Supporting Information

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SI Text

SI Methods ***Virtual ligand screening.*** Docking was performed with GLIDE 3.5 (1, 2) in standard precision mode (SP). The E-model scoring function was used to select the top compounds. A grid box of default size (18 x 18 x 18 $Å^3$) was centered on three core amino acids in the SIM peptide (D3, V4 and I5) (3, 4). Default parameters were used and the following constraints were included during grid generation. Intermolecular hydrogen bond constraints included two of the three sites on SUMO: K32 and K34. In addition, hydrophobic interactions with F31 and I33 of SUMO-3 were also included in the constraints. The Maestro user interface (Schrodinger, LLC) was used to prepare the GLIDE docking calculations and visualize the results. To eliminate molecules that adopt excessively strained conformations in their docked pose, all top compounds with internal energy greater than 1.5 kcal/mol/rotatable bond were eliminated.

NMR studies. All NMR samples contained 10 mM phosphate buffer (pH 7.0) in 90% H_2O/10%D_2O. NMR spectra were acquired at 25°C on a Bruker 500 MHz NMR spectrometer. All chemicals were dissolved in DMSO. HSQC spectra were acquired at SUMO:compound ratios of 1:3 or 1:10 for the top scored compounds from virtual ligand screening. Both SUMO-1 and SUMO-2 were examined for their interactions with the compounds. The HSQC spectra were acquired for the protein alone, the protein with addition of 2% DMSO, and the protein in complex with a compound and 2% DMSO. The dissociation constant K_d was estimated by titrating the compound to SUMO-2, followed by recording HSQC spectra at protein:compound ratios of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7 and 1:20.

Synthesis of derivatives 2 and 3. All amino acids, *O*-(6-Chlorobenzotriazol-1-yl)-*N,N,N',N'*-tetramentyluronium hexafluorophosphate (HCTU), and 2-chloro chlorotrityl resin were obtained from EMD Biosciences (Novabiochem). All other synthesis reagents were obtained from commercial sources. ^{1}H NMR spectra were obtained on a Varian 400 MHz spectrometer and TMS was used as an internal reference. Derivative **2** (Fig. 2) was constructed on 2-chlorotrityl resin loaded with Fmoc-Leu-OH at 0.8 mmol/g. Fmoc-Ile-OH, Fmoc-cinnaminic acid (5), and 3-(*t*-butoxycarbonylthio)propanioc acid (6) were sequentially coupled on using standard Fmoc synthesis conditions using HCTU (3 eq) and diisopropylethylamine (8 eq). The compound was cleaved from resin and deprotected with 95% trifluoroacetic acid/2.5% H_2O/2.5% triisopropylsilane. The cleaved derivative **2** was purified via reverse phase HPLC (Agilent C-18 prep column 21.5x150mm) using 20-60% acetonitrile in a water gradient and lypholized. The negative control, **3**, was constructed in a similar manner as **2** with 2-chlorotrityl resin loaded with Fmoc-Gly-OH, Fmoc -7-aminoheptanoic acid and 3-(t-butoxycarbonylthio)propanioc acid.

Loading derivative 2 and 3 onto AuNP. The AuNP-ligand conjugate was synthesized according to a procedure described by You et al. (36): C4-AuNP (7 mg) was combined with of derivative **2** or **3** (14 mg) in 1 mL of dichloromethane and equilibrated for 4 d. The precipitated solid was collected via filtration (fine fritted funnel), rinsed with chloroform and dried in vacuo. The nanoparticle suspension was air-dried on the specimen grid and observed using a TECNAI 12 transmission electron microscope (Fig. S2*A*). To conjugate a small amount of biotin onto AuNP **4** or **5**, C4-AuNP (6 mg) was combined with derivative **2** (10 mg) or **3** (8 mg) and thiol-biotin (2 mg; Nanoscience Instruments, CMT015), equilibrated for 4 d, and purified as described above. Although NMR spectra demonstrated that derivative **2** or **3** was conjugated to AuNP, biotin signal was not observable by NMR, indicating that the ratio of derivative:biotin on AuNP was more than 9:1. Conjugated biotin was detected by FITC-conjugated streptavidin.

