Table of Contents

1. Materials:

All Fmoc-protected α- and β-amino acids, O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU), 1-Hydroxybenzotriazole (HOBt), Rink amide resin {(4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)phenoxy resin) with a 0.55 mmol/g substitution}, N,N- dimethylformamide (DMF), Pyridine and N,N-Diisopropylethylamine (DIPEA) were purchased from Chem-Impex International Inc, Ethynylferrocene and Cu(I)I was purchased from Sigma-Aldrich and used without further purification. Fmoc-cis-4-azidoproline was synthesized starting with commercially available trans-Hyp-OH. Gold(III) chloride hydrate and citric acid were purchased from Sigma-Aldrich, Bis(p-sulfonatophenyl) phenylphosphine dehydrate dipotassium salt (BSPP) from Strem Chemicals. Modified Human Osteosarcoma Cells (HOS.T4.R5) engineered to express CD4 and CCR5, as well as pNL4-3.Luc R-E- was from Dr. Nathaniel Landau[1]. The HOS.T4.R5 cells were grown in DMEM supplemented with 10% FBS, 2.5% HEPES, 1% Penicillin- Streptomycin, 2% L-Glut and 1mg of puromycin. 293T (Human Embryonic Kidney) cells were obtained from American Type Culture Collection and grown in the same growth medium as the HOS.T4.R5 cells but without the antibiotic (puromycin). The plasmid for HIV-1_{BaL} gp160 was a generous gift from Dr. Julio Martin-Garcia. Ten mM phosphate buffer was prepared using monosodium phosphate, monohydrate and disodium phosphate heptahydrate to reach pH 7.2. All other materials were obtained from Fisher Scientific.

2. Synthesis and Characterization of Products.

2.1. Synthesis of KR13 - Peptide Triazole Inhibitors:

Peptide KR-13 was synthesized by manual solid phase synthesis using Fmoc chemistry on a Rink amide resin at 0.25mmol scale. The [3 + 2] cyclo addition ,of azide and Ethynylferrocene was carried out by copper catalyzed, on resin method[2]. Once the synthesis was complete, the peptide was removed from solid-phase resin using a cleavage cocktail mixture of 95:2:2:1 trifluoroacetic acid (TFA)/1,2-ethanedithiol/water/thioanisole for 3 h. Crude peptide was isolated by precipitation into 20 volumes of cold ether and purified by reverse-phase HPLC (Beckmann Coulter) on a C18 column with a linear gradient of 5-95 % of acetonitrile/water in 0.1% TFA (**Figure S1**). The final purified peptide was confirmed by MALDI-TOF-MS, m/z of KR-13: 2084.79 [M+H]+ (Mcal = 2083.5 Da) (**Figure S2**).

2.2. Peptide Characterization and Optical Biosensor Binding Assays:

The effects of KR-13 peptide on gp120 binding of sCD4 and mAb 17b were measured by competition ELISA (Enzyme Linked Immunosorbent Assay), **Figure 1B**. In a typical assay, HIV-1_{YU-2} gp120 (100 ng) was immobilized on a 96-well microtiter plate overnight at 4 °C, followed by three times washing with PBST buffer (1x PBS with 0.1 % Tween-20 v/v) followed by blocking with 3 % BSA (Bovine Serum Albumin) in 1× PBS for 2 h. Serial dilutions of peptide (5 µM to down 0.001 µM) was premixed with 0.1 µg/ml sCD4, and the mixture was added to the plate in triplicate (65 µl/well) and incubated for 1 hour. The plate was washed three times with PBST

followed by 1 hour incubation with biotinylated anti-CD4 antibody (65 µl/well) (eBioscience). The PBST wash step was repeated followed by 1 hour incubation with streptavidin-bound horseradish peroxidase (AnaSpec) at 1:3000 dilution and 65 µl/well. The above experiment was repeated using serial dilutions of peptide (5 µM to down 0.001 µM) mixed with mAb 17b (protein A purified) at 0.1 µg/ml. After 1 h incubation followed by washing three times, goat-anti-human-HRP antibody (Chemcon) was added and incubated for 1 h. The extent of HRP conjugate binding was detected in both assays by adding o-phenylenediamine (200 µL/well) (Sigma–Aldrich) reagent for 30 min followed by measuring optical density (OD) at 450 nm using a microplate reader (Molecular Devices). All incubations were done at room temperature unless otherwise mention and the samples were loaded in triplicate.

