SUPPORTING INFORMATION

Multivalent Gold nanoparticle – Peptide Conjugates for Targeting Intracellular Bacterial Infection

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S1: Estimation of the number of peptide molecules bound per molecule of gold nanoparticle

First, the concentration (c) of gold nanoparticle was calculated using the Lambert-Beer's equation. Absorbance (A) of gold nanoparticle was measured at 520 nm using 1 cm pathlength (l) cuvette. Molar extinction coefficient value (ε) of gold nanoparticle of size about 20 nm is 8.78×10^{8} $M^{-1}cm^{-1}$ [1-2].

Using equation (1),

$A= \varepsilon.c.l$ eq. 1

$c= 8.15*10^{-10}$ M

Since we concentrated the gold nanoparticle solution five times for peptide conjugation, therefore, the final concentration of gold nanoparticle in peptide-nanoparticle conjugate solution is 4.075×10^{-9} M (~4.075×10^{-12} mol/ml).

Molecular weight of the peptide VG16KRKP is 1760.14 g/mol.

We have used 0.02 μg (i.e., 1.14×10^{-11} mol) of peptide for conjugation.

Therefore, the number of peptide molecule per gold nanoparticle is (1.14×10^{-11} mol)/ (4.075×10^{-12} mol) = 2.8 ≈ 3.

From the above calculation, it is concluded that each gold nanoparticle is conjugated with 3 peptide molecules.

Table S1: List of medium (i to i+2/3/4) and long range (i to ≥ i+5) NOEs used to calculate the LPS-bound three-dimensional structure of Au-VG16KRKP.

Gold nanoparticle conjugated VG16KRKP in *Salmonella* LPS	
Medium Range NOE	**Long Range NOE**
Val1C$^{\gamma}$H-Trp5H2	Leu11C$^{\delta}$H-Trp5H4
Val1C$^{\gamma}$H-Trp5H4	Leu11C$^{\delta}$H-Trp5H7
Val1C$^{\gamma}$H-Trp5H7	Leu11C$^{\gamma}$H-Trp5H7
Val1C$^{\gamma}$H-Trp5H5	Phe12C$^{\beta}$H-Trp5H7
Val1C$^{\gamma}$H-Trp5HE1	Phe12C$^{\varepsilon}$H-Trp5H7
Ala2C$^{\beta}$H-Gly4NH	Leu11C$^{\delta}$H-Trp5H5
Ala2C$^{\alpha}$H-Trp5H2	Leu11C$^{\gamma}$H-Trp5H5
Ala2C$^{\alpha}$H-Trp5H7	Phe12C$^{\beta}$H-Trp5H5
Ala2C$^{\beta}$H-Trp5H2	Leu11C$^{\delta}$H-Trp5H6
Ala2C$^{\beta}$H-Trp5H2	Leu11C$^{\delta}$H-Trp5HE1
Ala2C$^{\beta}$H-Trp5H7	Ala2C$^{\beta}$H-Phe12H2
Ala2C$^{\beta}$H-Trp5H4	Ala2C$^{\beta}$H-Phe12H3
Arg3C$^{\gamma}$H-Trp5NH	Pro10C$^{\gamma}$H-Trp5H4
Lys6C$^{\gamma}$H-Lys8NH	Pro10C$^{\delta}$H-Trp5H4
Cys9C$^{\beta}$H-Trp5H4	Pro10C$^{\delta}$H-Trp5H5
Cys9C$^{\beta}$H-Trp5H5	Val1C$^{\gamma}$H-Phe12H3
Leu11C$^{\beta}$H-Gly13NH	Val1C$^{\gamma}$H-Phe12H4
Gly16C$^{\alpha}$H-Phe12NH	Trp5HH2-Leu11NH
Gly16C$^{\alpha}$H-Phe12H3	Trp5HZ2-Phe12H3
Gly16C$^{\alpha}$H-Phe12H2	Trp5HE1-Phe12H3
Gly16C$^{\alpha}$H-Lys14NH	Trp5HZ3-Phe12H2
	Phe12C$^{\alpha}$H-Trp5H6
	Phe12C$^{\alpha}$H-Trp5H4
	Phe12C$^{\alpha}$H-Trp5H5
	Gly13C$^{\alpha}$H-Trp5H4
	Gly13C$^{\alpha}$H-Trp5H5
	Gly13C$^{\alpha}$H-Trp5H6
	Gly16C$^{\alpha}$H-Trp5NH