Poly-SUMO chain formation. Poly-SUMO chains were synthesized by an in vitro SUMOylation reaction (4.375 μM GST-SUMO-3 and His-SUMO-3, 40 nM Ubc9 and E1, and 5 mM ATP in a 100 μL reaction). The reaction was carried out at 37°C for 4 h and then diluted to 1 mL with phosphate buffered saline (PBS). Poly-SUMO-3 chains were first separated from the His-SUMO-3 monomer by glutathione affinity chromatography. The eluted poly-SUMO chains were subsequently separated from the GST-SUMO-3 monomer by Ni-NTA chromatography. The eluted poly-SUMO chains were dialyzed and concentrated.

Purification of GST-WT-SIM and GST-SC-SIM. GST-tagged wild-type (GST-WT-SIM) or scrambled SIM sequence (GST-SC-SIM) was subcloned into the pGex4T-S expression vector (Amersham) and overexpressed as an N-terminal GST fusion protein in *Escherichia coli* strain BL21 (DE3) (Invitrogen) at 37°C in LB media. When the OD A_{595} nm reached 0.6, isopropyl 1-thio–D-galactopyranoside (IPTG; 1 mM) was added to the media and cells were grown for an additional 4 h at 37°C. Harvested cells were resuspended in lysis buffer (PBS, pH 7.4, 150 mM sodium chloride, 5 mM β-mercaptoethanol [β-ME], 1x BugBuster and Benzonase Nuclease [Novagen, 1000X]), and the pellet was isolated by centrifugation (15,000 x g for 30 min). Proteins were dissolved in 8M urea and renatured in a buffer containing PBS, pH 7.4, 150 mM sodium chloride, 10 mM 2-mercaptoethanol and 5% glycerol. The GST-tagged proteins were then conjugated to glutathione agarose resin and eluted in a buffer containing 10 mM glutathione.

SIM pull-down. Individual wells in 96-well EIA/RIA plates (Costar) were pre-coated with GST-WT-SIM or GST-SC-SIM (5 μg) at 4°C for overnight. After pre-coating, the plate was blocked with 5% BSA at 37°C for 2 h. SUMO-1, SUMO-3 or polymer SUMO-3 (5 μg) was mixed with 0.5 % BSA and free ligand, AuNP or ligand-conjugated AuNP at various concentrations, as indicated (Fig. 3), before addition to the EIA plate. After the SUMO-chemical compound or SUMO-gold particle mixture was added to the wells, the plate was incubated at 37 °C for 3 h. Unbound protein was removed by washing the wells three times with 1x PBS containing 1% BSA. Anti-SUMO-1 rabbit polyclonal antibody or anti-SUMO-2 and 3 rabbit polyclonal antibody was then added to the wells and the plate was incubated at 4°C overnight. The wells were then washed three times with 1x PBS containing 1% BSA, after which, horseradish peroxidase (HRP) conjugated goat anti-rabbit antibody (1:2,000) was added to the wells and the plate was incubated at 37°C for 1 h. After three washes with 1x PBS containing 1% BSA, 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) (1:1) were added to the wells, generating a color reaction. The plate was read at 450 nm by an ELISA reader after the reaction was stopped by addition of phosphoric acid. Quantifications of all SIM pull-downs were scaled by measurement of GST-WT-SIM pull-down (100%) and GST-SC-SIM pull-down (0%).

Antibodies. All antibodies were obtained from commercial sources, including mouse anti-SUMO-1 (Abgent), rabbit anti-SUMO2 and 3 (Abcam), mouse anti-Flag M2 (Sigma), rabbit anti-PML (Santa Cruz Biotech), and mouse anti-β-actin (Sigma) antibodies.

Cell culture. The HeLa-SUMO2 cell line that stably expresses His-tagged SUMO2 was a kind gift from Dr. Ronald Hay (University of Dundee, UK). MCF-7, HeLa and HeLa-SUMO2 cells were maintained in DMEM (GIBCO) supplemented with 10% (v/v) fetal bovine serum (FBS) (Irvine Scientific), 1 mM sodium pyruvate, nonessential amino acids, and 100 μg/mL of penicillin/streptomycin (Irvine Scientific) and Normocin (InvivoGen). MCF-10A cells were maintained in DMEM with 10% (v/v) FBS, penicillin/streptomycin (100 μg/mL), nonessential amino acids, EGF (20 ng/mL), hydrocortisone (0.5 μg/mL), insulin (10 μg/mL) and Cholera toxin (100 ng/mL). PC3 cells were maintained in RPMI with 10% (v/v) FBS and penicillin/streptomycin (100 μg/mL). RWPE1 cells were maintained in Keratinocyte-SFM media from Invitrogen. All cells were incubated at 37°C in a humidified chamber supplemented with 5% CO_2.