Surface plasmon resonance (SPR) interaction analyses were performed on a Biacore 3000 optical biosensor (GE Healthcare) (**Figure 1C**). The experiment was carried out at 25°C using standard 1× PBS, pH 7.3, with 0.005% Tween-20. A CM5 sensor chip was derivatized by amine coupling by using N-ethyl-N-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide1 with HIV-1_{YU-2} gp120 (Fc2 cell) and as a control surface, mAb 2B6R (antibody to human IL-5 receptor α, Fc1 cell). For direct binding experiments, HIV-1_{YU-2} gp120 was immobilized on the sensor surface (~5500 RU); peptide analyte in PBS buffer (concentration range of 125 nM to 0.98 nM) was passed over the surface at a flow rate of 50 µL/min with a 5 min association phase and a 5 min dissociation phase. Regeneration of the surface was achieved by a single 5 s pulse of 10 mM glycine, pH 1.5. Data analysis was performed using BIAEvaluation 4.0 software (GE). A double reference subtraction was performed for each data set to account for non-specific binding. The steady state affinity analysis was performed by plotting data of association equilibrium value selected from 10 sec before dissociation phase for each concentration versus peptide concentration (**Figure 1A**).

2.3. General Methods Gold Nanoparticle (AuNP) Synthesis and Characterization: The citrate reduction method developed by Frens et al.[3] was modified in order to synthesis size-controlled, stable, monodispersed AuNPs (Experimental Section). The solution was then cooled to room temperature and BSPP (18 mg) was added into the synthesized particles and stirred overnight at room temperature. The produced particles were further washed with phosphate buffer at pH 7 and concentrated using the Millipore 100,000 kDa filter. The particle size was obtained using Dynamic Light Scattering (DLS) in the Zetasizer NS90 (Malvern Instruments), and the particle concentration was calculated using the absorbance reading at 450 nm, A_{450} and at the surface plasmon resonance absorbance A_{spr}. This method was adopted from Haiss et al.[4] (**Figure S3**). The 20 nm AuNP particle morphology was characterized using Transmission Electron Microscopy (TEM) (**Figure S4**). Sample was prepared by adding a drop of the AuNP solution onto a carbon grid film and allowed to evaporate. Bright field images were taken on a JEM 2100 operated at 200 kV.