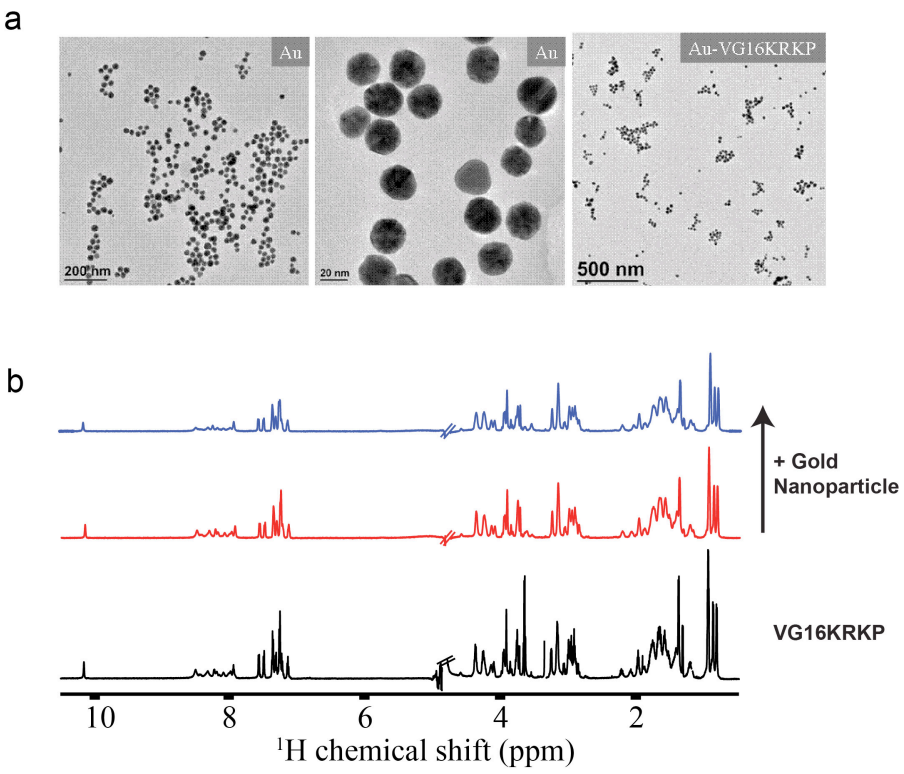


Figure S1. **Characterization of peptide-coated gold nanoparticle:** (a) TEM images of gold nanoparticle, before and after conjugation with the peptide, depicted that the size remains 20 nm even after tagging. (b) Compared to free VG16KRKP (**black**), one dimensional ^{1}H NMR spectrum of VG16KRP upon titration with increasing concentration of gold nanoparticle (**red and blue**) showed significant proton resonance broadening and chemical shift perturbation at all region of the spectrum, signifying binding of the peptide to the nanoparticle.

