

## **Protein-protein interaction between surfactant protein **D** and DC-SIGN via C-type lectin domain can suppress HIV-1 transfer**

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Running title: SP-D: DC-SIGN interaction and HIV-1 transfer

## Abstract

Surfactant protein SP-D is a soluble C-type lectin, belonging to the collectin (collagen-containing calcium-dependent lectin) family, which acts as an innate immune pattern recognition molecule in the lungs and other mucosal surfaces. Immune regulation and surfactant homeostasis are salient functions of SP-D. SP-D can bind to a range of viral, bacterial and fungal pathogens and trigger clearance mechanisms. SP-D binds to gp120, the envelope protein expressed on HIV-1, through its C-type lectin or carbohydrate recognition domain (CRD). This is of importance since SP-D is secreted by human mucosal epithelial cells and is present in the female reproductive tract including vagina. Another C-type lectin, Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), present on the surface of the dendritic cells, also binds to HIV-1 gp120 and facilitates viral transfer to the lymphoid tissues. Dendritic cells are also present at the site of HIV-1 entry, embedded in vaginal or rectal mucosa. In the present study, we report a direct protein-protein interaction between recombinant forms of SP-D (**rfhSP-D**) and DC-SIGN via their C-type lectin domains. Both SP-D and DC-SIGN competed for binding to immobilized HIV-1 gp120. Pre-incubation of Human Embryonic Kidney (HEK) cells expressing surface DC-SIGN with **rfhSP-D** significantly inhibited the HIV-1 transfer to activated PBMCs. *In silico* analysis revealed that SP-D and gp120 may occupy same sites on DC-SIGN, which may explain the reduced transfer of HIV-1. In summary, we demonstrate, for the first time, that DC-SIGN is a novel binding partner of SP-D, and this interaction can modulate HIV-1 capture and transfer to CD4<sup>+</sup> T cells. In addition, the present study also reveals a distinct mechanism of host defense by SP-D against HIV-1.

## Introduction

Surfactant protein D (SP-D) is a collagen-containing C-type lectin, belonging to the collectin family (1). The primary structure of human SP-D is composed of three subunits of polypeptide chains; each subunit consists of a short N-terminal region, a triple-helical collagen-like region, an  $\alpha$ -helical coiled-coil neck region, and a calcium-dependent highly conserved C-type lectin or carbohydrate recognition domain (CRD) at the C-terminus (2);(3). The primary structure can get cross-linked via the cysteine-containing N-terminal region to give rise to a cruciform structure, which can undergo further multimerization to yield a fuzzy ball, where the CRD regions are facing towards the exterior. SP-D, via its homotrimeric CRD region, is known to interact with a wide range of viral, bacterial and fungal pathogens and bring about clearance mechanisms that involve aggregation or agglutination, opsonisation, enhanced phagocytosis and super-oxidative burst (4);(3). Primarily found in the lungs as a part of pulmonary surfactant, SP-D has been localized at a range of extra-pulmonary sites as a part of mucosal defense system (5).

SP-D is present throughout the female genital tract, with likely involvement in the prevention of uterine infections (6). Epithelial linings of vagina, cervix, uterus, fallopian tubes and ovaries are positively stained for SP-D (7). SP-D has been shown to bind to different strains of HIV-1 (BaL and IIIB) at pH 7.4 (physiological) and 5.0 similar to the pH found in the female urogenital tract (8). Glycoprotein gp120, a highly conserved mannosylated oligosaccharide present on the envelope of HIV-1 virion, plays an important role in the viral entry and facilitates viral replication by activating the NF- $\kappa$ B pathway. SP-D has been shown to bind gp120 of various strains of HIV-1, and prevent HIV-1 interaction with CD4 receptor on T cells, thereby inhibiting viral entry and replication (9, 10).

Another pattern recognition immune regulatory molecule, DC-SIGN/CD209, a type-II transmembrane protein of 44kDa present on dendritic cell (DC) surface (11), plays a major role in mediating dendritic cell (DC) adhesion, migration, inflammation and activation of T cell. DC-SIGN serves as a route of immune escape for pathogens and tumors (12) and is a known receptor for many viruses including HIV-1 and HIV-2. DC-SIGN is expressed by both mature and immature DCs in lymphoid tissues (11, 13), but not on follicular DCs, plasmacytoid DCs or CD1a<sup>+</sup> Langerhans cells (14), monocytes, T cells, B cells, thymocytes, and CD34<sup>+</sup> bone marrow cells. It is also expressed by polarized (M2) macrophages that infiltrate tumours (15), and on antigen presenting cells such as macrophages, and in chorionic

villi of placenta (16). Cells expressing DC-SIGN are located in T cell area of lymph nodes, tonsils and spleen, and dermal DCs in skin (CD14<sup>+</sup> macrophages) (17). DC-SIGN expressing cells are seen in mucosal tissue of rectum (18) (with high antigen-presenting capacities), cervix and uterus, and in foetal tissues endothelial cells in hepatic sinusoid and lymphatic sinus (19, 20).

HIV-1 virus, when exposed to genital and anal mucosal tissues, binds to DC-SIGN on tissue embedded DCs (21, 22) and gets transmitted to CD4<sup>+</sup> T cells, activating adaptive immune response (23, 24). DC-SIGN facilitates HIV-1 transmission in both *cis* and *trans* fashion (25). Expression of DC-SIGN is regulated by IL-4 during monocyte-DC differentiation pathway, along with GM-CSF (26). TGF- $\beta$  and IFNs are known to be inhibitors of DC-SIGN expression, and, thus, indirectly regulate HIV-1 transmission (26).

The interaction of HIV-1 with DC-SIGN takes place in the mucosal tract where SP-D is present. Since both SP-D and DC-SIGN can bind gp120, we set out to examine if interplay between these proteins can modulate DC-SIGN mediated viral transfer of HIV-1. This view was further substantiated by observations that SP-D levels are increased in the bronchoalveolar fluid of HIV-1 patients (27); and rfhSP-D can bind to gp120 of HIV-1, acting as a potent inhibitor of viral infection *in vitro* via inhibition of the interaction between CD4 and gp120 (10). In this study, we show, using recombinant forms of tetrameric and monomeric forms of DC-SIGN and its homologue, DC-SIGNR, that there is a protein-protein interaction between the two C-type lectins via CRD regions. They compete for binding to HIV-1 gp120, and thus, SP-D suppresses DC-SIGN mediated transfer of HIV-1 to CD4<sup>+</sup> cells.

## Materials and Methods

### Recombinant expression and purification of soluble tetrameric and monomeric forms of DC-SIGN and DC-SIGNR

*E. coli* strain BL21 ( $\lambda$ DE3) (Invitrogen, UK) was transformed with pT5T plasmid encoding DC-SIGN sequences (inserted at the Bam HI restriction site into Plasmid construct) with and without multimerizing neck region. In the presence of neck region, the bacterial cells expressed tetrameric DC-SIGN and DC-SIGNR; without the neck region, the corresponding constructs produced monomeric forms of DC-SIGN and DC-SIGNR (28). *E. coli* strain BL21 ( $\lambda$ DE3) cells containing ampicillin (50  $\mu$ g/ml) (Sigma-Aldrich) resistant plasmids [except in the case of DC-SIGNR monomer expressing construct that was kanamycin (50  $\mu$ g/ml) resistant (Sigma Aldrich)] were sub-cultured overnight at 37°C. One liter LB medium containing ampicillin or kanamycin was inoculated with 10 ml of overnight bacterial culture was grown at 37°C until the OD<sub>600</sub> reached 0.7 and then induced with 0.5 mM isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG). After 3 h, the bacterial cells were centrifuged at 13,800  $\times$  g for 15 min to collect the bacterial pellet. Protein expression was analyzed on 12 % SDS-PAGE.

The cell pellet was treated with 22 ml of lysis buffer containing 100 mM Tris, pH 7.5, 0.5 M NaCl, lysozyme (50 $\mu$ g/ml), 2.5 mM EDTA, pH 8.0 and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and left to stir for 1 h at 4°C. Cells were then sonicated for 10 cycles for 30 seconds with 2 min intervals and the sonicated suspension was spun at 10,000  $\times$  g for 15 min at 4°C. The inclusion bodies, present in the pellet, were solubilized in 20 ml of 6 M Urea, 10 mM Tris-HCl, pH 7.0 and 0.01%  $\beta$ -mercaptoethanol ( $\beta$ -ME) by rotating on a shaker for 1 h at 4°C. The mixture was then centrifuged at 13,000  $\times$  g for 30 min at 4°C and the supernatant was drop-wise diluted 5-fold with loading buffer containing 25 mM Tris-HCl, pH 7.8, 1 M NaCl, and 2.5 mM CaCl<sub>2</sub> with gentle stirring. This was then dialysed against 2 liters of loading buffer with 3 buffer changes every 3 h. Following further centrifugation at 13,000  $\times$  g for 15 min at 4°C, the supernatant was loaded onto a Mannan agarose column (5ml; Sigma) pre-equilibrated with the loading buffer. The column was washed with 5 bed volumes of the loading buffer and the bound protein was eluted in 1 ml fractions using the elution buffer containing 25mM Tris-HCl, pH 7.8, 1M NaCl, and 2.5mM EDTA. The absorbance was read at 280 nm and the peak fractions were frozen at -20. Purity of protein was analyzed by 15% w/v SDS-PAGE.

