Supporting information. Patra and Bhattacharya et al. "Targeted delivery of gemcitabine to pancreatic using cetuximab as a targeting agent".

Preparation of cell lysates for Westernblot analysis

AsPC-1, PANC-1 and MIA Paca2 cells were washed twice with 10 ml of cold PBS, lysed with ice-cold lysis buffer [50 mM Tris (pH 7.5), 1% NP40, 150 mM NaCl, 1 mM Na_3VO_4, 2 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml leupeptin, 0.5% aprotinin, and 2 mM pepstatin A], incubated on ice for 10 min, and centrifuged at 4°C for 10 min.

Synthesis of gold nanoparticles

Gold nanoparticles (AuNPs) were synthesized from tetrachloroauric acid by wet chemical methods using sodium borohydride as a reducing agent as previously described. Briefly, a stock solution of tetrachloroauric acid (HAuCl4) was prepared by dissolving 1.0 g of HAuCl4 in 250 ml of deionized double distilled water (NANOpure). A 1:100 dilution of the stock in distilled H_2O was mixed with an aqueous solution of sodium borohydride ($NaBH_4$) under vigorous stirring. Stirring continued for 12 h to obtain the GNPs used in this study. GNPs thus formed was characterized using UV-Vis and TEM.

X-ray photo electron spectroscopy

XPS was performed on a PHI 5400 instrument using a Mg Kα X-ray (1253.6 eV) anode source operated at 250 W under a pressure below 2 $x10^{-9}$ torr as described previously. The electron pass energy on the hemispherical analyzer was set at 89.45 eV for survey scans and 17.9 eV for high-resolution scans. The binding energy scale was referenced to that of C1s (285.0 eV). Samples were prepared by drop-coating the gold nanoconjugates solution on a clean silicon wafer and the drops were allowed to air dry before the measurement.

Western Blot analysis

The expression of EGFR protein was determined in untreated AsPC-1, PANC-1 and MIA Paca2 cells. Cells were grown in a 100 mm tissue culture dish in RPMI medium containing serum, L-glutamine and antibiotics. Confluent cells were harvested and cell lysates were collected using NP40 lysis

buffer. Protein concentration in the cell lysates was measured using Bradford assay kit (Bio-rad). The proteins were loaded on a 10% SDS-PAGE gel for separation and transferred to a membrane. The membrane was incubated with EGFR antibody (Santa cruz, sc-03). Blot was washed with thrice with TBS-Tween 20 (0.1%) and incubated with secondary antibody conjugated with peroxidase.

Non-invasive optical imaging

In vivo optical imaging (biolumiscence) for -luc was done approximately 20 minutes after intraperitoneal injection of 3 mg n-Luciferin into each animal using a Xenogen-IVIS cooled CCD optical system (Xenogen-IVIS, Alameda, CA). Photons emitted from the implanted luc-expressing cells were collected and integrated for a period of 15 seconds. Images were obtained by superimposing gray-scale photographs and -luc color images using the overlay option of the Living Image software (Xenogen).

Transmission electron microscopy of cells treated with nanoconjugates

TEM sample preparation involving cells were performed as previously described. After the incubation with nanoconjugates, cells were trypsinized and centrifuged initially at 1500 rpm for 5 min. The resultant cell pellets were then washed thrice with PBS, and fixed in Trump's fixative containing 1% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer at pH 7.2. and processed as described in the literature. Thin (90 nm) sections were cut on a Reichert Ultra cut E ultramicrotome, placed on 200 mesh copper grids, and stained with lead citrate. Micrographs were taken on a TECNAI 12 operating at 120KV.

Inductively coupled plasma (ICP) analysis

ICP were performed as previously reported. In brief, separate aqueous acidic calibrating standards were diluted with cell suspensions/plasma containing gold or aqueous acidic diluent containing internal standards. Blanks were diluted with aqueous acidic diluent containing internal standards but no cells/plasma. Quality control specimens and experimental specimens were diluted in an identical manner. All diluted blanks, calibrating standards, quality control samples, and experimental samples

were aspirated through a pneumatic nebulizer into hot, anular argon plasma. Instrument response was defined by a linear relationship of gold concentration versus ion count ratio (Gold mass 197/Internal Standard Platinum mass 195). Analyte concentrations were derived by reading the ion count ratio for each mass of interest and determining its corresponding concentration from the calibration curve.

Supporting Figure 1. TEM picture of AsPC-1 cells treated with goldnanoconjugates; 1a) treated with Au-C225 (inset showing higher magnification image of a portion of the cell). 1b) treated with Au-IgG showing minimal gold uptake.