Supplemental Information
Multifunctional Gold Nanoparticle-Peptide Complexes for Nuclear Targeting

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Synthesis and characterization of gold particle/biomolecule conjugates

To prepare BSA-peptide conjugates, BSA (Roche, USA) was mixed in aqueous solution with a 10 to 40-fold molar excess of MBS linker (MBS is 3-maleimido benzoic acid N-hydroxysuccinimide ester; Pierce Chem, Co., Rockford, IL). Excess MBS was removed by gel filtration on Sephadex G50. BSA contains lysine residues, which react with the succinimide ester moiety of MBS (Figure S1). The maleimide group remains exposed for functionalization with a cysteine—terminated peptide (20:1 molar excess of peptide was used). After purification by centricon or dialysis the efficiency of peptide conjugation was estimated by mobility shift assay on SDS-PAGE (7.5 %) and by isoelectric focusing electrophoresis (IEF) (Figure S2). Fluorescein-labeled peptides were also used to confirm the peptide:BSA ratio for a given reaction stoichiometry (Table S2). BSA-peptide conjugates were complexed in aqueous solution to 20 nm diameter citrate-stabilized gold particles (Ted Pella Inc). Typically, nanoparticles were coated with a combination of BSA-peptide conjugates by slow addition of 200 µL of BSA-peptide conjugate (1 mg/mL) to 500 µL of 20 nm diameter gold sol at pH 11. Dynamic light scattering (Malvern Zetasizer 1000HS) revealed that BSA-peptide conjugates form only thin skins around the gold particle (Table S1).

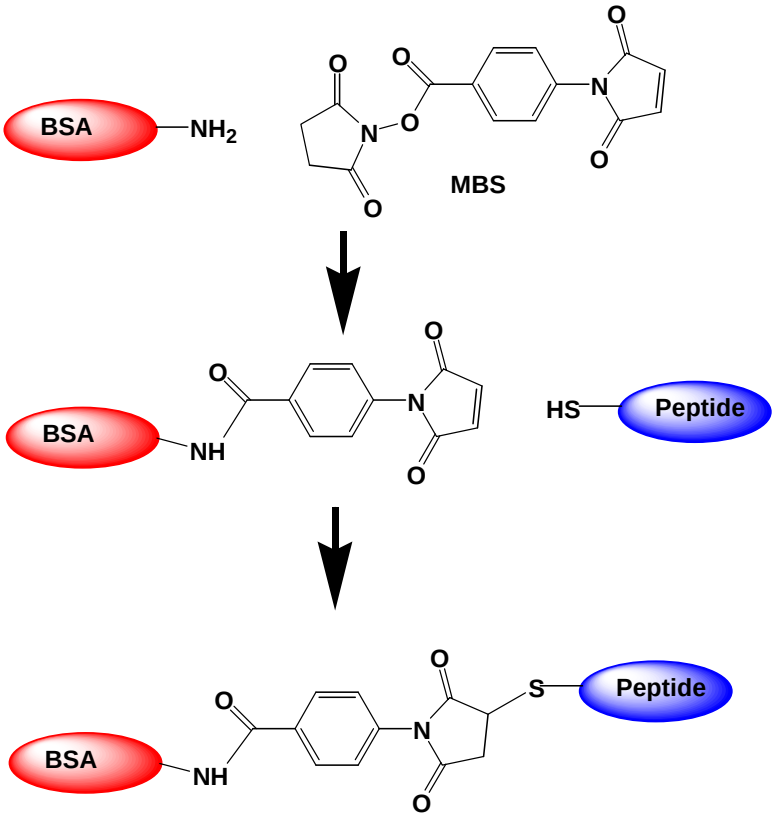

Figure S1. Synthesis of a BSA-peptide bioconjugate.

Table S1. Dynamic light scattering analysis of gold-conjugate complexes. Analysis was performed in filtered aqueous solutions by Malvern Zetasizer 1000HS using NNLS algorithm.

