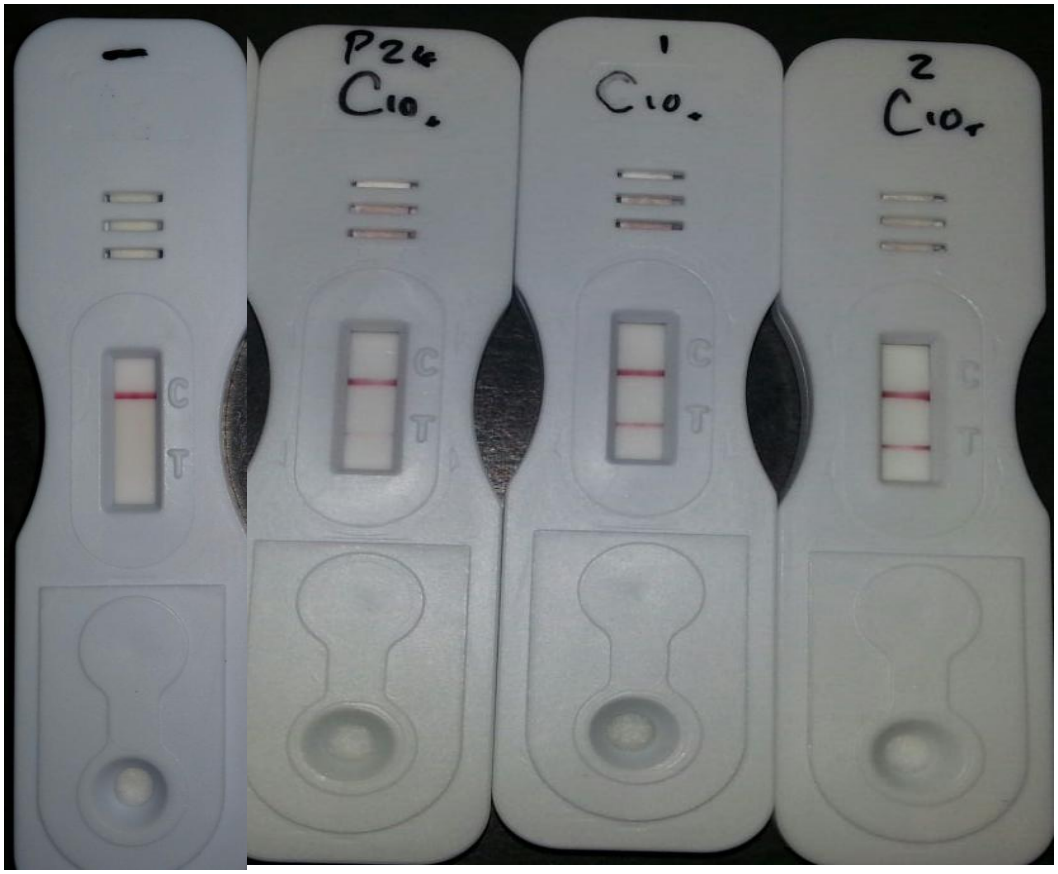
Figure 12: G-Rating of "in-house" binding assay



(A) (B)

Figure 13: AMP 1/ 1.1 LFD binding assay testing (A) p24 negative sample and (B) recombinant p24 protein

LFD prototype using AMP 1 and AMP 1.1 for HIV detection



A B C D

Figure 14: AMP 1/ AMP 1.1 LFD Prototype testing samples (A) HIV negative sample, (B) p24 antigen, (C) Global HIV-1 standard and (D) Global HIV-2 standard

Conclusion and Future work

- Identification of novel AMPs
- Identified AMP prototype which accurately detects HIV-1 and HIV-2
- Surface Plasmon Resonance
- Elucidate structural binding interactions: NMR
- Therapeutic capacity of AMPs
- Field study of LFD prototype testing at least 500 patients.

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