Cell proliferation assay. MCF-7, MCF-10A, PC3, or RWPE1 cells (1×10^3) were plated in a single well of a 96-well plate one day before treatment with AuNPs to allow cells to reach 50% confluency. One hour before radiation or doxorubicin treatment, C4-AuNP or AuNP 4 was added to the cells at 0, 2, or 4 M. For the radiation sensitization assay, cells were irradiated at 4 Gy and then returned to incubator for another 48 h. For the doxorubicin sensitization assay, cells were exposed to 2 μM doxorubicin for 48 h. The CellTiter 96 Aqueous One Solution Cell Viability Assay kit (Promega) was used to analyze cell viability.

Clonogenic assay. One day before AuNP treatment, 2×10^2 or 2×10^3 MCF-7 cells were seeded for non-irradiation or irradiation, respectively in a single well of a six-well plate. C4-AuNP or AuNP **4** (0, 0.1, 1, or 2 μM) was added to the cells. The cells were either irradiated at 4 Gy or not after treatment with AuNP for 1 h and then returned to the incubator and incubated for 12 d until the colonies formed. Colonies were fixed and visualized by a mixture of 6.0% glutaraldehyde and 0.5% crystal violet (7).

Arsenic treatment and immunofluorescence assay. For arsenic treatment and immunofluorescence experiments, HeLa-SUMO2 cells were seeded on coverslips one day before treatments with AuNPs and arsenic C4-AuNP, AuNP **4** or **5** was added to cells 1 h before exposure to 1 μM As_2O_3 for 1, 6, and 24 h. Cells were then fixed with 4% formaldehyde for 10 min at room temperature. Subsequently, cells were permeabilized with aceton and methanol mix (1:1) for 10 min and blocked in PBS containing 5% BSA and 0.1% Tween for 30 min. The mouse anti-PML antibody was diluted in PBS with 1% BSA (1:200; Abcam) and then added to the cells for 1 h. After three washes with PBS containing 1% BSA, cells were incubated with Alexa Fluor 555 donkey anti-mouse IgG (Invitrogen) and 0.1 μg/mL DAPI for another hour. After three washes with PBS containing 1% BSA, cells were mounted in Vectashield mounting medium. Images were collected using a fluorescence microscope (Axio; Zeiss). Protein samples from cells treated as described above were denatured by Laemmli sample buffer and then separated by SDS-PAGE. The proteins were then transferred to PVDF membrane and detected by Western blot.

Flag-RNF4 protein expression and coimmunoprecipitation. pCMV-Flag-RNF4 plasmid DNA, gift from Dr. Peter Schultz (The Scripps Research Institute), was transfected into HeLa-SUMO2 cells using Lipofectamine LTX (Invitrogen) according to the manufacturer's instruction. Twenty-four hours after DNA transfection, the Flag-RNF4 expressing cells were first treated with 4 μM of C4-AuNP, AuNP **4** or **5** for 24 h and then exposed to 1 μM arsenic trioxide for 1 h. Whole-cell extracts were obtained in cell lysis buffer (300 mM NaCl, 50 mM Tris (pH 7.5), 0.5% NP-40, 1 mM EDTA, protease inhibitor cocktail (Roche), 100 mM iodoacetamide and 1 mM PMSF). For co-immunoprecipitation (co-IP), the whole-cell extract was first pre-cleared by incubation with protein G Dynabeads (Invitrogen) for 2 h at 4 °C, and then the pre-cleared lysate (containing 2.5 mg of total cellular protein) was incubated with a mouse anti-Flag antibody (5 μg) overnight at 4 °C. After brief centrifugation, the anti-Flag antibody and its associated proteins were isolated by using protein G Dynabeads. The precipitates were then washed six times with co-IP buffer (50 mM Tris-HCl pH7.5, 150 mM NaCl, 0.05% Triton-X100, 10 mM iodoacetamide) and analyzed by Western blot.