## **Expression and Purification of rfhSP-D**

*E. coli* BL21 ( $\lambda$ DE3) pLysS plasmid pUK-D1 (containing cDNA sequences for Gly-X-Y repeat, neck and CRD region of human SP-D) was cultured in ampicillin (100 $\mu$ g/ml) (Sigma Aldrich) and chloramphenicol (50  $\mu$ g/ml) (Sigma Aldrich) at 37°C overnight. Expression and purification was carried out as described earlier (29);(30). Bacterial cells were grown until the OD<sub>600</sub> reached 0.6 to 0.8, then induced with 0.4mM IPTG and allowed to grow for 3 additional hours. Cells were then pelleted via centrifugation and bacterial pellet was re-suspended in 50ml of lysis buffer (50mM Tris-HCl, pH 7.5, 200mM NaCl, 5mM EDTA with freshly added 0.1 mM PMSF and 100  $\mu$ g/ml lysozyme) at 4°C for 1 h. Cells were then sonicated at 4 kHz for 30 s with 2 min interval for 15 cycles. The sonicate was centrifuged at 13,800  $\times$ g for 15 min at 4°C to collect the rfhSP-D rich pellet containing inclusion bodies. 25 ml of solubilization buffer (50mM Tris-HCl, pH 7.5, 100mM NaCl, 5mM EDTA, 6M urea) was used to re-suspend the pellet and incubated at 4°C for 1 h. The suspension was then centrifuged at 13,800  $\times$ g, at 4°C for 15 min and the supernatant was dialysed against solubilization buffer containing 4M urea and 10mM  $\beta$ -ME for 2 h at 4°C. The dialysis buffer was serially changed to solubilization buffer containing 2 M, 1 M and 0 M urea at 4°C, 2 h each. Final dialysis was done in solubilization buffer containing 5mM CaCl<sub>2</sub>) for 3 h to completely remove any traces of urea. The dialysate was centrifuged at 13,800  $\times$ g, 4°C for 15 min and the clear supernatant containing rfhSP-D was affinity-purified using maltosyl-agarose column (Sigma-Aldrich). The bound protein was eluted with solubilization buffer containing 10mM EDTA, pH 7.5. Endotoxin levels were removed by passing the purified protein fractions through Polymyxin B column (Detoxi-Gel, Peirce & Warriner, UK) and the levels were measured using the Limulus Amoebocyte Lysate Assay (BioWhitaker, UK). The endotoxin level was found to be <5 pg/ $\mu$ g rfhSP-D.

## **SDS-PAGE and Western blot analysis**

Proteins were separated on a 12% (w/v) SDS-PAGE under reducing conditions. After electrophoresis, the polyacrylamide gels were stained with Coomassie Brilliant Blue. For the western blotting, proteins were electro-transferred onto polyvinylidene difluoride nitrocellulose membrane (Sigma) and blocked with 5% w/v milk in PBS. The membrane bound proteins were probed with primary antibodies (anti-DC-SIGN (1:5000) (ProSci) and anti-SP-D (1:5000) (Medical Research Council Immunochemistry Unit, Oxford) polyclonal antibodies. The blot was then probed with Protein A-HRP Conjugate (1:1000) (Sigma),

followed by color development with Diaminobenzidine (DAB) as a substrate (Sigma-Aldrich, UK).

## **ELISA**

DC-SIGN and DC-SIGNR proteins were coated in carbonate/bicarbonate buffer, pH 9.6 in decreasing double dilutions (5µg/well to 0.625µg/well) in duplicates and left overnight at 4°C. The microtiter wells were blocked with 2% w/v BSA in PBS for 2 h at 37°C. The wells were then washed 3 times with PBS + 0.05% v/v Tween 20 and incubated with a constant concentration (2.5µg) of rfhSP-D in 20mM Tris-HCl, pH 7.5, 100mM NaCl, 5mM CaCl<sub>2</sub> or 5mM EDTA at 37°C for 1 h, followed by 1h at 4°C. Following PBS + Tween 20 wash, the bound rfhSP-D was detected using anti-SP-D (1:5000) polyclonal antibody and Protein A-HRP conjugate (1:5000). Colour was developed using o-Phenylenediamine (OPD) as a substrate and absorbance was measured at 490 nm.

## **Competitive ELISA**

The ability of rfhSP-D to compete with and DC-SIGN for binding to gp120 was analyzed by competitive ELISA. Gp120 was coated at decreasing double dilutions from 250 ng/well in duplicates and left overnight at 4°C. Wells were blocked with 2% BSA in PBS for 2 hours at 37°C. The wells were washed 3 times with PBS + 0.05% v/v Tween 20. We used two formats, one with a constant high concentration of SP-D (5µg/well) with decreasing concentrations of DC-SIGN tetramer (from 5µg/well double diluting to 0.312µg/well) and the other with constant high concentration of DC-SIGN tetramer (5µg/well) and decreasing concentrations of rfhSP-D (from 5µg/well double diluting to 0.312µg/well) in calcium buffer for 1h at 37°C and 1h at 4°C. Two identical plates of each format were probed with anti-DC-SIGN (1:5000) and anti-SP-D (1:5000) to evaluate the respective binding of DC-SIGN and rfhSP-D to gp120. Following washes, the wells were incubated with Protein HRP conjugate (1:1000). Colour was developed using OPD as a substrate.

## **Fluorescence Microscopy**

A human embryonic kidney cells 293 (HEK 293), transfected with DC-SIGN construct (DC-HEK) (31), were grown and maintained in DMEM (Life technologies, UK) containing 10% v/v fetal calf serum (FCS), 2mM L-glutamine, penicillin (100 units/ml)/streptomycin (100 µg/ml) (Thermo Fisher), and blasticidin (5 µg/ml) (Gibco). HEK 293 cells were grown and maintained in DMEM (Life technologies) containing 10% FBS. Both cell lines were

incubated under standard conditions (37°C, 5% v/v CO<sub>2</sub>) until 80-90% confluency was reached. HEK 293 and DC-HEK cells (0.5 x 10<sup>5</sup>) were grown on the coverslips in a 24-well plate (Nunc) overnight to perform three different sets of immunofluorescence experiments; DC-SIGN expression (primary antibody: rabbit anti-DC-SIGN, 1:500 and secondary antibody: anti-rabbit/CY3, 1:500, Thermo Fisher), rfhSP-D (10 µg/ml) binding to DC-SIGN (primary antibody: monoclonal anti-SP-D, 1:500 and secondary antibody: anti-mouse conjugated/CY5, 1:500, Abcam) and co-localization of DC-SIGN and rfhSP-D (primary antibodies: anti-DC-SIGN polyclonal and anti-SP-D monoclonal, 1:500 and secondary antibodies: anti-rabbit/CY3 and anti-mouse/FITC, 1:500) on the cell surface of DC-HEK cells. HEK 293 cells were used as a control for all experiments and DC-HEK cells were incubated with secondary antibody alone as an additional control. Hoechst (1:10,000, Thermo Fisher) was used to stain the nucleus for all the staining experiments. The cells were incubated for 1 h with primary antibody followed by 1 h incubation with secondary antibodies as described earlier with three times phosphate-buffered saline (PBS, Thermo Fisher) washes between each step. For rfhSP-D binding with DC-SIGN analysis, the rfhSP-D was incubated with the cells for 1 h at 4°C. The cells were fixed with 4% paraformaldehyde (PFA, Sigma) before mounting on the coverslips to visualize under a HF14 Leica DM4000 microscope.

### **Viral transfer assay**

Pooled human Peripheral Blood Mononuclear Cells (PBMCs) (HI media Laboratories, India) were stimulated in RPMI 1640 medium (Sigma Aldrich) containing 10% v/v FBS, 1% Penicillin-Streptomycin and 5µg/ml phytohemagglutinin (PHA) and 10 U/ml of rhIL-2 (Gibco) for 24 h. PHA/IL-2 was washed off and activated PBMCs were cultured further in complete RPMI medium. For the experiment, DC-HEK cells were grown in a 12-well tissue culture plate until 80% confluence in complete DMEM/F12 (Sigma Aldrich, USA) containing 10% FBS (Gibco) and blasticidin. Indicated concentrations of rfhSP-D containing 5mM CaCl<sub>2</sub> was added to each well and incubated for 2 h to allow binding to DC-SIGN. The wells without rfhSP-D were used as controls. Excess protein was removed and cells were challenged for 1 h with 5 ng/ml p24 of HIV-1 SF-162 strain (kindly provided by Dr. Jay Levy, AIDS Program, National Institutes of Health, U.S.A.). 5mM EDTA was added along with the virus in EDTA controls. Unbound virus was washed off and DC-HEK cells were co-cultured with PHA/IL-2 activated PBMCs for 24 h to facilitate transfer. PBMCs along with the medium were then separated (siphoned off) from the DC-HEK monolayer and were



transferred to fresh plates. They were cultured further in RPMI 1640 medium containing 10% FBS for 7 days and viral titers were determined in supernatants on day 4 and 7 using HIV-1 p24 antigen ELISA kit (XpressBio Life Science Products, Frederick, MD).

### **Molecular modelling and Bioinformatics**

The crystal structures of trimeric human lung surfactant protein D (PDB ID: 1PW9), CD4 bound to HIV-1 envelope glycoprotein gp120 (PDB ID:1GC1) and homo 10-mer DC-SIGN complexed with sugars (PDB ID:1K9I) were retrieved from Protein Data Bank. The tetrameric form of non-glycosylated DC-SIGN was used for docking studies as this structure was found to bind to rfhSP-D *in vitro* experiments. DC-SIGN tetramer was docked to CD4 already bound to HIV-1 envelope glycoprotein gp120 (PDB ID: 1GC1) using Patch Dock server with default parameters.