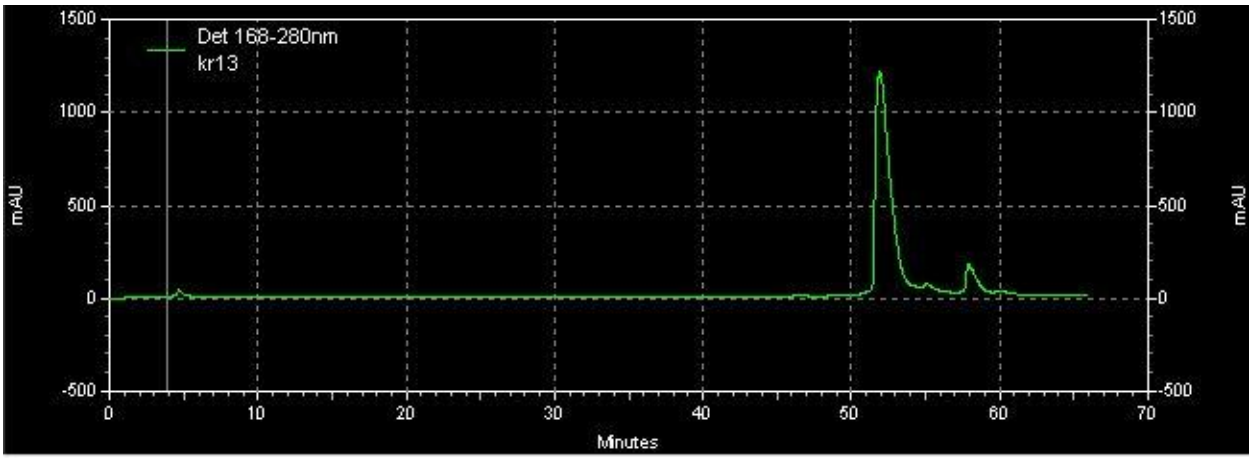

Figure S1: HPLC profile of KR13: VYDAC-C18 analytical column: 5-95% Acetonitrile/water in 0.1%TFA.

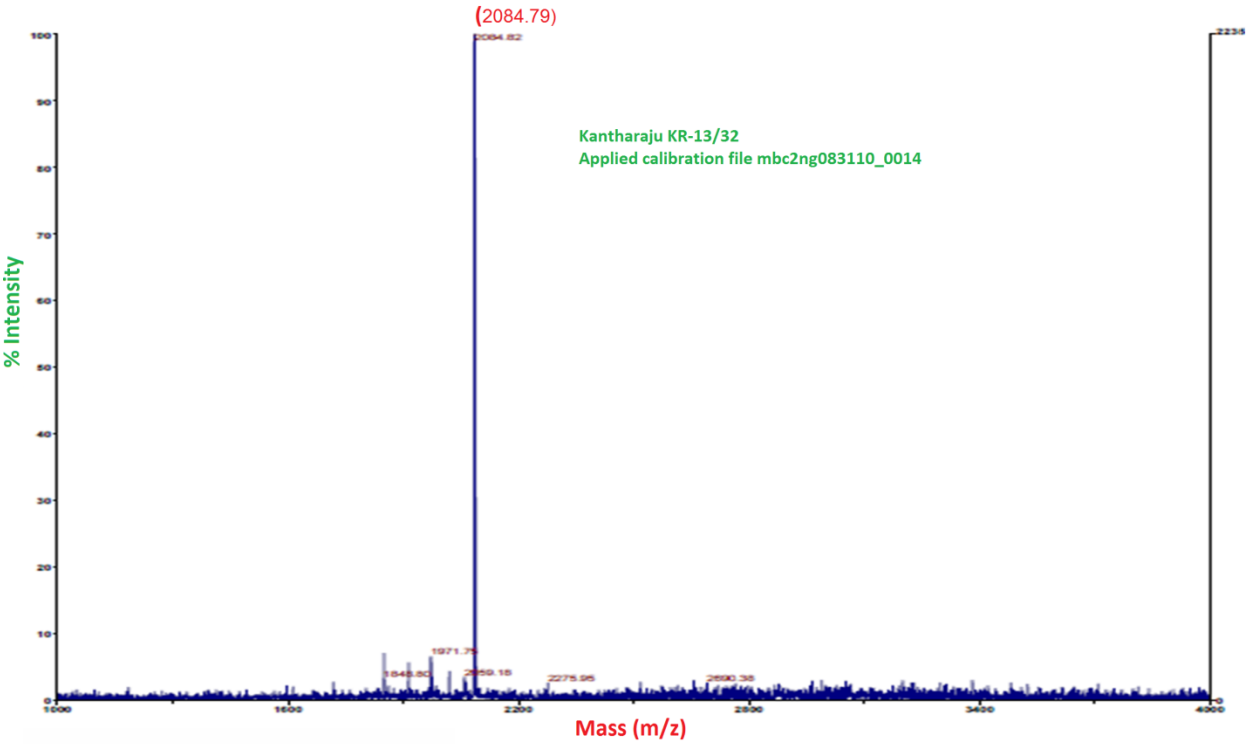

Figure S2: MALDI-TOF spectrum of KR13; m/z Obs : 2084.79 [M+H]+ (*Mcal* = 2083.5 Da) (data from Wistar Inc.)