Sample	Mean Hydrodynamic Diameter (D_H), nm	R^2 of fit
20nm gold only	24.5	0.99926
20nm gold + BSA	28	0.99922
20nm gold + BSA + peptide	29.5	0.99812

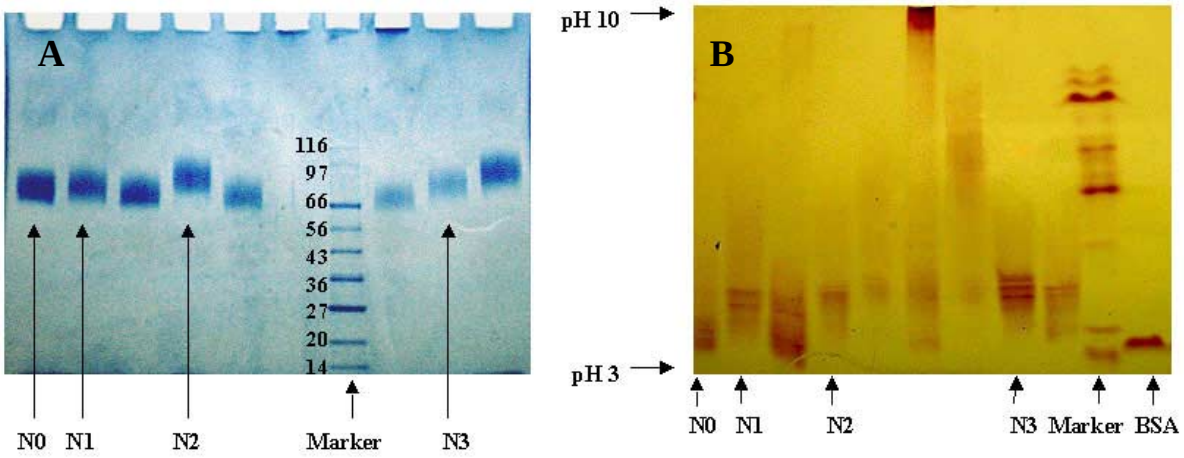

Figure S2. Gel shift assay of BSA/peptide conjugates. (A) 7.5% SDS PAGE 66K marker band corresponds to unreacted bovine serum albumin (BSA). Molecular weights of BSA-peptide conjugates were calculated according to the band shift relative to the marker. The molecular weights were used to determine the number of peptides per BSA. (B) pH 3-10 isoelectrofocusing electrophoresis (IFE). IFE provides qualitative confirmation of BSA-peptide binding, and it distinguishes the number of BSA-peptide species present by resolving single wide bands obtained by SDS-PAGE. For example the lane marked N3 shows three distinct bands shifted from the native-BSA sample, whereas a single wide band was observed in SDS-PAGE (Figure S2A). The three bands indicate three different ratios of peptide N3-BSA conjugates. Electrophoretic analysis was performed using BioRad Ready Gel system. Lanes marked N0, N1, N2, and N3 are peptides #1-#4 described in this manuscript. Unmarked lanes are for peptides to be included in a manuscript in preparation.

Table S2. Sample characterization data of the number of peptides per BSA using fluorescein-labeled peptides.

Molar ratio of peptide #2 added per BSA	Peptide:BSA Ratio
2	1.5
5	2.7
10	7.3

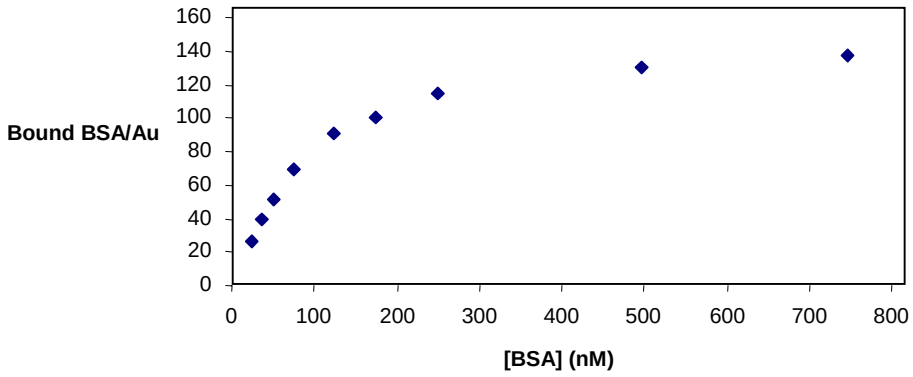

Figure S3. Number of native BSAs bound per particle *vs*. concentration of BSA added to solution.