The CRD mediated protein-protein interaction between trimeric SP-D and tetrameric DC-SIGN, as observed in this study was further examined by docking these two molecules using ZDOCK algorithm of Discovery Studio (Accelrys Inc.). The best pose of these two molecules was subsequently docked into gp120 using Patch Dock server. The shortlisted poses from PatchDock and ZDOCK were further refined using Fire Dock and RDOCK, respectively.

### **Results**

#### **rfhSP-D and DC-SIGN can interact with each other via their C-type lectin domains**

Structurally, DC-SIGN is composed of an extracellular domain which exists as a tetramer, stabilized by an N-terminal  $\alpha$ -helical neck region, followed by a Carbohydrate Recognition Domain (CRD). DC-SIGN and DC-SIGNR comprising of the entire extracellular domain (ECD) (tetramer) (Figure 1a) and the CRD region alone (monomer) (Figure 1a) were expressed in *E. coli* and affinity-purified on Mannose-agarose (28). The CRD regions of DC-SIGN and SIGN-R bound mannose weakly as majority of the proteins appeared in the flow through. The ECD domains of both DC-SIGN and DC-SIGNR bound to mannose with much greater affinity in the presence of  $\text{Ca}^{2+}$  and eluted with EDTA. A recombinant form of human SP-D, containing 8 Gly-X-Y repeats of the collagen, neck and CRD regions were expressed and purified as homotrimeric molecules, as described earlier (29);(30) (Figure 1b). Tetrameric and monomeric forms of DC-SIGN and DC-SIGNR were checked for their respective interactions with rfhSP-D via ELISA (Figure 2), which showed a calcium- and dose-dependent interaction between the two lectins; tetrameric forms bound rfhSP-D better

that the monomers. This was confirmed by a far western blot (Figure 3a), which revealed that rfhSP-D was able to bind to DC-SIGN and DC-SIGNR proteins immobilized on PVDF membrane. The CRD-mediated protein-protein interaction between trimeric SP-D and tetrameric DC-SIGN was further studied by docking these two molecules. The docked pose showed that the two molecules likely interact via their CRD regions (Figure 3b).

### **rfhSP-D: DC-SIGN interaction leads to competition for binding to HIV-1 gp120**

To examine if rfhSP-D can inhibit the binding of DC-SIGN to gp120, we carried out a competitive ELISA. As expected, both rfhSP-D and DC-SIGN tetramer bound gp120 in a dose and calcium-dependent manner (data not shown) (32). In order to assess a likely interference by rfhSP-D in DC-SIGN: gp120 interaction, a constant concentration of rfhSP-D was used against different concentrations of DC-SIGN and added to solid-phase gp120 (Figure 4). With decreasing concentration of DC-SIGN tetramer, rfhSP-D was able to inhibit DC-SIGN-gp120 interaction, suggesting that the binding sites on these two C-type lectins for gp120 may be overlapping.

### **rfhSP-D co-localizes with DC-SIGN on the surface of transected HEK293 cells**

HEK cells transfected with DC-SIGN (DC-HEK cells) were shown to express DC-SIGN via immunofluorescence microscopy. The DC-SIGN expression was seen on the cell surface on DC-HEK cells distributed evenly as compared to HEK293 cells, which were used as a control, using anti-DC-SIGN polyclonal antibody (Figure 5a). DC-HEK cells, incubated with secondary antibody, alone did not show any expression (Figure 5a). rfhSP-D binding was visible on the cell surface of DC-HEK cells, whereas rfhSP-D binding could not be detected in either HEK293 cells or DC-HEK cells incubated with secondary antibody alone as controls (Figure 5b). rfhSP-D and DC-SIGN co-localised on the HEK cell surface transfected with DC-SIGN construct (Figure 5c).

### **rfhSP-D inhibits DC-SIGN mediated viral transfer to PBMCs in a dose dependent manner**

To understand whether interaction between rfhSP-D and DC-SIGN impacted upon DC-SIGN- mediated HIV-1 transfer to T cells, we performed a co-culture assay using DC-HEK cells and mitogen-activated PBMCs. Presence of rfhSP-D led to a significantly ( $p < 0.005$ ) reduced HIV-1 p24 levels in day 4 and day 7 PBMC culture supernatants in a dose-dependent manner (Figure 6). This suggested that in presence of rfhSP-D, the viral uptake by DC-HEK

was significantly inhibited resulting in reduced transfer and replication of HIV-1 in PBMC cultures. It is likely that rfhSP-D may have occupied sites on both DC-SIGN as well as HIV-1 gp120 that resulted in reduced DC-SIGN interaction with HIV-1 gp120. EDTA significantly inhibited DC-HEK mediated viral transfer, as reported previously (33).

### **Bioinformatics analysis revealed that HIV-1 gp120 and rfhSP-D may occupy the same site on the CRDs of DC-SIGN**

To strengthen our hypothesis that DC-SIGN once bound to rfhSP-D may not interact with gp120, we performed *in silico* analyses. The best-docked pose of rfhSP-D and DC-SIGN was subsequently docked to gp120 using Patch Dock server. The shortlisted poses from Patch Dock and ZDOCK were further refined using Fire Dock and RDOCK, respectively. Two poses suggesting that HIV-1 gp120 and rfhSP-D possibly occupy the same site on CRD of DC-SIGN (Figure 7). Thus, in the presence of rfhSP-D, it is likely that interaction of DC-SIGN with gp120 could be inhibited. To validate our bioinformatics strategy, we evaluated the known interaction of gp120 with DC-SIGN followed by docking with CD4. DC-SIGN binds to gp120 at a site distant from its CD4 binding site, and hence, DC-SIGN bound HIV-1 possibly interacts with CD4 for viral transmission (Figure 8). The global energy of these docked complexes has also been presented (Table 1).

### **Discussion**

In this study, we report, for the first time, an interaction of DC-SIGN and Surfactant Protein D (SP-D), two C-type lectins and pattern recognition receptors; both proteins are known to bind to HIV-1 gp120. We demonstrate that this interaction involves their CRD domains, which is relevant in inhibiting DC-SIGN mediated HIV-1 trans-infection of CD4<sup>+</sup> T cells. Interaction of HIV-1 gp120 with DC-SIGN not only increases the affinity of gp120 for CD4 (34), but also leads to a productive infection via reactivation of provirus involving NF- $\kappa$ B pathway (35);(36). This interaction also results in down-regulation of Nef-induced release of IL-6 (37) and leads to Ask-1 dependent activation leading to induction of apoptosis of human DCs (38). Simultaneous binding of rfhSP-D to both gp120 and DC-SIGN, thus, may result in blockade of DC-SIGN mediated viral transmission and inhibition of replication.

Structure-function studies have revealed that the CRD region of DC-SIGN is the specific ligand binding site that is reliant on the neck region within the ECD (39). This notion was validated in our binding ELISA type assays when we used the tetrameric forms of DC-SIGN

and DC-SIGNR (comprising of the extracellular domain and CRD region) as well as the monomeric forms, which only consist of the CRD region. The binding studies involving rfhSP-D highlighted that multimeric forms bind better, not surprisingly, due to multivalent nature of interactions. Since DC-SIGN promotes HIV-1 infection, we examined if rfhSP-D by virtue to its ability to bind gp120 as well as DC-SIGN can potentially interfere with HIV-1, similar to earlier studies (40); (41); (42). We also included DC-SIGNR (DC-SIGN-Related), a homolog of DC-SIGN, in our study. DC-SIGN-R, expressed on endothelium including liver sinusoidal, lymph node sinuses and placental capillary, can also bind gp120 to facilitate HIV-1 viral infection (43).

The current study provides the first evidence that DC-SIGN is a novel immune receptor or adaptor for the CRD region of SP-D, modulating the HIV-1 infection. Interaction of gp120 and rfhSP-D is calcium dependent as reported earlier (8-10). Tetrameric DC-SIGN also efficiently binds gp120 in a dose-dependent manner, which is not significantly inhibited in presence of sugars similar to previous reports (28, 44). The recombinant rfhSP-D has been shown to inhibit the gp120-CD4 interaction (10) while, DC-SIGN bound trimeric gp140 interacts with CD4 more avidly (34). In vitro competitive assays and the bioinformatics analysis confirmed that rfhSP-D and DC-SIGN compete for gp120. The reduced p24 levels confirmed that rfhSP-D significantly inhibits the DC-SIGN mediated viral transfer.

The rfhSP-D molecule (a recombinant fragment of human SP-D comprising homotrimeric C-type lectins), with part of collagen region,  $\alpha$ -helical coiled-coil neck and CRD region, has been extensively studied via in vitro, in vivo and ex vivo experiments. In a number of studies, rfhSP-D has worked at par with full-length SP-D, as evident from its ability to be therapeutic in murine models of allergic bronchopulmonary aspergillosis (45); (46)), invasive pulmonary aspergillosis (45), and dust mite allergy (47). It can also induce apoptosis in activated eosinophils (29); (48) and PBMCs (49). Thus, rfhSP-D is an excellent well-tested therapeutically active molecule.