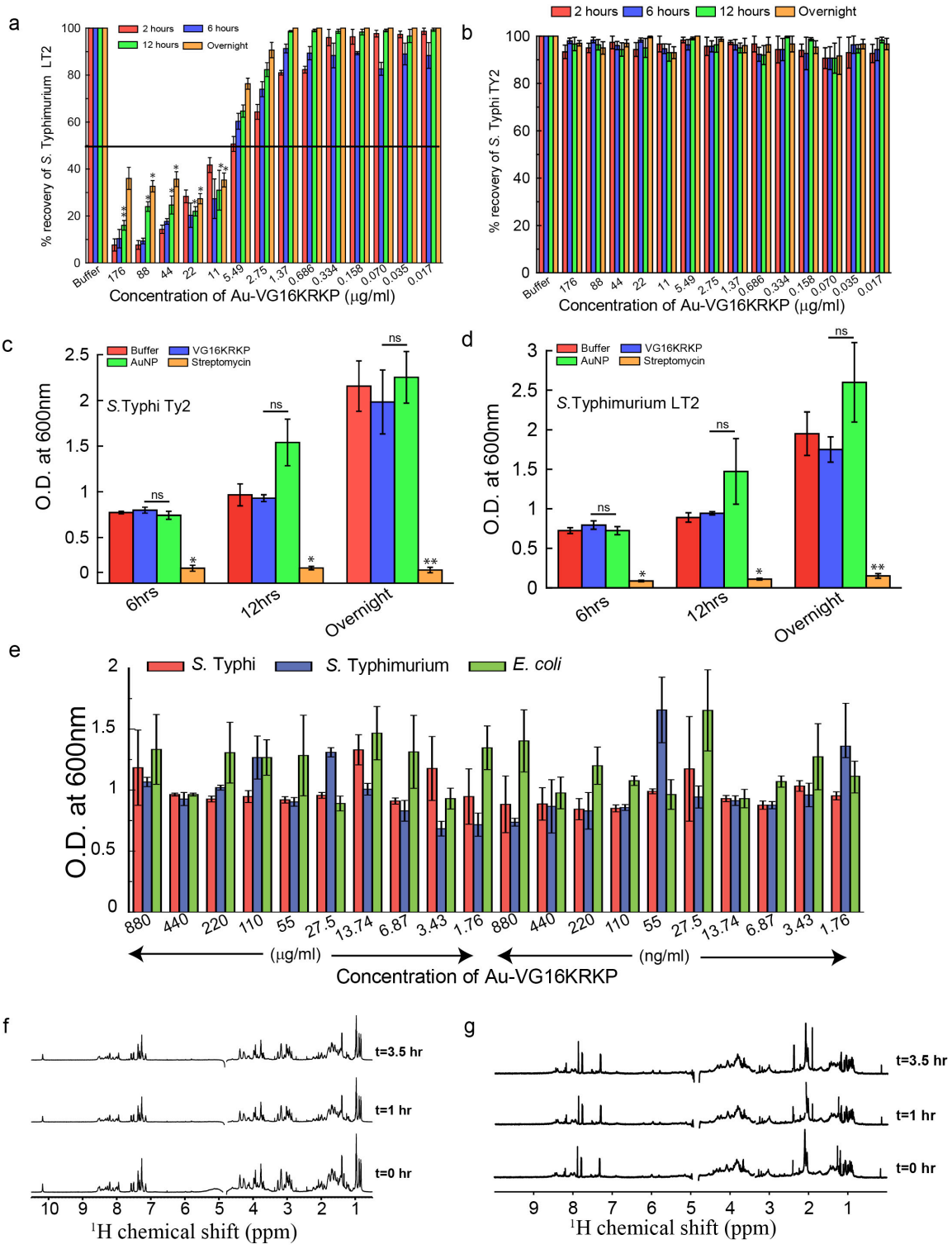


Figure S2. **Antimicrobial assay and live cell NMR of control sets, free VG16KRKP and AuNP**: (a-b) *S.* Typhimurium LT2 showed significant reduction in the CFU counts when incubated with Au-VG16KRKP while *S.* Typhi Ty2 was not inhibited under similar experimental conditions, due to shielding effect by the V_i polysaccharide coat. Statistical significance was calculated by comparing % recovery of bacteria in buffer vs. Au-VG16KRKP treated fractions at various time points, by Mann Whitney test. (c-d) The ability of free peptide, VG16KRKP and only gold nanoparticle (AuNP) to kill *Salmonella* serovars was also tested. Results clearly depicted that when compared to control, both free

VG16KRKP as well as gold nanoparticle were unable to lyse the cells. Statistical significance was calculated by comparing OD_{600} of cultures treated with buffer vs. other treatment fractions at individual time points, by Kruskal Walis test with Dunn's post-test comparison. (e) Even at higher concentrations, free VG16KRKP failed to show any lytic activity thus ruling out the possibility of concentration dependent ability of cell killing. (f) One dimensional ^{1}H NMR spectrum of free VG16KRKP treated *S.* Typhi Ty2 cells showed similar pattern. No metabolite release was observed when the cells were treated for same duration of time, as with Au-VG16KRKP. (g) Live cell one dimensional ^{1}H NMR spectrum of only *S.* Typhi Ty2 cells clearly depicted that there was no metabolite release from untreated cell during the same time period. All experiments were performed in triplicates and more than 6 times. ** $p<0.01$, * $p<0.05$; rest were found to be non-significant (ns).