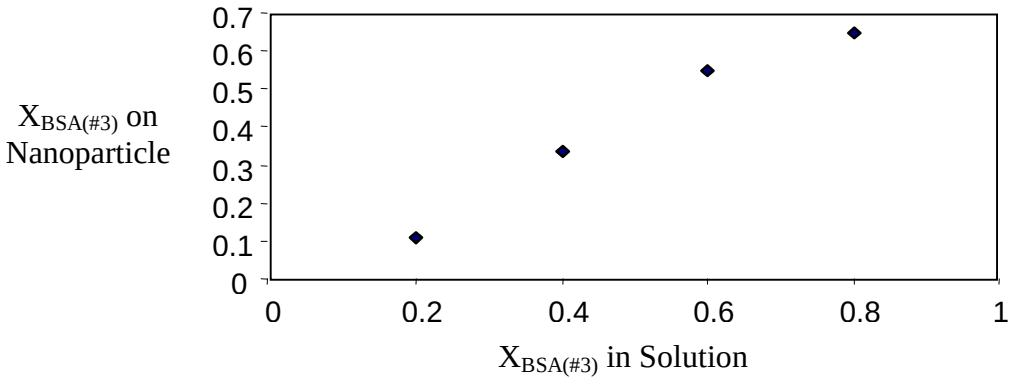

Figure S4. Mol fraction X of BSA-peptide #3 bound to gold nanoparticles vs. mol fraction added to solution. (Mol fraction is relative to BSA-peptide #2).

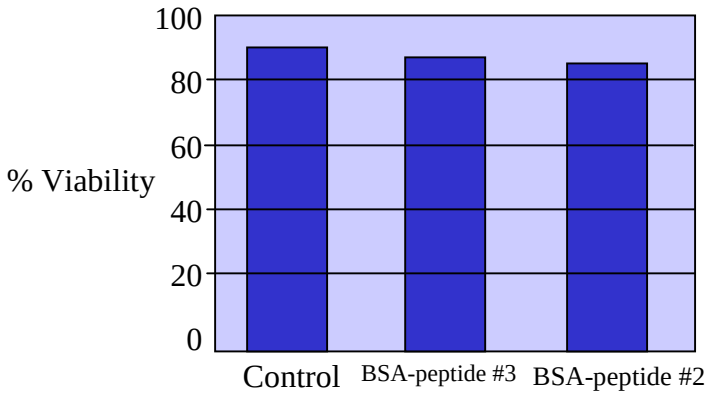

Figure S5. Results of the LDH colorimetric cell viability assay.

Cell cultures and Cellular Delivery

HepG2 cell line was obtained from the American Type Culture Collection (Rockville, MD) and maintained in EMEM medium (BioWhittaker) without addition of antibiotics at 37°C in controlled 5 % CO_2 atmosphere. For investigating the cellular localization of peptides, cells were plated on glass coverslips and grown to 85 % confluency in multiwell plates and then incubated with nanoparticle delivery vectors for various times. At indicated time points the coverslips were rinsed extensively with phosphate buffered saline and cells were fixed with 5% paraformaldehyde in PBS for 20 min at room temperature and then rehydrated in PBS. Once all cells were fixed the cover slip with the cells were mounted onto glass slides with FluorSave mounting media (Calbiochem, CA) and then examined using a Leica DMLB DIC equipped microscope with 100X/1.3 NA objective. Images were taken and processed with Nikon DMX-1200 digital color CCD camera.

