



SELECTION OF SPECIFIC HUMAN SINGLE CHAIN ANTIBODIES AGAINST  
GLYCOPROTEIN 120 OF HIV

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**ABSTRACT**

*The development of human specific antibodies with broadly neutralizing properties is needed for preventing of HIV-1 infection. The production of neutralizing antibodies against HIV-1 envelope glycoproteins have shown effective neutralization of different strains of the virus. Due to several advantages of single chain antibodies (scFvs), these antibodies have been introduced in anti-viral targeted therapy. In this investigation we describe the selection of specific scFvs against RGPGRAFVTI sequence, conserved neutralizing epitope of gp120. After four rounds of panning a specific clone was selected with the frequency of 65%. The selected scFv was tested in ELISA. Results demonstrated the reactivity and specificity of the selected scFv. The OD obtained from reaction of the antibody with the corresponding epitope was significantly higher than the negative control. The isolation of such a specific anti-gp120 of HIV suggest further evaluation of the selected specific scFv for its application in clinical use.*

**Introduction**

Rapid increase in the number of human immunodeficiency virus (HIV)-infected individuals has become one of the most serious challenges of worldwide health and decreasing or preventing the progression of this pandemic disease has met little success (1). According to estimation of World Health Organization, more than 6800 people become infected with HIV and more than 5700 persons die from AIDS every day (2). The first anti-HIV drug that licensed in 1987 for treatment of HIV infection was Zidovudine (AZT) which interferes with viral replication (1). Currently five different groups of anti-HIV drug are used including nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside Reverse Transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), entry/fusion Inhibitors and integrase Inhibitors (3). The virus is

changing permanently due to high mutation rate and generates different HIV mutant strains that are resistant to immune responses and anti-retroviral drugs. However, nowadays innovative strategies including immunotherapy through neutralizing antibodies and vaccination have been developed as the best hope for effective HIV therapy and prevention (4). Among these, some of neutralizing antibodies against viral envelope proteins have shown effective neutralization of different strains of the virus *in vitro* (5).

The glycoprotein spikes of HIV-1 envelope such as external glycoprotein 120 (gp120) and trans-membrane protein gp41 play main roles in virus entrance and initiation of infection (6). These glycoproteins have conserved epitopes that are common among different HIV-1 strains. Although the presence of variable regions on envelope gp120 enables the virus to escape from immune response, production of neutralizing antibodies against conserved epitopes mediates prevention of different strains of entry into the cells and eradicate the infection (7-9).

Recently, new monoclonal antibodies (mAbs) have been designed that target the gp 120. These mAbs include the PG9/PG16 Ab that recognizes an epitope involving V2 and V3 loops created by the trimeric structure, the PGT Abs against a glycan at position 332 in the C3 region of gp120, the VRC01 mAb that targets the CD4 binding site on gp120 and also the membrane proximal external region of gp41 (10). One of the basic problems for the use of these neutralizing antibodies is that the majority of them are largely strain-specific and therefore would not create protective response against globally circulating viral variants (11). The conserved epitopes of gp120 are poorly immunogenic and/or inaccessible to mAbs. In addition usually no cross-neutralizing activity occurs and the antibodies typically bind to determinants that either varies from virus to virus due to high mutation rate (12)

Single-chain variable fragment (scFv) antibodies which are composed of VH and VL domains linked by a flexible linker offer several advantages over monoclonal antibodies. They can carry radioactive materials and toxic drugs to the target cells, due to their small size they have greater penetration, low kidney uptake, rapid blood clearance, and less immunogenicity. The high affinity and specificity of these recombinant antibodies are additional advantage of scFvs that offer their clinical potential as new and effective agents (13,14). ScFv fragments could gain access to neutralizing epitopes and provide highly virus neutralization effect (9).

In this study we selected specific scFvs against gp120 of HIV-1 using phage display technology and evaluated the reactivity and specificity of the selected antibodies against the gp120 epitope by ELISA.