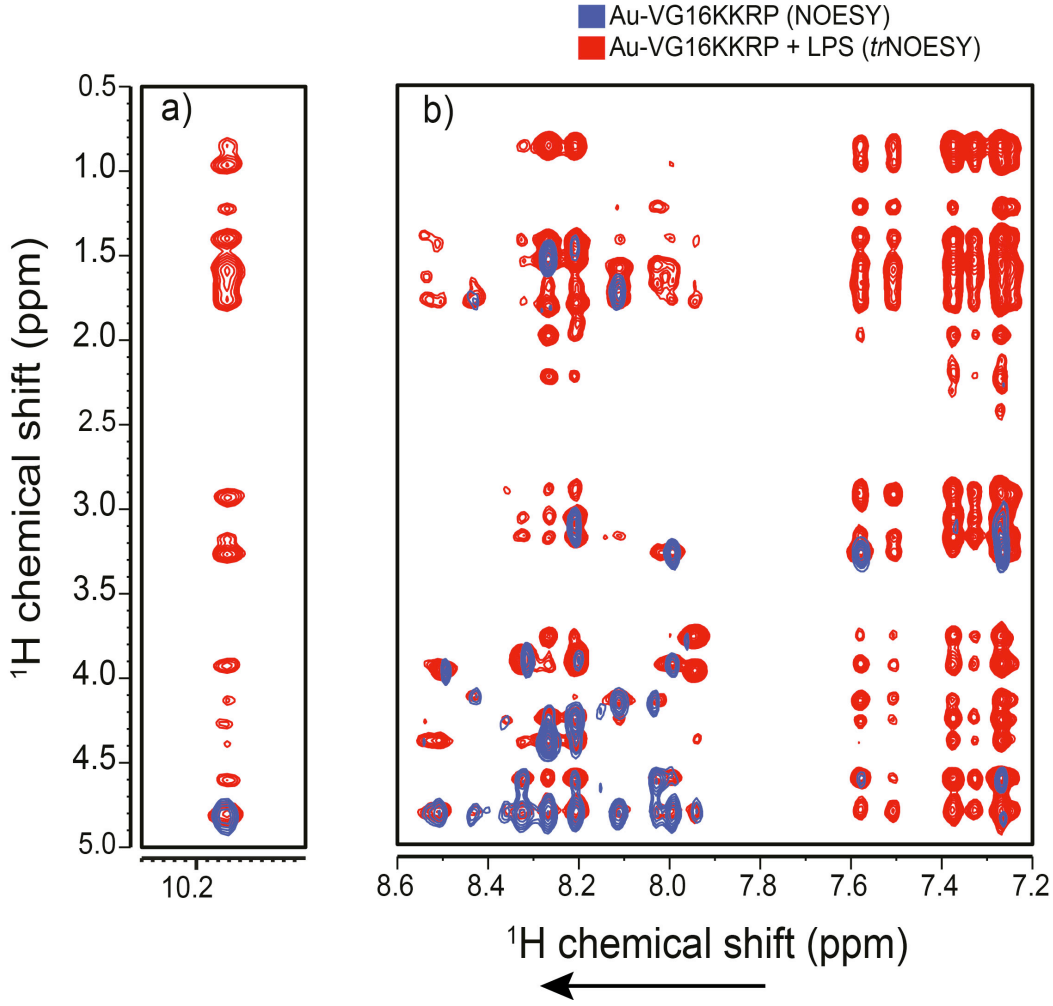


Figure S3. **Overlaid NOESY and trNOESY spectra of Au-VG16KRKP in the absence and presence of LPS:** (a-b) Two-dimensional ^{1}H-^{1}H Nuclear Overhauser Spectroscopy (2D-NOESY) was recorded for Au-VG16KRKP in free solution (**blue contour**) and upon titration with LPS (**red contour**). Au-VG16KRKP remained in an unfolded conformation (**blue**), similar as VG16KRKP, indicated by the lack of NOEs in the NOESY spectrum in aqueous solution, while large number of NOE peaks appeared upon titration with LPS, indicating that nanoparticle tagged VG16KRKP adopts well-defined conformation when bound to LPS. Both the experiments were performed using Bruker Avance III 700 MHz NMR spectrometer, 150 ms mixing time and at 298 K.