Biotin-AuNP pull-down. HeLa cells were collected by centrifugation (500 *g*, 4 °C, 15 min) and washed four times in ice-cold PBS containing 200 mM iodoacetamide. Next, cells were incubated on ice in cell extraction buffer (10 mM Hepes, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl_2, 0.07% NP40, complete protease inhibitor cocktail tablets (Roche) and 100 mM iodoacetamide) for 15 min. Cells were disrupted by sonication on ice (6 × 20 s with an 18% amplitude by a Virtis Sonicator). Cellular debris was removed by centrifugation at 17,000 × *g* and the supernatant was pre-cleared with streptavidin agarose beads before incubation with biotinylated AuNPs. Biotin-AuNP 4 or biotin-AuNP 5 (5 μg) was incubated with 10 mg of pre-cleared HeLa whole-cell extract at 4 °C overnight. After a brief centrifugation, streptavidin agarose beads (20 μg) were added to the solution, which was then incubated at 4 °C for 2 h. After incubation, the agarose was centrifuged (4,500 × *g*, 5 min) to remove the unbound proteins. The agarose was then washed three times in cell extraction buffer and the isolated proteins were eluted with Laemmli sample buffer. The protein samples were separated by SDS-PAGE and analyzed by Western blot according to standard procedures.

Subcellular location of AuNPs. HeLa cells were seeded on an 8-well chambered coverglass (LabTekII) one day before AuNP treatment. Biotin-AuNP **4** and -AuNP **5** (4 μM) were incubated with HeLa cells for 24 h at 37 °C in a humidified chamber supplemented with 5% CO_2. Cells were then washed twice with PBS and fixed with 4% formaldehyde for 10 min at room temperature. Subsequently, cells were permeabilized with 0.1% Triton X-100 for 20 min and blocked in PBS containing 5% BSA and 0.1% Tween for 30 min. Streptavidin-FITC was diluted in PBS with 1% BSA (1:1,000, EMD Millipore) and incubated with the cells for 1 h at room temperature. After three washes with PBS containing 1% BSA, cells were incubated with 0.1 g/mL DAPI for 20 min. After three washes with PBS containing 1% BSA, cells were then mounted in Vectashield mounting medium and images were collected using a fluorescence microscope (AX10, Zeiss).

Comet assay. MCF-7 cells were grown on 24-well plates to reach 60% confluency. Each well was then treated with either 0.2% DMSO, 2 M C4-AuNP, or 2 M AuNP **4** and incubated at 37 °C for 24 h before irradiation. Cells received either no irradiation or 4 Gy irradiation (Cs137 irradiator) followed by incubation at 37 °C for 30 min or 2 h. Cells were gently removed using sterile rubber scrapers. Suspended cells were centrifuged at 700 × *g* for 5 min, aspirated, and resuspended in ice-cold autoclaved PBS. Cells were then prepared using the OxiSelect™ Comet Assay Kit (Cell Biolabs, Inc.) following the manufacturer's protocol. The cell suspension was re-centrifuged and resuspended again in ice-cold autoclaved PBS at 1×10^5 cells/mL. Cell samples were combined with molten OxiSelect™ Comet Agarose at a 1:10 ratio

(v/v), and 75 μL added per well. An overnight lysis in chilled Lysis Buffer at 4 °C was done instead of the 1 h lysis suggested in the manufacturer's protocol. Slides were transferred to chilled Alkaline Solution for 30 min at 4 °C, and run on a horizontal electrophoresis apparatus (1V/cm) for 30 min. The slides were then washed three times with deionized water and rinsed with ice-cold 70% ethanol. Slides were allowed to completely dry, and 100 L Vista Green DNA Dye was added to each well. Twenty comet images from each well were imaged by fluorescence microscopy (Olympus Inverted IX81). The tail moment (Tail DNA% x Length of Tail) was quantified for each cell using Comet Assay IV (Perceptive Instruments).