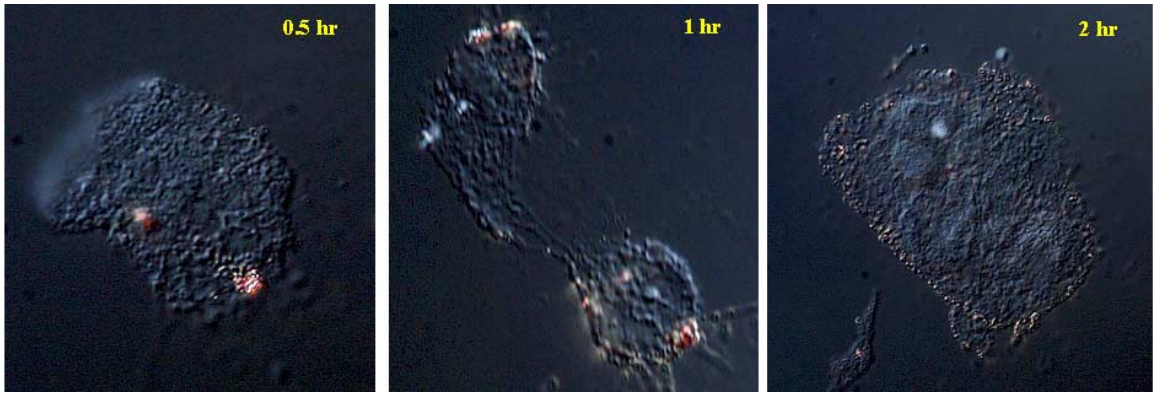

Figure S5. Incubation of Nanoparticle–peptide complex #1 with HepG2 cells (0.5-2hours) observed by video enhanced color differential interference contrast microscopy.

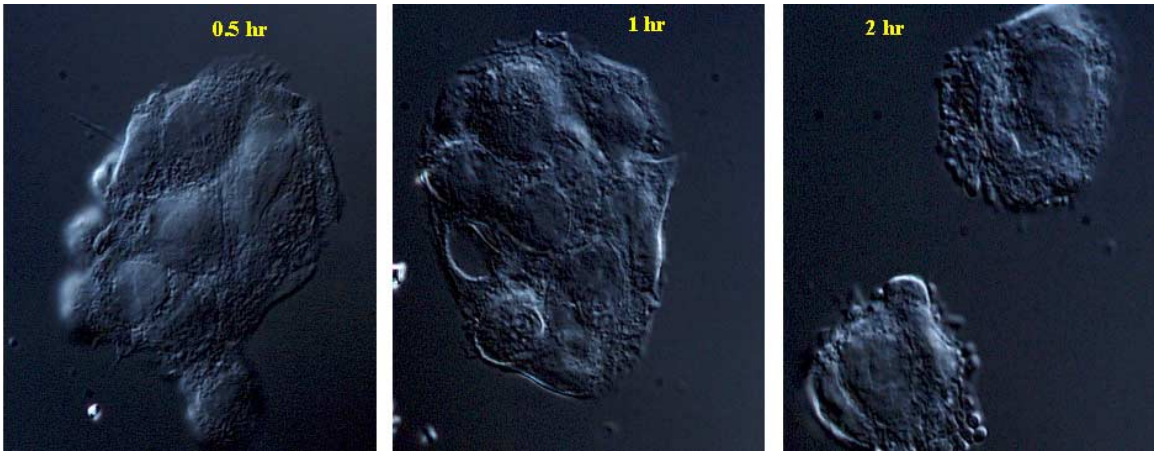

Figure S6. Incubation of Nanoparticle–peptide complex #2 with HepG2 cells (0.5-2hours) observed by video enhanced color differential interference contrast microscopy.

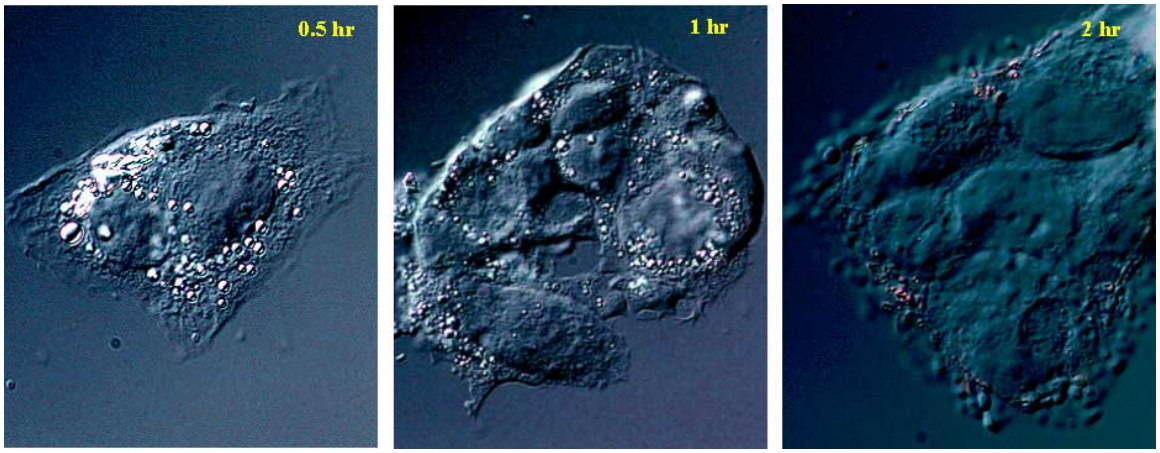

Figure S7. Incubation of Nanoparticle–peptide complex #3 with HepG2 cells (0.5-2hours) observed by video enhanced color differential interference contrast microscopy.