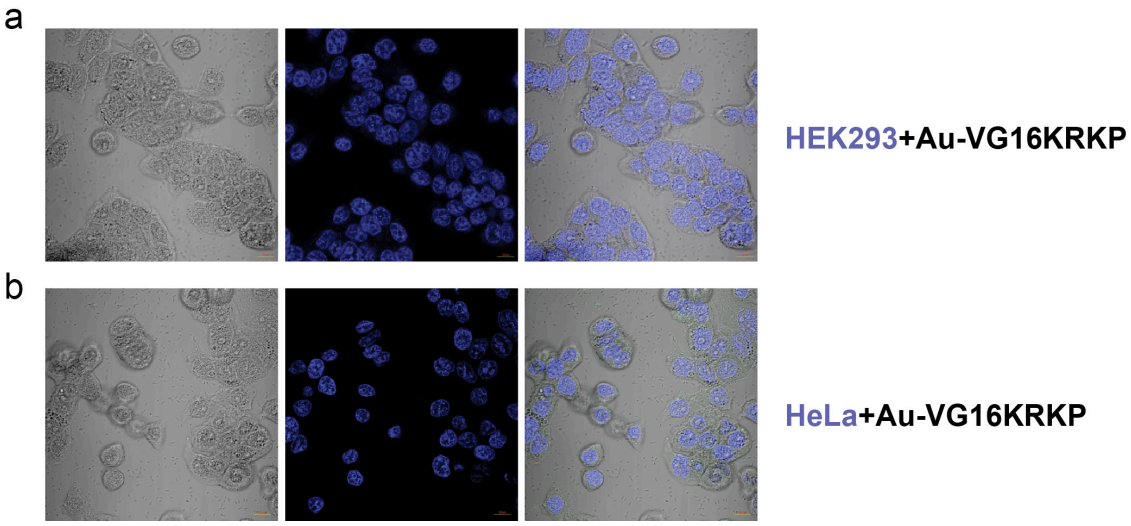
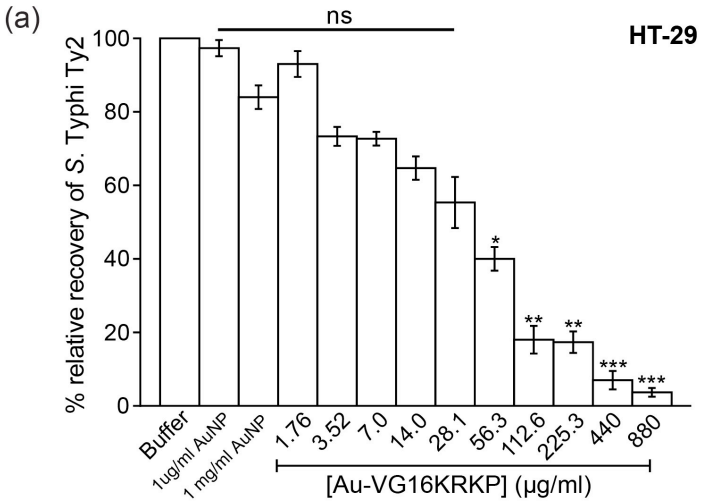


Figure S4. **Au-VG16KRKP can penetrate other epithelial cell lines:** (a-b) Cells were treated with 30µg/ml Au-VG16KRKP and visualized by confocal microscopy. Both **(a)** HEK293 and **(b)** HeLa cells, showed internalization of Au-VG16KRKP thus confirming that the internalization of the tagged peptide is not cell lineage specific.



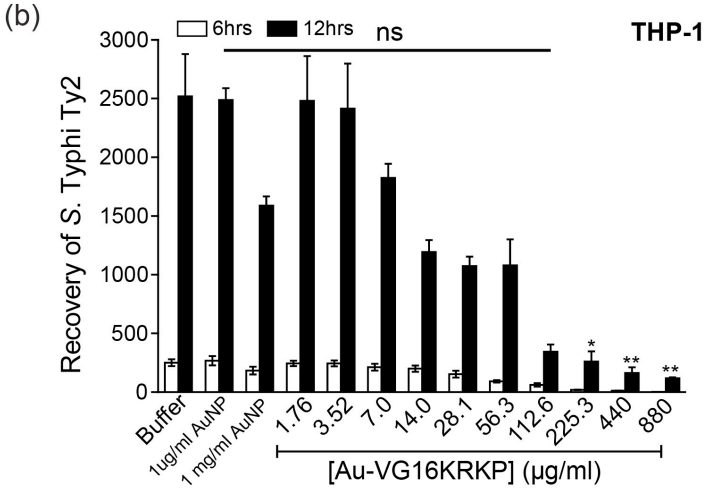


Figure S5. **Bacterial Recovery assay:** (a-b) Gentamicin protection assay was repeated by changing the order of AuNP or Au-VG16KRKP addition to depict specificity of action. It showed similar results, significant reduction in the bacterial recovery and hence, lysis of the intracellular *Salmonella* by Au-VG16KRKP. However, gold nanoparticle (AuNP) failed to cause significant lysis of internalised bacteria in both epithelium (HT29) and macrophage (THP-1) cell lines. Statistical significance in difference of CFU counts between buffer treated and Au-VG16KRKP or AuNP treated cells were calculated by Kruskal Wallis and Mann Whitney test respectively. All experiments were performed in triplicates and more than 6 times. ***, p<0.001 ** p<0.01, * p<0.05; rest were found to be non-significant (ns).