## Materials and method

### Selection of specific scFv antibody against gp120

Phage rescue was performed on a library of phage transformed E. coli scFv developed previously (15) clones displaying scFv were selected from the library after four rounds of panning. Briefly, immunotube (Nunc, Roskilde, Denmark) was coated with the gp 120 peptide as the epitope (amino acids 311-320 of gp120 of HIV) at 4°C overnight. The phage-rescued supernatant ( $10^{10}$  PFU/mL) diluted with blocking solution (skimmed milk 2%), added to the tube and incubated for 1h at room temperature. Following washing log phase E. coli TG1 cells were added and incubated at RT for 1hr with occasional shaking. The tube was centrifuged and the pellet was grown and rescued with helper phage M13KO7 (Amersham, Biosciences). Four rounds of panning were performed to remove nonspecific scFvs and select the specific and high affinity binders.

### PCR and DNA Fingerprinting of the selected clones

The existence of VH-Linker-VL inserts of the selected clones obtained after panning was tested by PCR on the clones and the single chain fragments were amplified. DNA fingerprinting of the selected clones were determined by using MvaI restriction enzyme (Roche Diagnostic GmbH, Mannheim, Germany). The common patterns were revealed by electrophoresis. One clone with the most frequent pattern was selected against the epitope and phage-rescued for further evaluations.

### Determination of phage-antibody concentration

Phage antibody supernatant was added to 1ml of log phase TG1 E. coli and incubated with shaking at 37°C for 1 h. Serial dilution of bacteria was cultured on 2TY Agar/Ampicillin medium at 30°C overnight. Number of colonies per dilution was determined and phage concentration titer per milliliter was calculated.

### Evaluation of reactivity of scFvs by phage ELISA

Specificity of the selected scFv was assessed by phage ELISA. The 96 well ELISA plate was coated with the peptide (dilution: 100µg/ml in PBS) at 4°C overnight. An unrelated peptide was used as a negative control. The wells were blocked with 2% skimmed milk for 2 h at 37°C. The plate was washed with PBS/Tween 20 and PBS, the phage-rescued supernatant of each clone containing the selected scFvs was added to the wells. M13KO7 helper phage was added to coated wells as a negative antibody control. After incubation and washing, anti-fd bacteriophage antibody (Sigma, UK) was added and incubated for 1 hr at Rt. Following washing, HRP-conjugated anti-Rabbit IgG (Sigma, UK) was added and left at room temperature for 1 h. The plate was washed and 150µl of the substrate (1µl H<sub>2</sub>O<sub>2</sub> with 0.5 mg/ml ABTS in citrate buffer) was added and the optical density of each well was detected at 405 nm by an ELISA reader (BP-800, Biohit, USA).

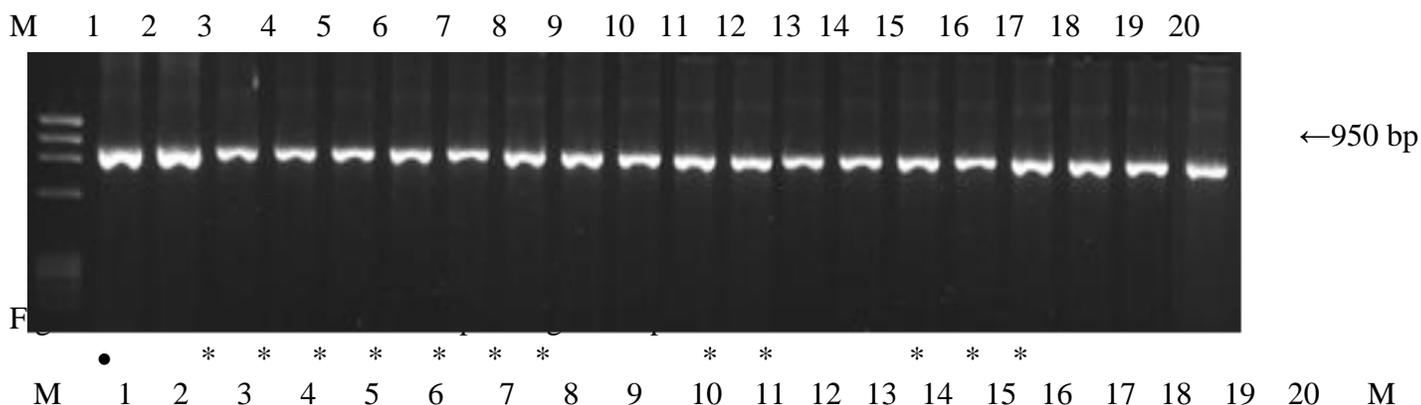
### Statistical analysis

To compare the mean ratio of the phage ELISA results using phage display scFvs against the peptide and of the controls (unrelated peptide, M13KO7, Unrelated scFv and no peptide), Mann-Whitney test was used.