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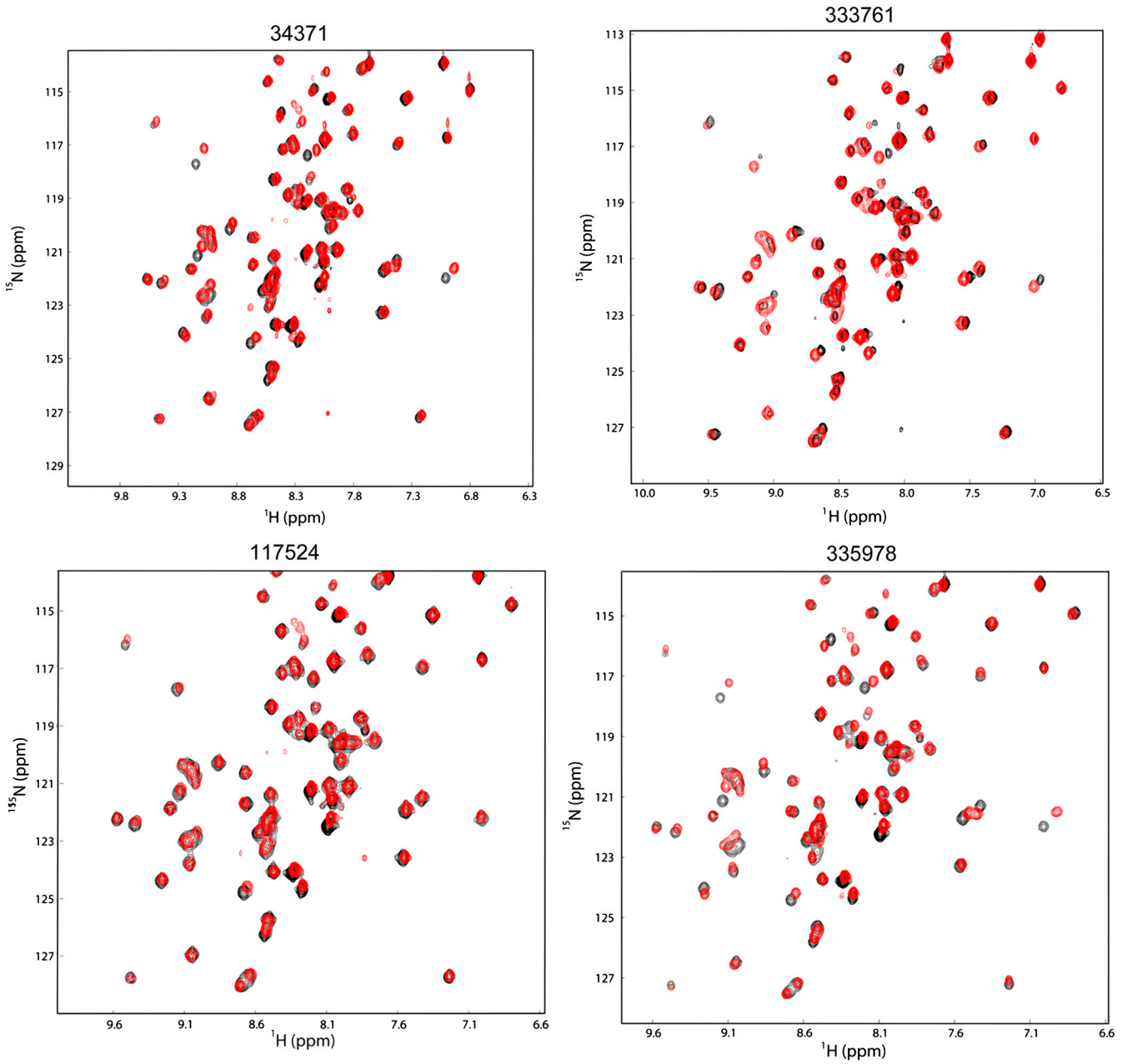

Fig. S1. Overlay of the HSQC spectra of SUMO-3, free (black) and in complexes with each of the compounds (red) shown in Fig. 1*A* at ligand:protein ratios of approximately 3:1.