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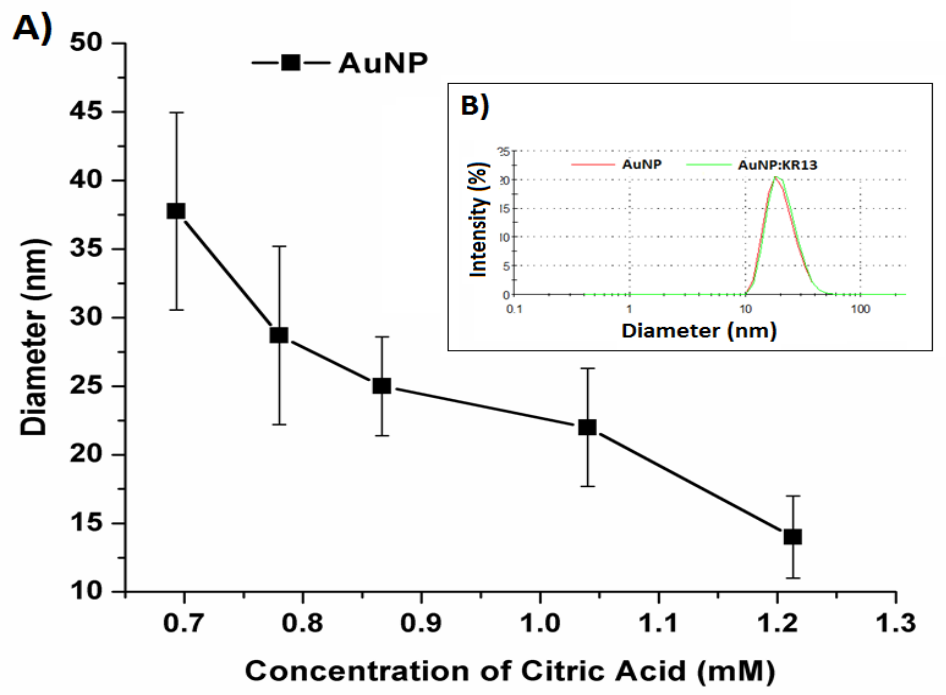

Figure S3: **A)** Dynamic Light Scatterning (DLS) measurements of the produced AuNPs (Malvern Instruments- Zetasizer NS90). Showing that the concentration of Citric and the size of the particles have a linear dependency. **B) (inset)** Shows the DLS measurement for the 20 nm AuNP particles in comparison to the AuNP-KR13 conjugate showing a 2 nm shift in diameter.

3. Production of Single-Round Recombinant Luciferase Producing HIV-1 Virus Like Particles (VLPs)

The recombinant virus consisted of the pro-viral envelope plasmid sequence corresponding to the CCR5 targeting HIV-1_{BaL} strain or a VSV (Vesicular Stomatitis Virus) pseudotype and the backbone sequence corresponded to an envelope-deficient pNL4-3-Fluc+env– provirus developed by N. Landau[5]. Envelope DNA (4 µg) and the backbone DNA (8 µg) were co-transfected into the 293T (Human Embryonic Kidney) cells using FuGene 6 as the transfection reagent following the manufacturer's protocol. Fourteen hours post-transfection the medium was changed and subsequently the pseudovirus- containing medium supernatants were collected at 24 hour intervals for 72 hours. The pseudovirus-containing supernatant was cleared of cell debris by filtration using a 0.45 µm pore size filter followed by low speed centrifugation. Purification of the pseudovirus was conducted by loading filtered cell supernatant in a Beckman UltraClear TM Tube followed by an underlay of 20% sucrose (1ml) cushion using a hamilton syringe. The samples were centrifuged at 30,000 rpm for 120 minutes at 4°C (Beckman rotor SW41). The viral titers were determined by measuring viral infection on a monolayer of HOS.T4.R5 cells (Modified Human Osteosarcoma Cells (HOS.T4.R5) engineered to express CD4 and CCR5) (data not shown).