Mannose binding lectin (MBL), another serum collectin, is also shown to inhibit DC-SIGN-mediated trans infection of HIV-1 T cells (50) whereas SP-A and SP-D facilitate this transfer (51);(8). Madsen et al incubated SP-D-HIV-1 complexes with iMSDDCs and demonstrated increased viral uptake and transfer from DCs to PM-1 cells. However, the assay system employed in the two studies (Madsen and ours) significantly differed, thus the observed variation in the results. Further studies in appropriate animal models will help to determine the overall effects of SP-D and DC-SIGN binding during virus infections. Our findings have

revealed a new phenomenon in SP-D mediated viral transfer through DCs as rfhSP-D occupies similar sites as gp120 on DC-SIGN. Hence, pre-incubation of rfhSP-D may have occupied gp120 binding site on DC-SIGN (displacement of gp120 via ELISA and in silico analysis), resulting in poor uptake. This must have resulted in reduced transfer of viral particles to activated PBMCs, adding another aspect to rfhSP-D mediated anti-HIV activity.

To summarise, rfhSP-D has the ability to directly inhibit the viral entry by interacting with gp120 and to significantly inhibit the DC-SIGN mediated viral transfer. Importantly, these molecular interactions inhibit the immuno-modulation mediated by gp120 and DC-SIGN further disfavoring the HIV-1 pathogenesis. DC-SIGN binding to SP-D could be one of the ligand-receptor interactions that in turn could play a major role in the inhibition of viral entry. Further, in vivo assays and clinical trials can elucidate the physiological conditions for therapeutic purposes against the infection.

## Reference:

- (1) Holmskov U, Thiel S, Jensenius JC. Collections and ficolins: humoral lectins of the innate immune defense. *Annu Rev Immunol* (2003) **21**:547-578. doi: 10.1146/annurev.immunol.21.120601.140954 [doi].
- (2) Kishore U, Reid KB. Structures and functions of mammalian collectins. *Results Probl Cell Differ* (2001) **33**:225-248.
- (3) Kishore U, Greenhough TJ, Waters P, Shrive AK, Ghai R, Kamran MF, et al. Surfactant proteins SP-A and SP-D: structure, function and receptors. *Mol Immunol* (2006) **43**:1293-1315. doi: S0161-5890(05)00316-0 [pii].
- (4) Tino MJ, Wright JR. Surfactant protein A stimulates phagocytosis of specific pulmonary pathogens by alveolar macrophages. *Am J Physiol* (1996) **270**:L677-88.
- (5) Nayak A, Dodagatta-Marri E, Tsolaki AG, Kishore U. An Insight into the Diverse Roles of Surfactant Proteins, SP-A and SP-D in Innate and Adaptive Immunity. *Front Immunol* (2012) **3**:131. doi: 10.3389/fimmu.2012.00131 [doi].
- (6) Madhukaran SP, Alhamlan FS, Kale K, Vatish M, Madan T, Kishore U. Role of collectins and complement protein C1q in pregnancy and parturition. *Immunobiology* (2016) **221**:1273-1288. doi: 10.1016/j.imbio.2016.06.002 [doi].
- (7) Leth-Larsen R, Floridon C, Nielsen O, Holmskov U. Surfactant protein D in the female genital tract. *Mol Hum Reprod* (2004) **10**:149-154. doi: 10.1093/molehr/gah022 [doi].
- (8) Madsen J, Gaiha GD, Palaniyar N, Dong T, Mitchell DA, Clark HW. Surfactant Protein D modulates HIV infection of both T-cells and dendritic cells. *PLoS One* (2013) **8**:e59047. doi: 10.1371/journal.pone.0059047 [doi].
- (9) Meschi J, Crouch EC, Skolnik P, Yahya K, Holmskov U, Leth-Larsen R, et al. Surfactant protein D binds to human immunodeficiency virus (HIV) envelope protein gp120 and inhibits HIV replication. *J Gen Virol* (2005) **86**:3097-3107. doi: 86/11/3097 [pii].
- (10) Pandit H, Gopal S, Sonawani A, Yadav AK, Qaseem AS, Warke H, et al. Surfactant protein D inhibits HIV-1 infection of target cells via interference with gp120-CD4 interaction and modulates pro-inflammatory cytokine production. *PLoS One* (2014) **9**:e102395. doi: 10.1371/journal.pone.0102395 [doi].
- (11) Geijtenbeek TB, Krooshoop DJ, Bleijs DA, van Vliet SJ, van Duijnhoven GC, Grabovsky V, et al. DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking. *Nat Immunol* (2000) **1**:353-357. doi: 10.1038/79815 [doi].
- (12) van Kooyk Y, Geijtenbeek TB. DC-SIGN: escape mechanism for pathogens. *Nat Rev Immunol* (2003) **3**:697-709. doi: 10.1038/nri1182 [doi].
- (13) Bleijs DA, Geijtenbeek TB, Figdor CG, van Kooyk Y. DC-SIGN and LFA-1: a battle for ligand. *Trends Immunol* (2001) **22**:457-463. doi: S1471-4906(01)01974-3 [pii].
- (14) Engering A, Geijtenbeek TB, van Vliet SJ, Wijers M, van Liempt E, Demaurex N, et al. The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. *J Immunol* (2002) **168**:2118-2126.
- (15) Dominguez-Soto A, Sierra-Filardi E, Puig-Kroger A, Perez-Maceda B, Gomez-Aguado F, Corcuera MT, et al. Dendritic cell-specific ICAM-3-grabbing nonintegrin expression on M2-polarized and tumor-associated macrophages is macrophage-CSF dependent and enhanced by tumor-derived IL-6 and IL-10. *J Immunol* (2011) **186**:2192-2200. doi: 10.4049/jimmunol.1000475 [doi].

- (16) Soilleux EJ, Morris LS, Lee B, Pohlmann S, Trowsdale J, Doms RW, et al. Placental expression of DC-SIGN may mediate intrauterine vertical transmission of HIV. *J Pathol* (2001) **195**:586-592. doi: 10.1002/path.1026 [pii].
- (17) Ochoa MT, Loncaric A, Krutzik SR, Becker TC, Modlin RL. "Dermal dendritic cells" comprise two distinct populations: CD1+ dendritic cells and CD209+ macrophages. *J Invest Dermatol* (2008) **128**:2225-2231. doi: 10.1038/jid.2008.56 [doi].
- (18) Kamada N, Hisamatsu T, Honda H, Kobayashi T, Chinen H, Kitazume MT, et al. Human CD14+ macrophages in intestinal lamina propria exhibit potent antigen-presenting ability. *J Immunol* (2009) **183**:1724-1731. doi: 10.4049/jimmunol.0804369 [doi].
- (19) Jameson B, Baribaud F, Pohlmann S, Ghavimi D, Mortari F, Doms RW, et al. Expression of DC-SIGN by dendritic cells of intestinal and genital mucosae in humans and rhesus macaques. *J Virol* (2002) **76**:1866-1875.
- (20) Zhou T, Chen Y, Hao L, Zhang Y. DC-SIGN and immunoregulation. *Cell Mol Immunol* (2006) **3**:279-283.
- (21) Royce RA, Sena A, Cates W, Jr, Cohen MS. Sexual transmission of HIV. *N Engl J Med* (1997) **336**:1072-1078. doi: 10.1056/NEJM199704103361507 [doi].
- (22) Masurier C, Salomon B, Guettari N, Pioche C, Lachapelle F, Guigon M, et al. Dendritic cells route human immunodeficiency virus to lymph nodes after vaginal or intravenous administration to mice. *J Virol* (1998) **72**:7822-7829.
- (23) Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. *Annu Rev Immunol* (2000) **18**:767-811. doi: 18/1/767 [pii].
- (24) Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* (1998) **392**:245-252. doi: 10.1038/32588 [doi].
- (25) Soilleux EJ, Coleman N. Expression of DC-SIGN in human foreskin may facilitate sexual transmission of HIV. *J Clin Pathol* (2004) **57**:77-78.
- (26) Relloso M, Puig-Kroger A, Pello OM, Rodriguez-Fernandez JL, de la Rosa G, Longo N, et al. DC-SIGN (CD209) expression is IL-4 dependent and is negatively regulated by IFN, TGF-beta, and anti-inflammatory agents. *J Immunol* (2002) **168**:2634-2643.
- (27) Jambo KC, French N, Zijlstra E, Gordon SB. AIDS patients have increased surfactant protein D but normal mannose binding lectin levels in lung fluid. *Respir Res* (2007) **8**:42. doi: 1465-9921-8-42 [pii].
- (28) Mitchell DA, Fadden AJ, Drickamer K. A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. *J Biol Chem* (2001) **276**:28939-28945. doi: 10.1074/jbc.M104565200 [doi].
- (29) Mahajan L, Madan T, Kamal N, Singh VK, Sim RB, Telang SD, et al. Recombinant surfactant protein-D selectively increases apoptosis in eosinophils of allergic asthmatics and enhances uptake of apoptotic eosinophils by macrophages. *Int Immunol* (2008) **20**:993-1007. doi: 10.1093/intimm/dxn058 [doi].
- (30) Dodagatta-Marri E, Qaseem AS, Karbani N, Tsolaki AG, Waters P, Madan T, et al. Purification of surfactant protein D (SP-D) from pooled amniotic fluid and bronchoalveolar lavage. *Methods Mol Biol* (2014) **1100**:273-290. doi: 10.1007/978-1-62703-724-2\_22 [doi].
- (31) Lang SM, Bynoe MO, Karki R, Tartell MA, Means RE. Kaposi's sarcoma-associated herpesvirus K3 and K5 proteins down regulate both DC-SIGN and DC-SIGNR. *PloS one* (2013) **8**:e58056.