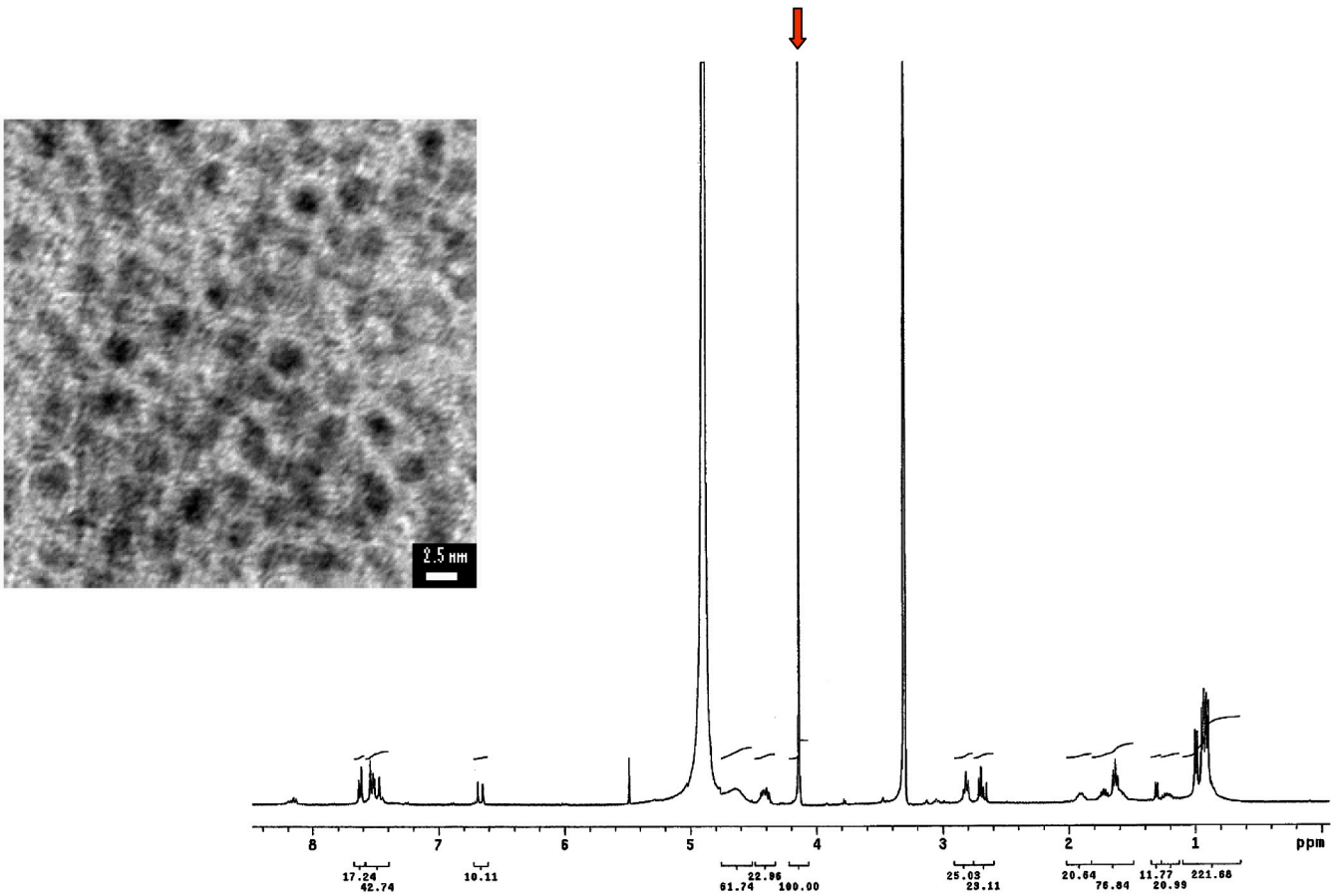

Fig. S2. Transmission electron microscopy image of the AuNP (*Left*) and the NMR spectrum of ligand conjugated AuNP with ferrocene as an internal standard (*Right*). The ferrocene signal is indicated by a red arrow.

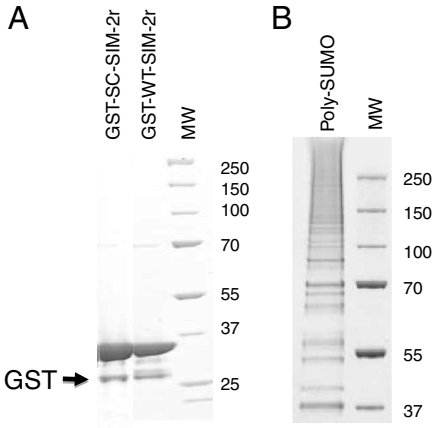

Fig. S3. (*A*) Purification of GST-WT-SIM and GST-SC-SIM. A small amount of GST was copurified with the GST-fusion proteins. (*B*) Poly-SUMO-3 chains were purified by glutathione affinity chromatography and then by Ni-NTA affinity chromatography. Molecular weight markers (MW) are shown to the right of each gel.