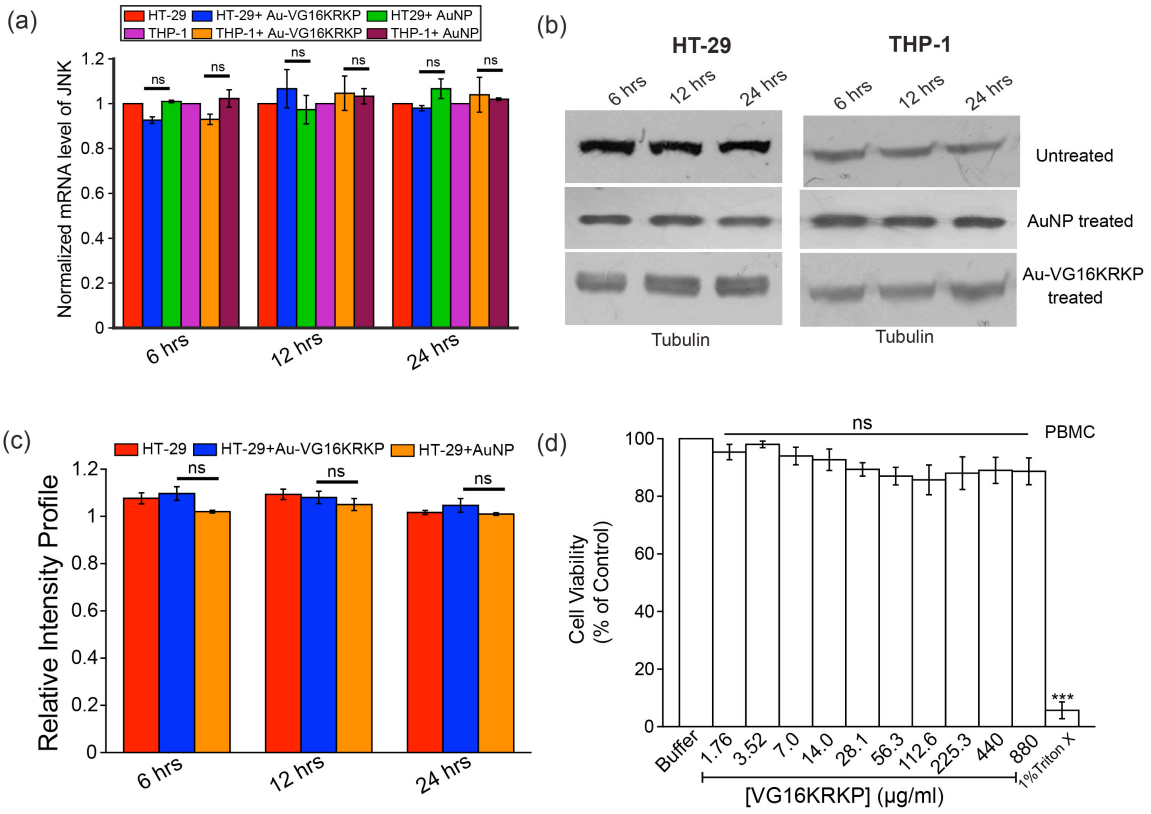


Figure S6. Au-VG16KRKP is non-cytotoxic to the host cells: (a) Real-time PCR of stress marker JNK showed no significant change in relative mRNA profile when compared to untreated control, signifying no stress induction. **(b-c)** Total protein content also remained unaffected when compared to untreated cells, at various time points, as seen by western blot analysis of constitutive protein Tubulin **(b)** and reflected in the intensity profile. **(d)** Cell viability of PBMC freshly isolated from human blood and treated with various concentrations of VG16KRKP was checked and compared to control cells treated only with buffer. Compared to Triton-X, no significant loss of cell viability was observed in any dose of VG16KRKP treatment. Statistical significance was calculated by comparing cell viability of buffer treated cells with VG16KRKP and Triton-X treated cells by Kruskal Wallis Test with Dunn's post-test comparison**.** All experiments were performed in triplicates and more than 6 times. ***, p<0.001 ** p<0.01, * p<0.05; non-significant (ns).

References

[1] Colloids and Surfaces B: Biointerfaces 2007, **58**, 3-7.

[2] Langmuir 2001, **17**, 6782-6786.