- (32) Hong PW, Nguyen S, Young S, Su SV, Lee B. Identification of the optimal DC-SIGN binding site on human immunodeficiency virus type 1 gp120. *J Virol* (2007) **81**:8325-8336. doi: JVI.01765-06 [pii].
- (33) Pohlmann S, Leslie GJ, Edwards TG, Macfarlan T, Reeves JD, Hiebenthal-Millow K, et al. DC-SIGN interactions with human immunodeficiency virus: virus binding and transfer are dissociable functions. *J Virol* (2001) **75**:10523-10526. doi: 10.1128/JVI.75.21.10523-10526.2001 [doi].
- (34) Hijazi K, Wang Y, Scala C, Jeffs S, Longstaff C, Stieh D, et al. DC-SIGN increases the affinity of HIV-1 envelope glycoprotein interaction with CD4. *PLoS One* (2011) **6**:e28307. doi: 10.1371/journal.pone.0028307 [doi].
- (35) Gringhuis SI, Geijtenbeek TB. Carbohydrate signaling by C-type lectin DC-SIGN affects NF-kappaB activity. *Methods Enzymol* (2010) **480**:151-164. doi: 10.1016/S0076-6879(10)80008-4 [doi].
- (36) van der Sluis RM, Jeeninga RE, Berkhout B. Establishment and molecular mechanisms of HIV-1 latency in T cells. *Curr Opin Virol* (2013) **3**:700-706. doi: 10.1016/j.coviro.2013.07.006 [doi].
- (37) Sarkar R, Mitra D, Chakrabarti S. HIV-1 gp120 protein downregulates Nef induced IL-6 release in immature dendritic cells through interplay of DC-SIGN. *PLoS One* (2013) **8**:e59073. doi: 10.1371/journal.pone.0059073 [doi].
- (38) Chen Y, Hwang SL, Chan VS, Chung NP, Wang SR, Li Z, et al. Binding of HIV-1 gp120 to DC-SIGN promotes ASK-1-dependent activation-induced apoptosis of human dendritic cells. *PLoS Pathog* (2013) **9**:e1003100. doi: 10.1371/journal.ppat.1003100 [doi].
- (39) Lozach PY, Lortat-Jacob H, de Lacroix de Lavalette A, Staropoli I, Fong S, Amara A, et al. DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. *J Biol Chem* (2003) **278**:20358-20366. doi: 10.1074/jbc.M301284200 [doi].
- (40) Balzarini J, Van Damme L. Microbicide drug candidates to prevent HIV infection. *Lancet* (2007) **369**:787-797. doi: S0140-6736(07)60202-5 [pii].
- (41) Balzarini J, Van Laethem K, Daelemans D, Hatse S, Bugatti A, Rusnati M, et al. Pradimicin A, a carbohydrate-binding nonpeptidic lead compound for treatment of infections with viruses with highly glycosylated envelopes, such as human immunodeficiency virus. *J Virol* (2007) **81**:362-373. doi: JVI.01404-06 [pii].
- (42) Hoorelbeke B, Xue J, LiWang PJ, Balzarini J. Role of the carbohydrate-binding sites of griffithsin in the prevention of DC-SIGN-mediated capture and transmission of HIV-1. *PLoS One* (2013) **8**:e64132. doi: 10.1371/journal.pone.0064132 [doi].
- (43) Bashirova AA, Geijtenbeek TB, van Duijnhoven GC, van Vliet SJ, Eilering JB, Martin MP, et al. A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. *J Exp Med* (2001) **193**:671-678.
- (44) Geijtenbeek TB, van Kooyk Y. DC-SIGN: a novel HIV receptor on DCs that mediates HIV-1 transmission. *Curr Top Microbiol Immunol* (2003) **276**:31-54.
- (45) Madan T, Kishore U, Singh M, Strong P, Clark H, Hussain EM, et al. Surfactant proteins A and D protect mice against pulmonary hypersensitivity induced by *Aspergillus fumigatus* antigens and allergens. *J Clin Invest* (2001) **107**:467-475. doi: 10.1172/JCI10124 [doi].
- (46) Madan T, Kaur S, Saxena S, Singh M, Kishore U, Thiel S, et al. Role of collectins in innate immunity against aspergillosis. *Med Mycol* (2005) **43 Suppl 1**:S155-63.



- (47) Singh M, Madan T, Waters P, Parida SK, Sarma PU, Kishore U. Protective effects of a recombinant fragment of human surfactant protein D in a murine model of pulmonary hypersensitivity induced by dust mite allergens. *Immunol Lett* (2003) **86**:299-307. doi: S0165247803000336 [pii].
- (48) Mahajan L, Gautam P, Dodagatta-Marri E, Madan T, Kishore U. Surfactant protein SP-D modulates activity of immune cells: proteomic profiling of its interaction with eosinophilic cells. *Expert Rev Proteomics* (2014) **11**:355-369. doi: 10.1586/14789450.2014.897612 [doi].
- (49) Pandit H, Thakur G, Koippallil Gopalakrishnan AR, Dodagatta-Marri E, Patil A, Kishore U, et al. Surfactant protein D induces immune quiescence and apoptosis of mitogen-activated peripheral blood mononuclear cells. *Immunobiology* (2016) **221**:310-322. doi: 10.1016/j.imbio.2015.10.004 [doi].
- (50) Spear GT, Zariffard MR, Xin J, Saifuddin M. Inhibition of DC-SIGN-mediated trans infection of T cells by mannose-binding lectin. *Immunology* (2003) **110**:80-85. doi: 1707 [pii].
- (51) Gaiha GD, Dong T, Palaniyar N, Mitchell DA, Reid KB, Clark HW. Surfactant protein A binds to HIV and inhibits direct infection of CD4+ cells, but enhances dendritic cell-mediated viral transfer. *J Immunol* (2008) **181**:601-609. doi: 181/1/601 [pii].

Table 1. Energy for docked complexes of DC-SIGN and gp120 bound to CD4 refined using FireDock

Rank No.	Global Energy (Kcal/mol)
1	-27.01
2	-21.83
3	-11.99
4	-10.94

## Figure Legends

**Figure 1. SDS-PAGE analysis of purified recombinant forms of DC-SIGN, DC-SIGNR and rfhSP-D.** (a) 12% SDS-PAGE of affinity-purified tetrameric and monomeric forms of DC-SIGN and DC-SIGNR under reduced conditions. (b) 12% v/v SDS-PAGE of affinity-purified rfhSP-D.

**Figure 2. Direct binding ELISA showing interaction between rfhSP-D and DC-SIGN/DC-SIGNR.** DC-SIGN tetramer (a), DC-SIGNR tetramer (b), DC-SIGN monomer (c) and DC-SIGNR monomer (d) were coated at decreasing double dilutions from 5µg/well to 0.625µg/well and then probed with 2.5 µg of rfhSP-D in either in calcium or EDTA buffer. The binding was detected using anti-human SP-D polyclonal antibodies (1:1,000 dilutions). The data represents mean and SD values of at least five experiments.

**Figure 3. Far western blot to detect binding of rfhSP-D to PVDF bound DC-SIGN and DC-SIGNR.** (a) Tetrameric and monomeric variants of DC-SIGN and DC-SIGNR were run on a SDS-PAGE and was transferred to a PVDF membrane followed by incubation with 5 µg/ml rfhSP-D and then probed with anti-SP-D polyclonal antibody. (b) Docked structure of trimeric SPD (yellow cartoon) and tetrameric DC-SIGN (blue cartoon). The 2 molecules interact via their carbohydrate recognition domains

**Figure 4. Competitive inhibition ELISA to show that rfhSP-D inhibits DC-SIGN binding to immobilized HIV-1 gp120.** HIV-1 gp120 tetramer (500 ng per well) was first coated to which 5µg/well to 0.625µg/well of rfhSP-D and a constant concentration (5µg/well) of DC-SIGN tetramer were added. Bound DC-SIGN tetramer was detected by anti-DC-SIGN polyclonal antibodies. Protein A-HRP conjugate (1:1000) was used to detect the antibodies bound and colour was developed using OPD. Zero in the graph represents the control where only PBS was used instead of gp120 and the experiments were repeated 3 times.