## Results

### Anti-gp 120 selected scFv

Figures 1 and 2 show PCR and DNA-Fingerprinting of 20 panned clones against gp 120 peptide respectively. The presence of VH-Linker-VL are shown by 950 bp PCR product. One common pattern obtained against the peptide, lanes 1, 3, 4, 5, 6, 7, 8, 9, 12, 13, 16, 17, 18 (Fig 2). The frequency of the common pattern was 65% while the other patterns frequencies were 10% and 5%. One clone from the common pattern was used for further evaluation.



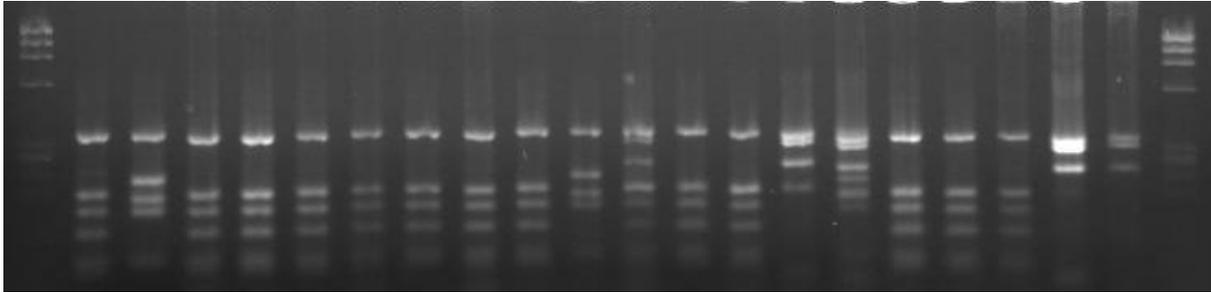


Fig2. Fingerprinting patterns of the selected scFvs. The frequent common patterns are shown in Lanes 1, 3, 4, 5, 6, 7, 8, 9, 12, 13, 16, 17, 18.

### Phage ELISA

Phage ELISA assay demonstrated the binding specificity of the selected scFv to the related peptide.

The obtained OD showed that the scFv antibody reacted with related peptide significantly higher than the wells with no peptide, OD= 1.126 versus OD= 0.208, (Pvalue<0.05). Also the M13KO7 helper phage, unrelated peptide and unrelated scFv showed no reactivity to the peptide (Fig 3).

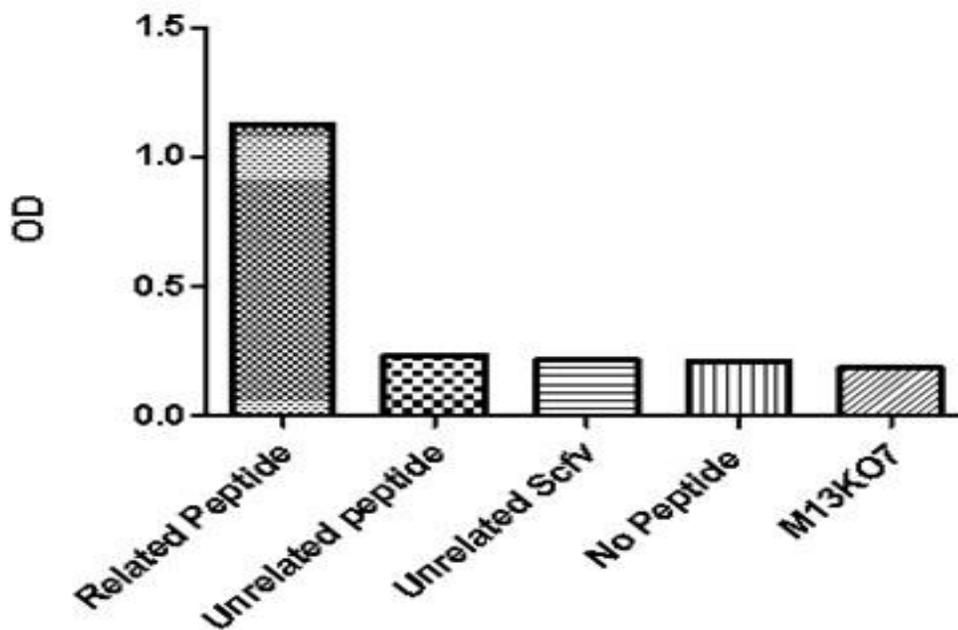


Fig3. ELISA results of the selected scFv against gp120 peptide, unrelated peptide and no peptide control. M13kO7 and unrelated scFv as negative antibody controls showed no reactivity with gp120 peptide.