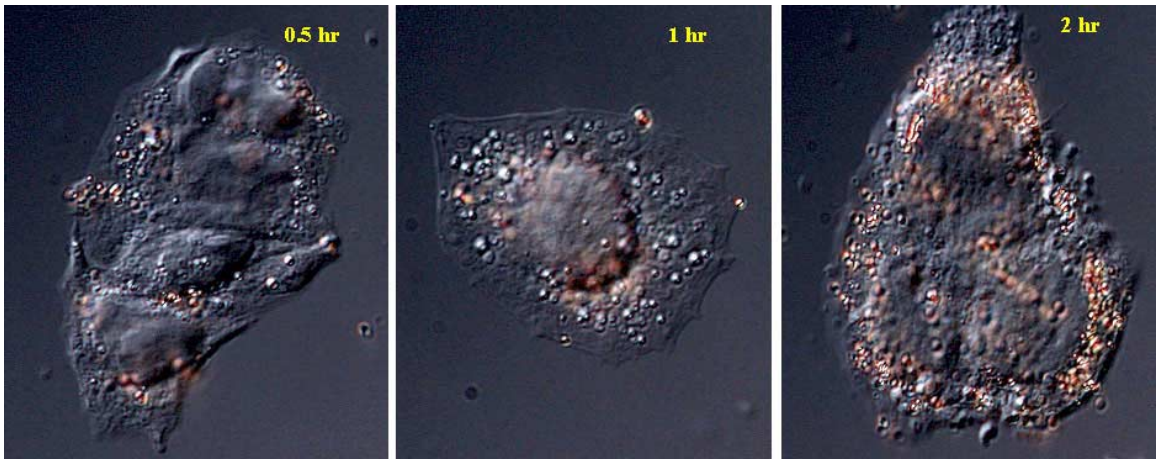

Figure S8. Incubation of Nanoparticle–peptide complex #4 with HepG2 cells (0.5-2hours) observed by video enhanced color differential interference contrast microscopy.

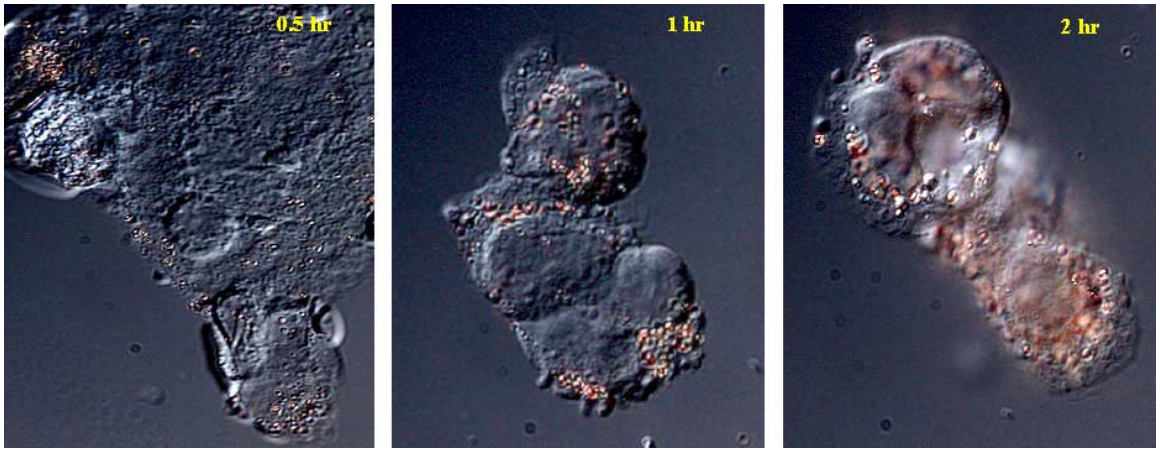

Figure S9. Incubation of nanoparticle–peptide complex #3/#4 with HepG2 cells (0.5-2hours) observed by video enhanced color differential interference contrast microscopy.