**Figure 5. Immunofluorescence microscopy to show rfhSP-D binding to DC-SIGN on the surface of the HEK cells transfected with DC-SIGN construct (DC-HEK cells).** (a) DC-HEK cells incubated with anti-rabbit/CY3 did not show DC-SIGN expression (control). DC-HEK and HEK cells incubated with anti-DC-SIGN followed by anti-rabbit conjugated with CY3 showed the DC-SIGN expression in DC-HEK cells only and not HEK cells. Hoechst was used to stain the nucleus. (b) Analysis of rfhSP-D binding to DC-SIGN on the DC-HEK

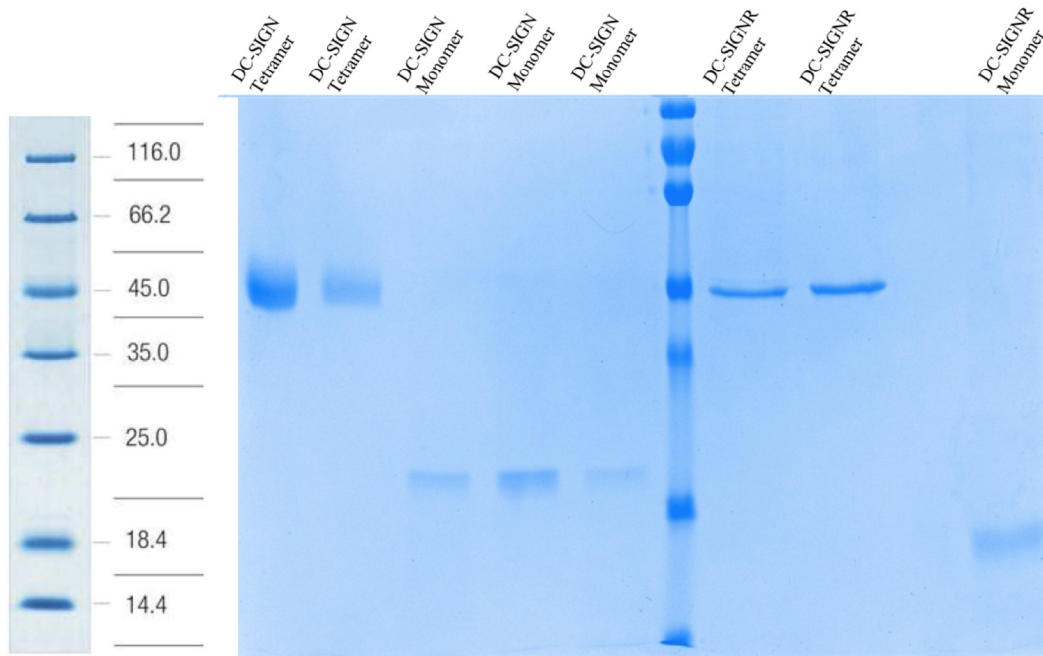
cells via immunofluorescence. DC-HEK cells incubated with anti-SPD for 1 h and then probed with anti-mouse/CY5 did not show binding. DC-HEK cells incubated with rfhSP-D (5 µg/ml) for 1 h, followed by anti-SPD for 1 h and then anti-mouse/CY5 showed the binding on the cell surface. (c) DC-SIGN expression and rfhSP-D binding co-localisation analysis via immunofluorescence microscopy. DC HEK cells incubated with secondary antibodies only (anti-mouse/FITC and anti-rabbit/FITC) for 1 h did not show immunofluorescence. DC-HEK and HEK cells incubated with rfhSP-D for 1 h prior to incubation anti-SP-D monoclonal and anti-DC-SIGN polyclonal for 1 h followed by anti-mouse/FITC and anti-rabbit/CY3 for 1 h showed co-localisation for rfhSP-D binding and DC-HEK expression.

**Figure 6. DC-SIGN-mediated HIV-1 transfer assay.** DC-HEK cells were grown in a 12 well plate until 80% confluence. 20 µg/ml, 10 µg/ml and 1 µg/ml of rfhSP-D concentrations were added to the cells and incubated for 2 h for binding. Unbound protein was removed and cells were challenged with 2.5ng/ml p24 of HIV-1 (SF-162 strain) for 1 hr (to bind to DC-SIGN). After 1h, unbound virus was washed off and cells were co-cultured with PHA-activated PBMCs for 24h. This allows the DC-SIGN captured virus to be transferred to CD4+ cells, where virus will multiply. PBMCs were separated from the monolayer and cultured separately for 4 days to determine viral titre.

**Figure 7. Two poses suggesting that HIV-1 gp120 and rfhSP-D possibly occupy the same site on CRD of DC-SIGN.** Selected docked poses of tetrameric DC-SIGN (blue cartoon) and HIV-1 envelope glycoprotein gp120 (cyan cartoon) bound to CD4 (pink cartoon). The sugars present in gp120 are shown as sticks. The calcium ions of DC-SIGN are represented as green spheres

**Figure 8. Known interaction of gp120 with DC-SIGN followed by docking with CD4.** Docked structures of SP-D trimer (yellow cartoon and calcium ions as red spheres) complexed with DC-SIGN tetramer (blue cartoon and calcium ions as green spheres) and HIV-1 envelope glycoprotein, gp120 (cyan cartoon). The sugars present in gp120 are shown as sticks

(a)



(b)

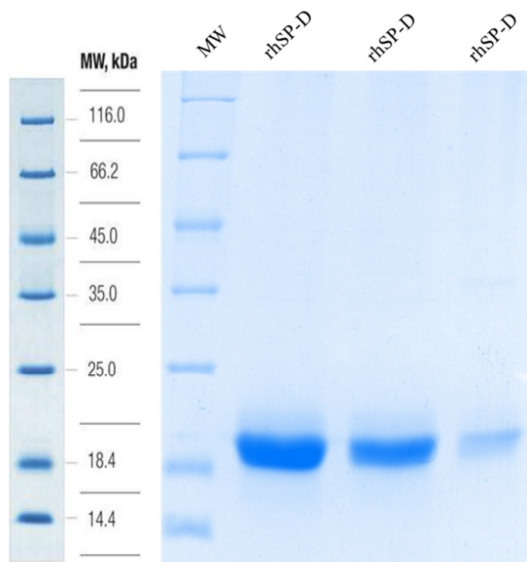


Figure 1

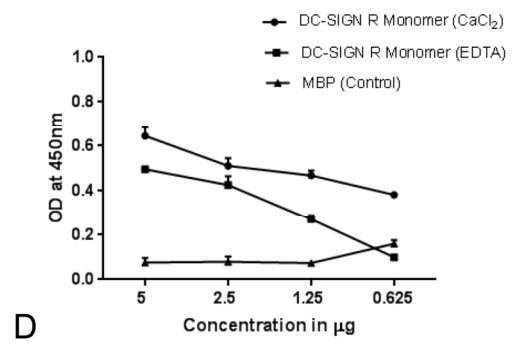
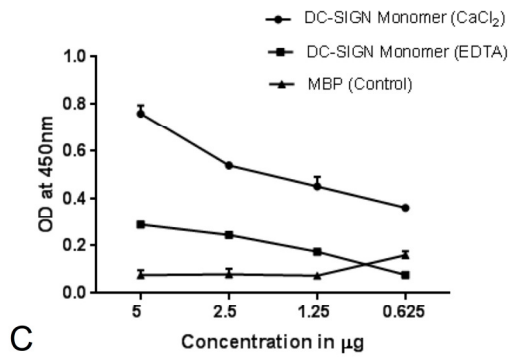
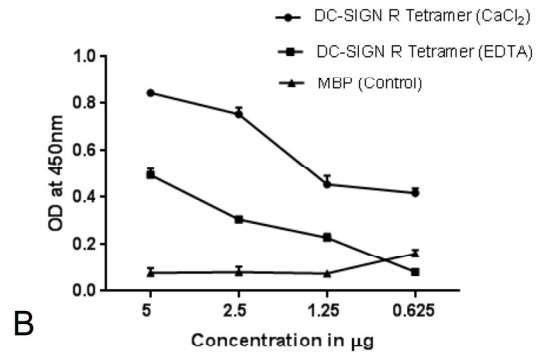
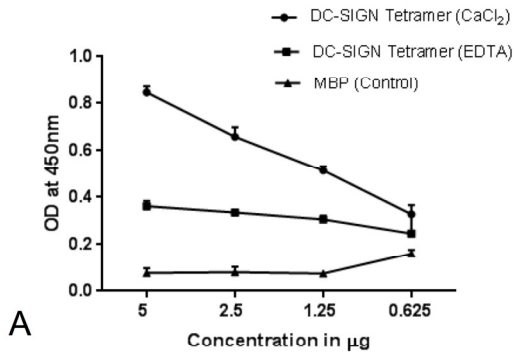