4. Cell-Culture and Cytotoxicity Test:

The AuNP-KR13 conjugates were tested for stability in physiological conditions (**Figure S5**), as well as cytotoxicity test in vitro using the using the tetrazolium salt premix reagent, WST-1 from Takara Bio Inc. following the manufacturer's protocol. The formazan product was measured using the microplate reader at absorbance wavelenght 460 nm (Molecular Devices) (**Figure 2B**).

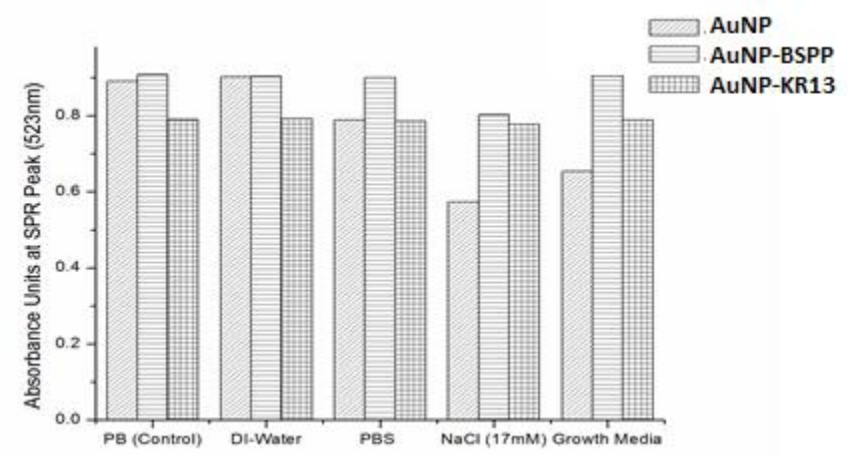

Figure S4: Figure showing the Absorbance peak of the AuNP, AuNP-KR13 as well as AuNP:BSPP at different physiological conditions, Phosphate Buffer (PB), DI-water, PBS, NaCl (17 mM) and HOS.T4.R5 growth media.

5. Additional p24 Release Experiments:

Two sets of additional p24 release assay experiments were conducted. First, in order to compare the virucidal effect of KR13 and AuNP-KR13 conjugate to other ligands, cell-free p24 release from the virion in the presence of sCD4 and HNG156[2b] were conducted (**Figure S5A and B**). Further the specificity of the virucidal effect was characterized by conducting p24 release assay using pseudovirus with VSV-G envelope with the same backbone as the HIV-1_{BaL} strain (**Figure S5C**). The inhibitor was incubated with purified virus (described above), either pseudotyped with HIV-1_{BaL} or VSV-G envelope for 30 minutes followed by a 2 hour centrifugation at 4 $^{\circ}$C and 13,200 rpm (Eppendorf Centrifuge 5415R). The supernant was collected and the p24 content was quantified by western blot analysis using the LiCor IR detection system (Experimental Section). Rabbit anti-p24 (abcam) and Goat Anti-Rabbit IgG conjugated with IRDye® 800CW (LiCor Biosciences) were used as the primary and secondary antibodies respectively. Lysed virus controls were prepared by heating the virus with 1% Triton X-100 for 5 minutes at 95 $^{\circ}$C followed by supernatant collection as the test samples. And further all the gels had a KR13 control which had three serial dilution of KR13 treated with the the same HIV-1_{BaL} strain of purified pseudovirus. **Figure S5** shows data with serial dilutions of the inhibitor incubated with HIV-1_{BaL} or VSV-G pseudovirus

followed by western blot analysis as described above. The results demonstrated that neither HNG156 nor sCD4 led to any p24 release even at the highest concentrations used. Further, the VSV-G control experiment which was conducted using inhibitors KR13 and AuNP-KR13, did not show any p24 leakage.