## Discussion

Current anti HIV treatments prevent cellular entry, viral transcription, and maturation of newly formed virus (16) but, these agents fail to full protection and also are limited by side effects, toxicities and emergence of resistant virus strains (17, 18)

Nowadays novel therapeutic approaches are being tested and shown the potential to improve the immune system against the virus. Immunotherapeutic approaches are one of the exciting areas in this regard. Immunotherapy aims to assist the natural immune system in achieving control over viral infection (19). Various immunotherapy formats have been evaluated in either therapy-naive or therapy-experienced HIV-infected patients over the last 20 years. These formats include non-antigen specific strategies such as cytokines that stimulate immunity or suppress the viral replication, as well as antibodies that block viral entry and infection (20).

In this study we used a conserved neutralizing epitope of HIV-1 gp120 V3loop (RGPGRAFVTI) to select specific scFvs against HIV virus. It has been reported that this epitope contributes to both eliciting neutralizing antibodies and CD8<sup>+</sup> T lymphocytes responses (21). Nino et al (22) reported that this epitope is capable to induce broad HIV-1 neutralizing antibody response when compared with other 12 gp120 V3 loop-derived epitope sequences and introduced as an effective immunogen for vaccine design against HIV. However, the difficulty in eliciting broadly neutralizing monoclonal Abs to gp120 has been attributed to either the lack of immunogenicity of conserved epitopes, or the inaccessibility of these epitopes to antibodies (23) but after introducing the conserved sequences of viral glycoproteins the possibility for producing highly effective antibodies is provided. In the current study after panning against a conserved sequence of gp120, one dominant common pattern with the frequency 65% was detected which represented the selection of a specific scFv against gp120. It has been shown that the specific scFvs are high effective antibodies and can be contribute in clinical applications for better immune-targeting results in comparison with mAbs (14, 15). In addition, mAbs have some disadvantages including high cost, time consuming preparation and inducing of immune reactions which reduce or eliminate their therapeutic efficiency and/or evoke allergic or hypersensitivity reactions in patients (24, 25).

In order to test the reactivity and specificity of the selected scFv, ELISA was done. The results demonstrated a significant higher OD for reaction of the scFv with the corresponding epitope in comparison with the no peptide well. Also the other negative controls showed significant lower OD than the related peptide ( $p < 0.05$ ). The negative reactivity of the selected scFv with the unrelated peptide in comparison with high reactivity with the gp120 antigen represents the specificity of the antibody. The main frequent immunogenic targets of the mAbs include structurally conserved or functionally important epitopes, such as CD4 and chemokine co-receptor binding sites on gp120 and proximal external region of gp41 (26). Current approaches for design vaccine against HIV, mainly is based on conserved sequences because these sequences are supposed as a promising effective immunogenic targets. The V3 loop of gp120 is a component of the chemokine receptor binding region. This region despite possess of highly variable sequences also have about 20% of the amino acid conserved parts and is a target for both mAbs in vivo (27, 28) We selected scFv against 10 amino acids located in crown of the V3 loop of the most of HIV-1 strains which has a major role in binding to cell-surface coreceptors CCR5 or CXCR4 which are involved in viral entry (29, 30). The results suggest further evaluation of the selected specific scFv for its application in clinical use. The absence of complete cure under current treatment underscores the great need for continued efforts in seeking innovative approaches for treatment of HIV/AIDS (31). Considering the well-known advantages of specific scFvs in anti-viral therapies, the neutralizing effects of the selected scFv should be investigated in vivo to provide information for their use in anti-HIV approaches.