Figure 2 Dodagatta-Marri et al

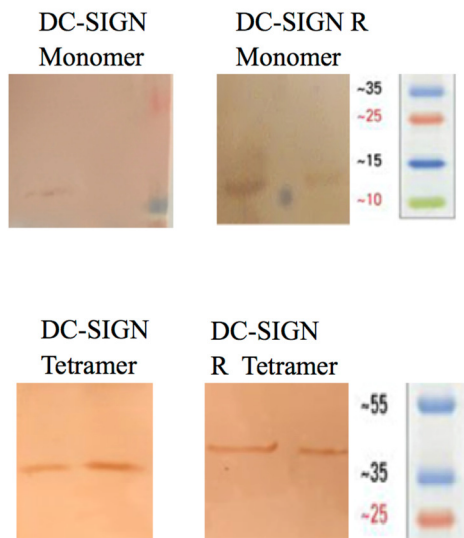


Figure 3a Dodagatta-Marri et al

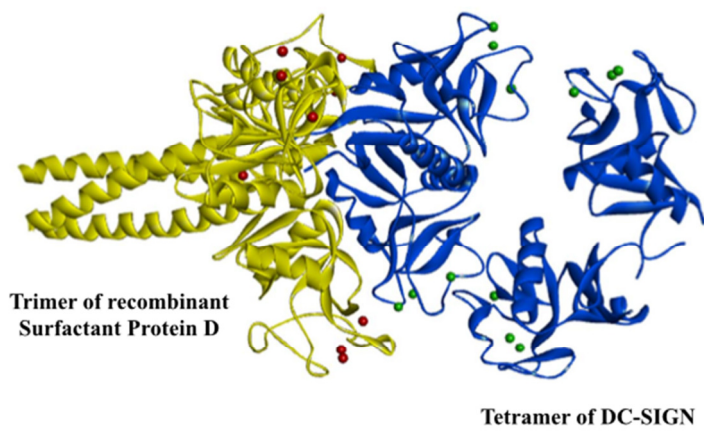


Figure 3b Dodagatta-Marri et al

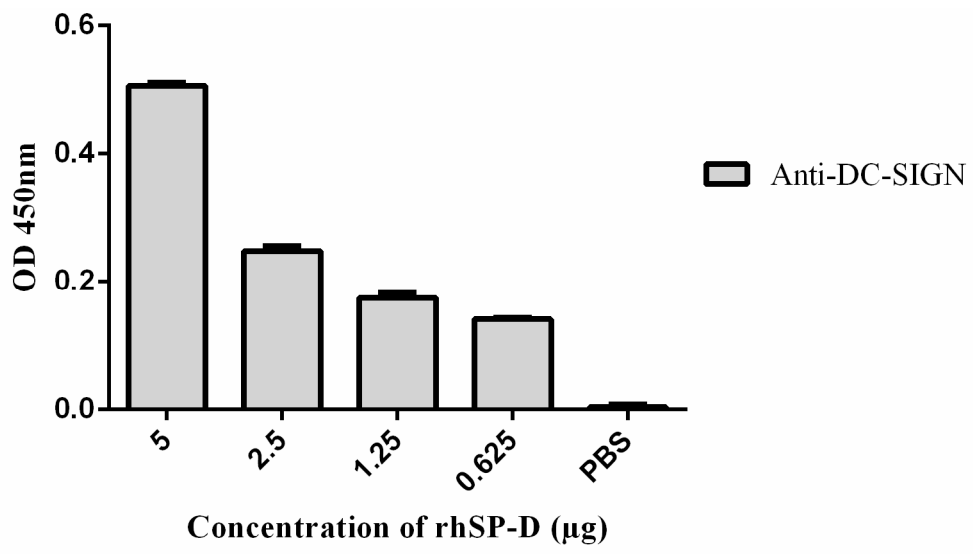


Figure 4

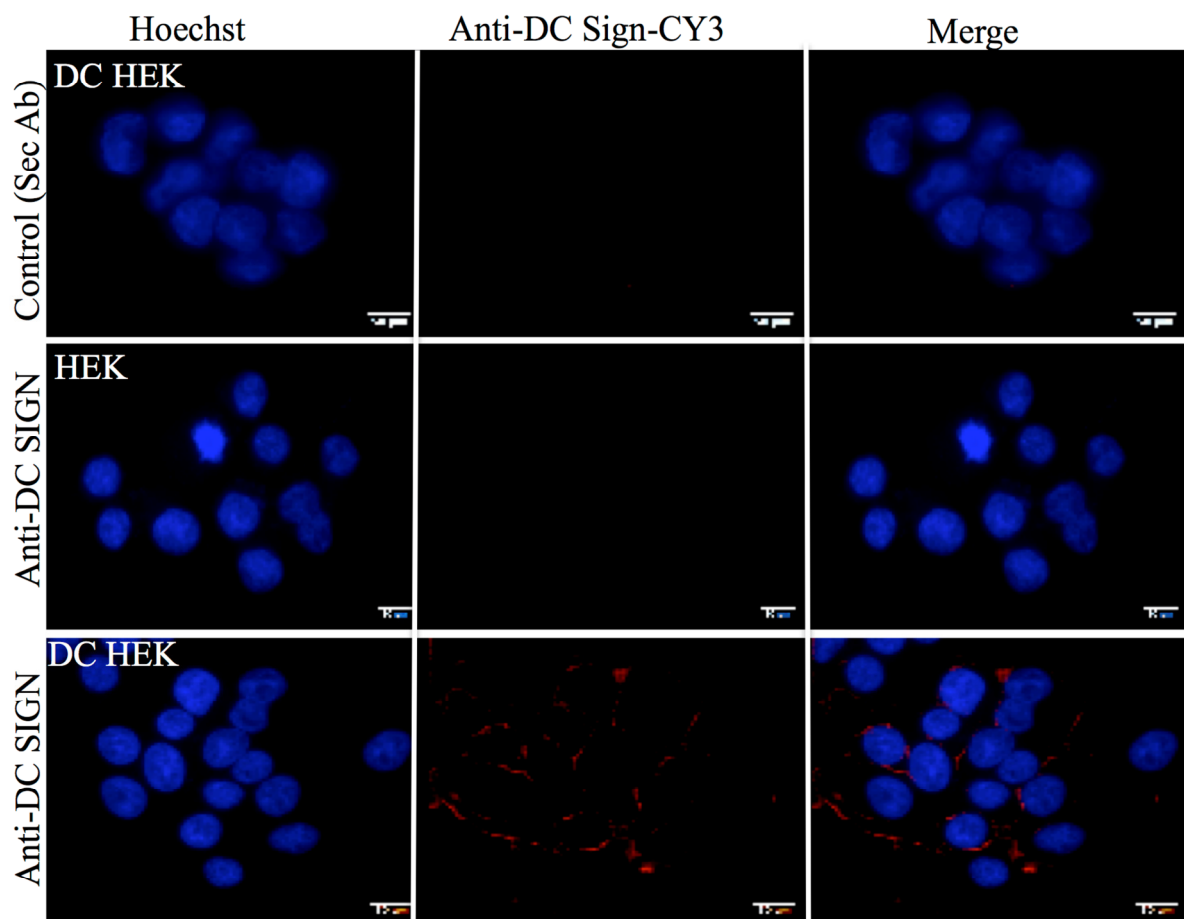


Figure 5a Dodagatta-Marri et al