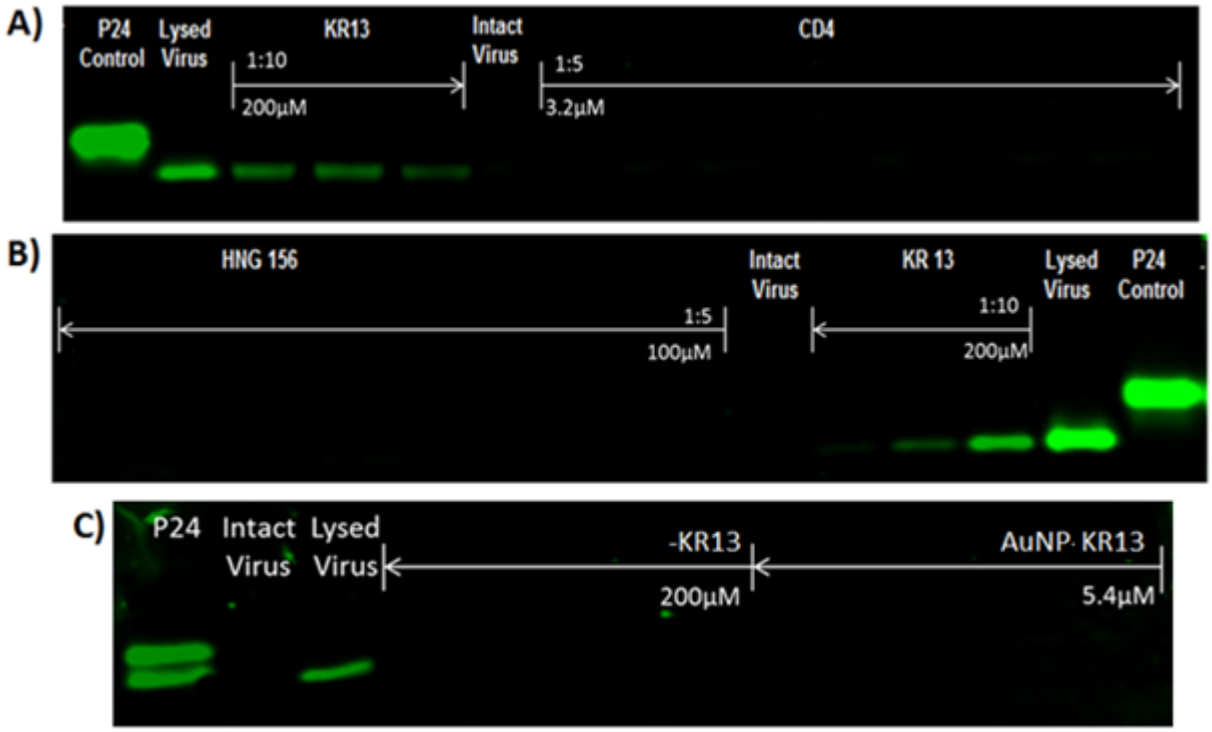

Figure S5: Western blot gel images showing p24 release as a function of dose of **A)** sCD4 and **B)** HNG156 from HIV-1_{BaL} envelope pseudovirus. **C)** The p24 release profile of pseudovirus with VSV-G envelope in the presence of KR13 and AuNP-KR13 as a function of dose. Controls shown are lysed virus (treated with 1% Triton X-100) intact virus (no treatments) and p24 control (5 µL of 20 µg/ml)

6. References

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