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## References

1. KUMAR, R., ANDRABI, R., TIWARI, A., PRAKASH, S. S., WIG, N., DUTTA, D., SANKHYAN, A., KHAN, L., SINHA, S. & LUTHRA, K.( 2012). A novel strategy for efficient production of anti-V3 human scFvs against HIV-1 clade C. *BMC biotechnology*, vol. 12, November, pp. 87.
2. WASTI, S. P., SIMKHADA, P., RANDALL, J. & VAN TEIJLINGEN, E.( 2009). Issues and challenges of HIV/AIDS prevention and treatment programme in Nepal. *Global Journal of Health Science*, vol. 1, October, pp.62.
3. DE CLERCQ, E.( 2009). Anti-HIV drugs: 25 compounds approved within 25 years after the discovery of HIV. *International journal of antimicrobial agents*, vol.33, April, pp. 307-320.
4. PHIMISTER, E. G. & WEISS, R. A. (2014). Immunotherapy for HIV Infection. *New England Journal of Medicine* , vol.370, January, pp. 379-380.
5. XIAO, Y., LU, Y. & CHEN, Y.-H. (2001). Epitope-vaccine as a new strategy against HIV-1 mutation. *Immunology letters*, vol.77, May, pp. 3-6.
6. WANG, H., COLE, D., JIANG, W., JIN, H., FU, N., CHEN, Z. & JIN, N. (2005). Engineering and functional evaluation of a single-chain antibody against HIV-1 external glycoprotein gp120. *Clinical & Experimental Immunology*, vol.141, July, pp. 72-80.
7. KRACHMAROV, C., PINTER, A., HONNEN, W. J., GORNY, M. K., NYAMBI, P. N., ZOLLA-PAZNER, S. & KAYMAN, S. C. (2005). Antibodies that are cross-reactive for human immunodeficiency virus type 1 clade a and clade B v3 domains are common in patient sera from Cameroon, but their neutralization activity is usually restricted by epitope masking. *Journal of virology*, vol. 79, October, pp. 780-790.
8. BALZARINI, J.( 2007). Targeting the glycans of glycoproteins: a novel paradigm for antiviral therapy. *Nature Reviews Microbiology*, vol.5, August, pp. 583-597.
9. WEST, A. P., GALIMIDI, R. P., GNANAPRAGASAM, P. N. & BJORKMAN, P. J.( 2011). Single chain Fv-based anti-HIV proteins: potential and limitations. *Journal of virology*, vol.86, October, pp.195-202.
10. LACERDA, M., MOORE, P. L., NGANDU, N. K., SEAMAN, M., GRAY, E. S., MURRELL, B., KRISHNAMOORTHY, M., NONYANE, M., MADIGA, M. & WIBMER, C. K. (2013). Identification of broadly neutralizing antibody epitopes in the HIV-1 envelope glycoprotein using evolutionary models. *Virology journal* ,vol. 10,December, pp.347.
11. PITISUTTITHUM, P., GILBERT, P., GURWITH, M., HEYWARD, W., MARTIN, M., VAN GRIENSVEN, F., HU, D. & TAPPERO, J. W.( 2006). Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *Journal of Infectious Diseases*, vol.194, December, pp. 1661-1671.
12. NEJATOLLAHI, F., RANJBAR, R., YOUNESI, V. & ASGHARPOUR, M. ( 2013). Deregulation of HER2 downstream signaling in breast cancer cells by a cocktail of anti-HER2 scFvs. *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics*, vol.20, pp. 333-340.
13. NEJATOLLAHI, F., ABDI, S. & ASGHARPOUR, M.( 2013). Antiproliferative and Apoptotic Effects of a Specific Antiprostata Stem Cell Single Chain Antibody on Human Prostate Cancer Cells. *Journal of oncology*, vol.2013, December, pp.1-8
14. NEJATOLLAHI, F., MALEK-HOSSEINI, Z. & MEHRABANI, D.( 2008). Development of single chain antibodies to P185 tumor antigen. *Iranian Red Crescent Medical Journal*, vol.10, October, pp. 298-302.
15. POWDERLY, W. G. 2000. Current approaches to treatment for HIV-1 infection. *Journal of neurovirology*, vol.6, pp. S8.