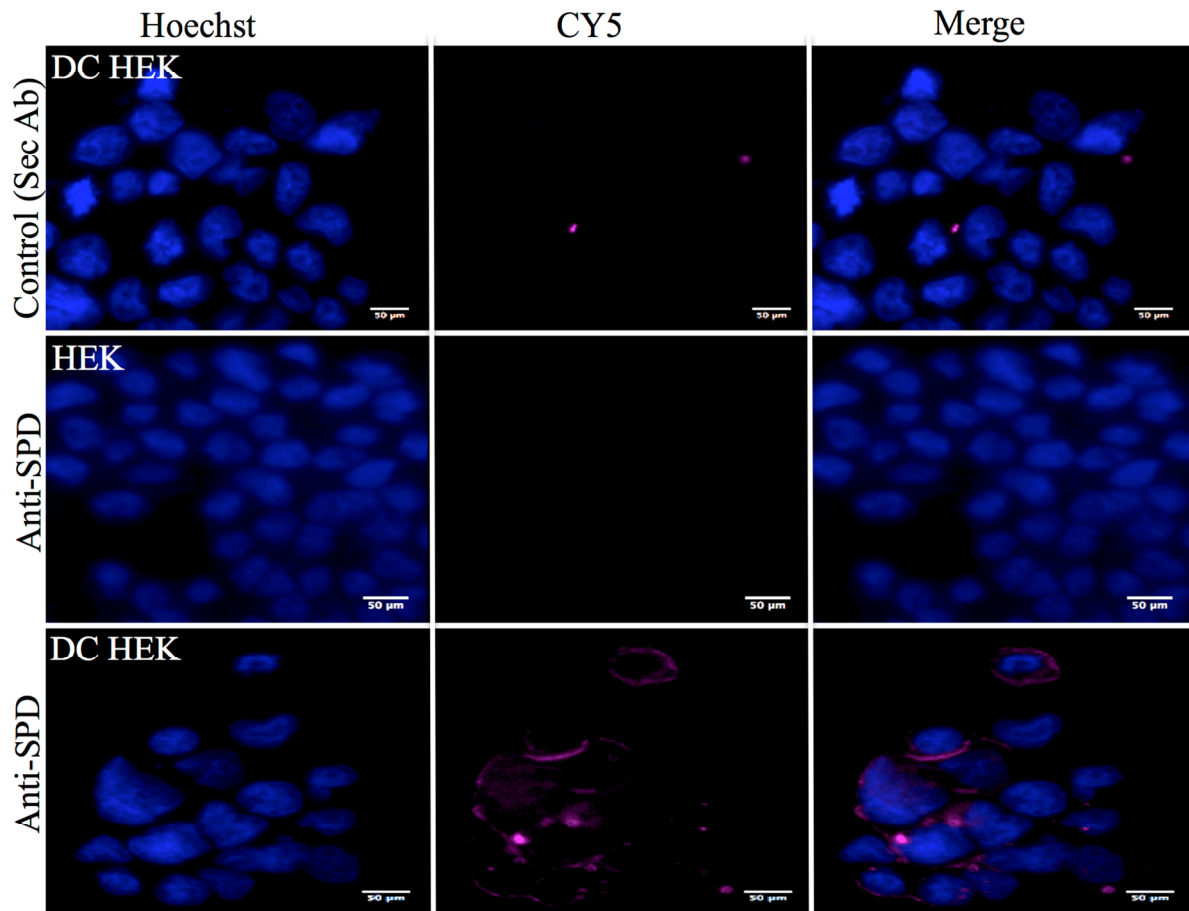


Figure 5b Dodagatta-Marri et al

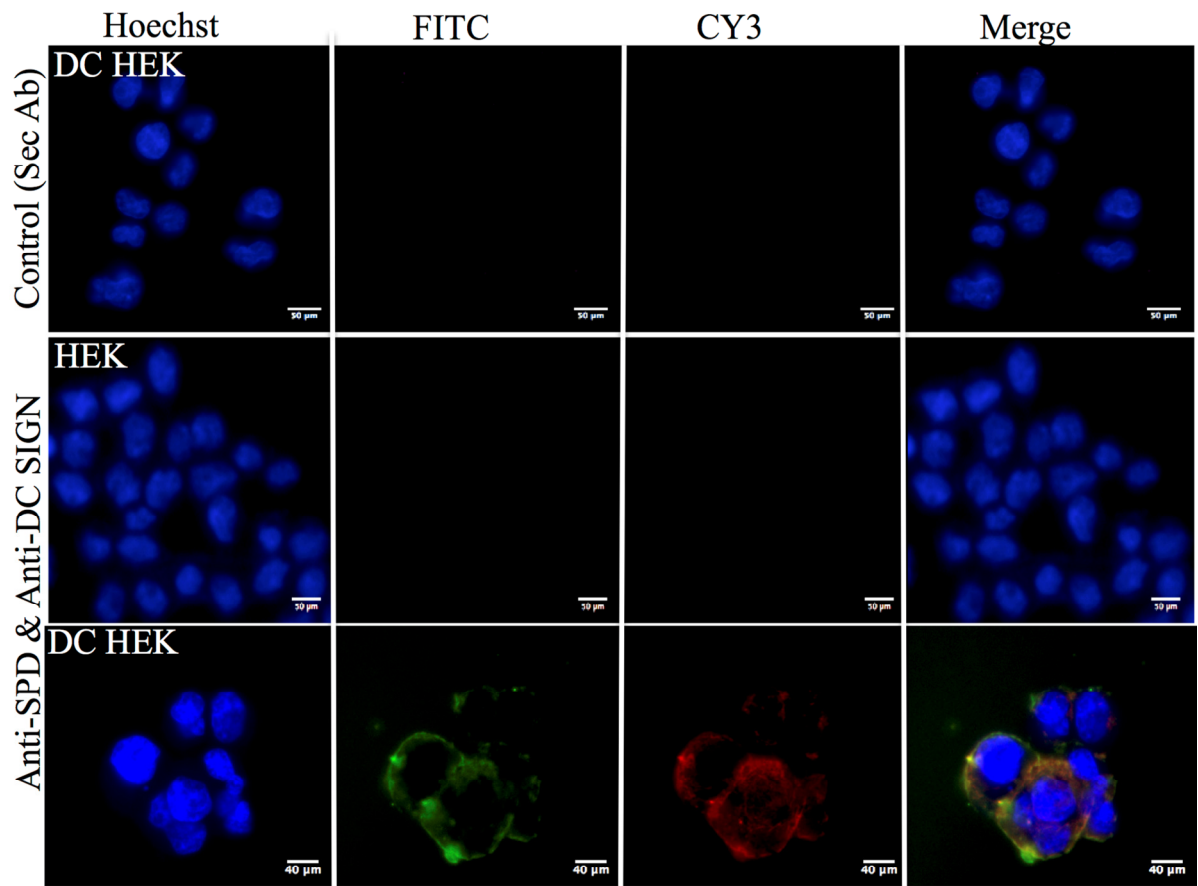


Figure 5c Dodagatta-Marri et al

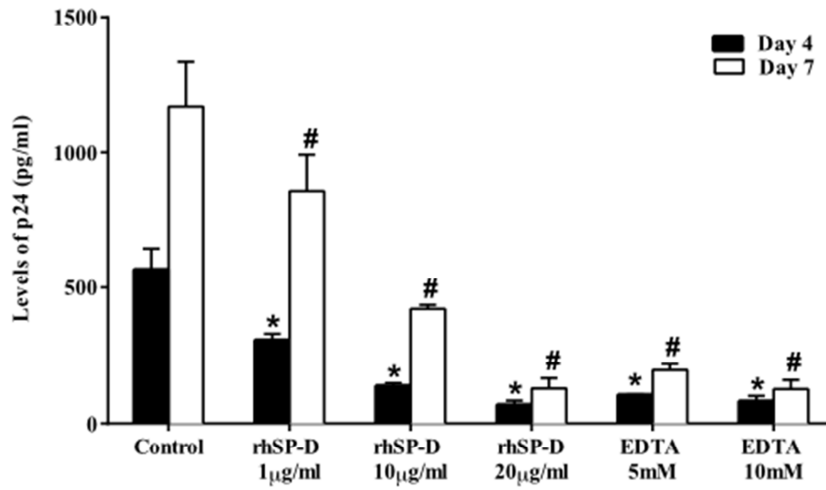


Figure 6.

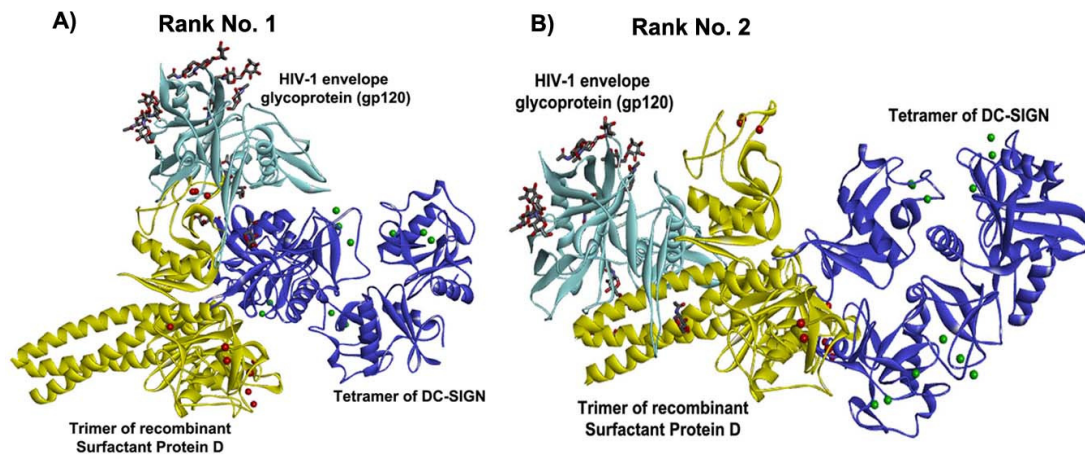


Figure 7.

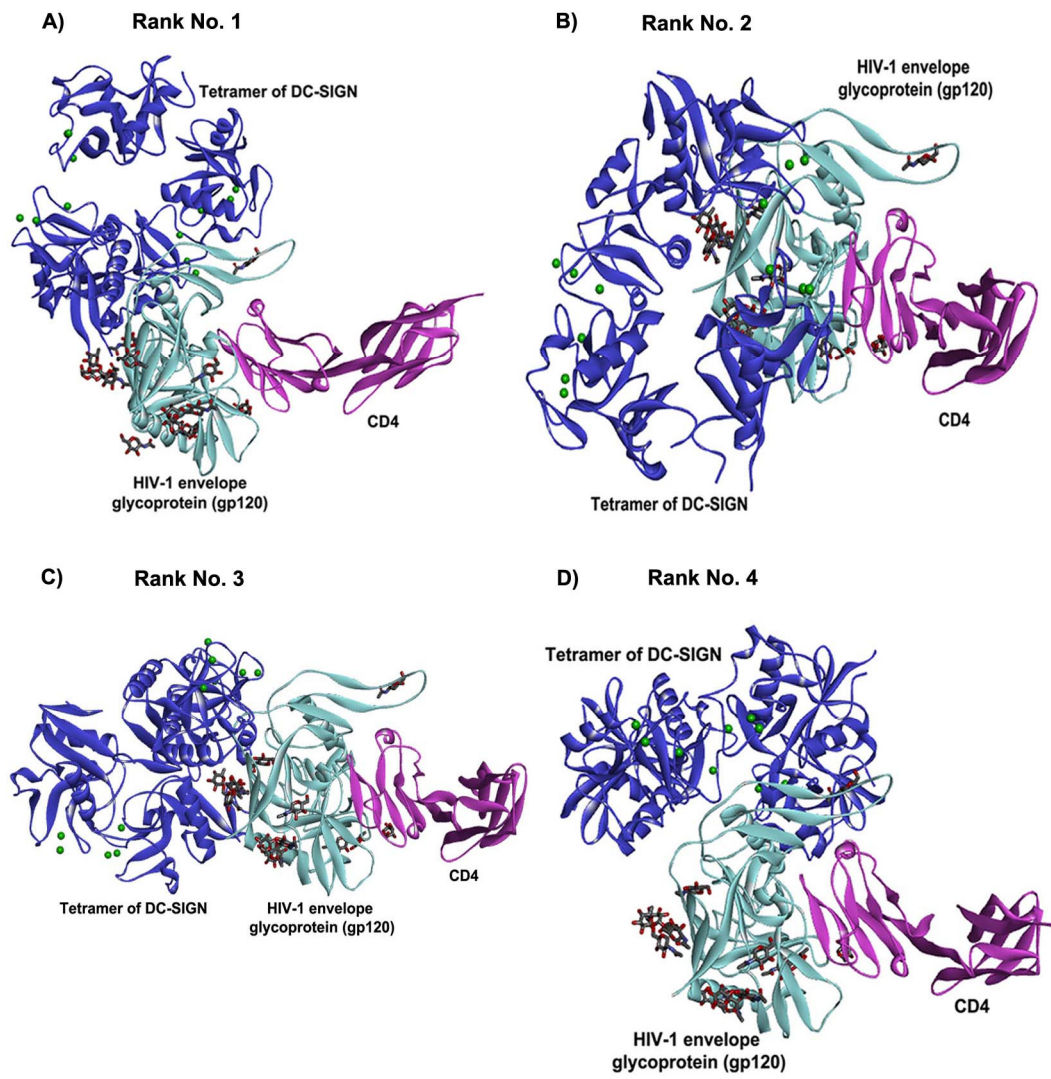


Figure 8