16. GIRARD, M. P., OSMANOV, S. K. & KIENY, M. P.( 2006). A review of vaccine research and development: the human immunodeficiency virus (HIV). *Vaccine*, vol.24, May, pp. 4062-4081.
17. RATHBUN, R. C., LOCKHART, S. M. & STEPHENS, J. R.( 2006). Current HIV treatment guidelines-an overview. *Current pharmaceutical design*, vol.12, March, pp. 1045-1063.
18. BURTON, D. R., DESROSIERS, R. C., DOMS, R. W., KOFF, W. C., KWONG, P. D., MOORE, J. P., NABEL, G. J., SODROSKI, J., WILSON, I. A. & WYATT, R. T.( 2004). HIV vaccine design and the neutralizing antibody problem. *Nature immunology* ,vol. 5, March, pp. 233-236.
19. SUMIDA, S. M., TRUITT, D. M., KISHKO, M. G., ARTHUR, J. C., JACKSON, S. S., GORGONE, D. A., LIFTON, M. A., KOUDSTAAL, W., PAU, M. G. & KOSTENSE, S. (2004). Neutralizing antibodies and CD8+ T lymphocytes both contribute to immunity to adenovirus serotype 5 vaccine vectors. *Journal of virology*, vol.78, March, pp.2666-2673.
20. CHARLES-NIÑO, C., PEDROZA-ROLDAN, C., VIVEROS, M., GEVORKIAN, G. & MANOUTCHARIAN, K.( 2011). Variable epitope libraries: New vaccine immunogens capable of inducing broad human immunodeficiency virus type 1-neutralizing antibody response. *Vaccine*, vol.29, july, pp.5313-5321.
21. COIA, G., HUDSON, P. J. & LILLEY, G. G. (1996). Construction of recombinant extended single-chain antibody peptide conjugates for use in the diagnosis of HIV-1 and HIV-2. *Journal of immunological methods*, vol.192, june, pp. 13-23.
22. SILVA, F. A. D., SANTA-MARTA, M., FREITAS-VIEIRA, A., MASCARENHAS, P., BARAHONA, I., MONIZ-PEREIRA, J., GABUZDA, D. & GONCALVES, J. (2004). Camelized rabbit-derived VH single-domain intrabodies against Vif strongly neutralize HIV-1 infectivity. *Journal of molecular biology*, vol.340, July, pp.525-542.
23. NELSON, P., REYNOLDS, G., WALDRON, E., WARD, E., GIANNOPOULOS, K. & MURRAY, P.( 2000). Monoclonal antibodies. *Molecular Pathology*, vol.53, June, pp.111.
24. PLANQUE, S., SALAS, M., MITSUDA, Y., SIENCZYK, M., ESCOBAR, M. A., MOONEY, J. P., MORRIS, M.-K., NISHIYAMA, Y., GHOSH, D. & KUMAR, A.( 2010). Neutralization of genetically diverse HIV-1 strains by IgA antibodies to the gp120 CD4 binding site from long-term survivors of HIV infection. *AIDS (London, England)*, vol.24, March,pp. 875.
25. JIANG, X., BURKE, V., TOTROV, M., WILLIAMS, C., CARDOZO, T., GORNY, M. K., ZOLLA-PAZNER, S. & KONG, X.-P.( 2010). Conserved structural elements in the V3 crown of HIV-1 gp120. *Nature structural & molecular biology*, vol.17, July, pp. 955-961.
26. PIETZSCH, J., SCHEID, J. F., MOUQUET, H., KLEIN F., SEAMAN, M. S., JANKOVIC, M., CORTI, D., LANZAVECCHIA, A. & NUSSENZWEIG, M. C.( 2010). Human anti-HIV-neutralizing antibodies frequently target a conserved epitope essential for viral fitness. *The Journal of experimental medicine*, vol.207, August, pp.1995-2002.
27. WYATT, R., KWONG, P. D., DESJARDINS, E., SWEET, R. W., ROBINSON, J., HENDRICKSON, W. A. & SODROSKI, J. G. 1998. The antigenic structure of the HIV gp120 envelope glycoprotein. *Nature*, vol.393, June, pp. 705-711.
28. MONNIER, P. P., VIGOUROUX, R. J. & TASSEW, N. G. (2013). In vivo applications of single chain Fv (variable domain)(scFv) fragments. *Antibodies*, vol.2, April, pp. 193-208.
29. MAMO, T., MOSEMAN, E. A., KOLISHETTI, N., SALVADOR-MORALES, C., SHI, J., KURITZKES, D. R., LANGER, R., ANDRIAN, U. V. & FAROKHZAD, O. C.( 2010). Emerging nanotechnology approaches for HIV/AIDS treatment and prevention. *Nanomedicine* , vol. 5, February, pp. 269-285.