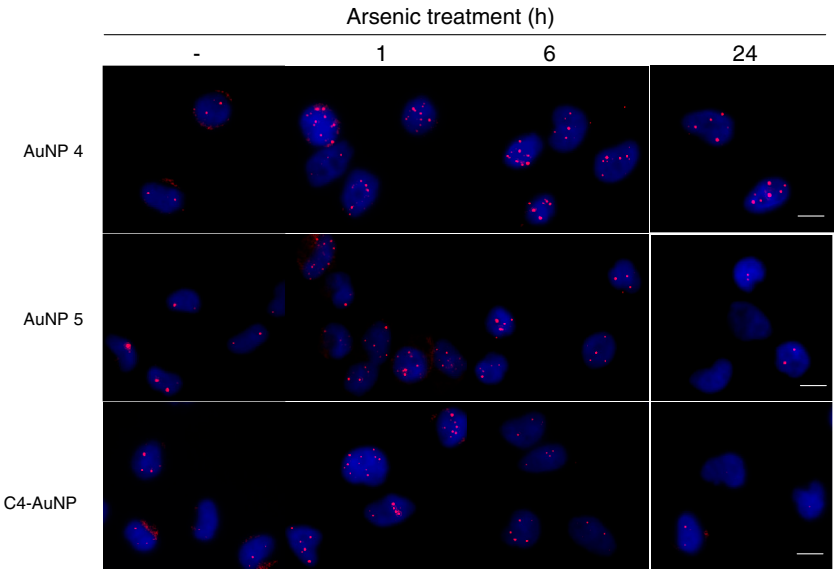

Fig. S4. Effect of AuNP 4, 5 and C4-AuNP on arsenic trioxide induced PML degradation. PML bodies were monitored in HeLa-SUMO2 cells treated with AuNPs (4 μM) for 1 h before exposure to 1 μM arsenic trioxide for 1, 6, or 24 h. Cells were then fixed and immunostained with an anti-PML antibody (red) and stained with DAPI (blue). Bar, 10 μm.

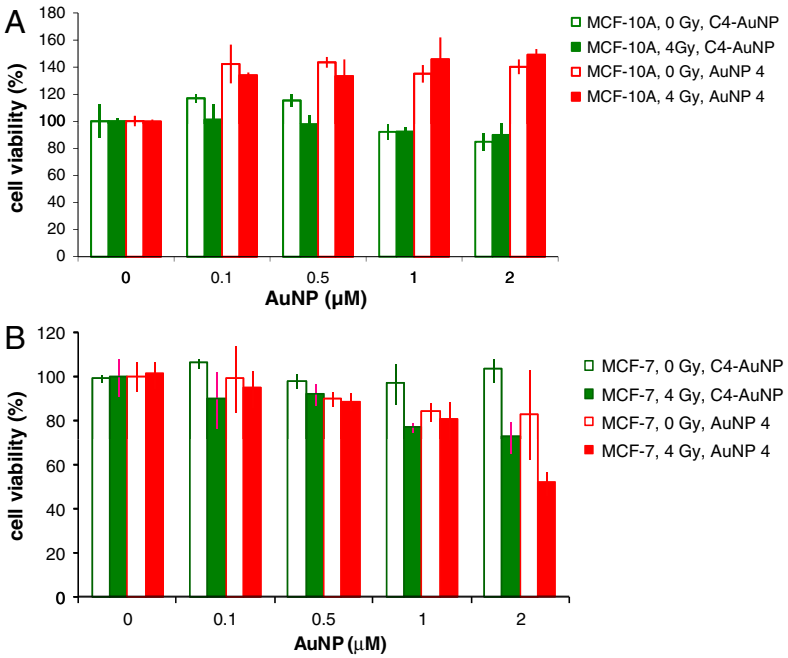

Fig. S5. Effects of AuNP **4** on cell toxicity and sensitivity to radiation. MCF-10A (*A*) or MCF-7 (*B*) cells were plated in 96-well plates one day before nanoparticle treatment and grown to 50% confluency. C4-AuNP or AuNP **4** were added to the cells at the indicated concentrations. One hour after the AuNP addition, cells were irradiated (4 Gy) or not and returned to the incubator for an additional 48 h. Shown is cell viability as analyzed by MTS assay.

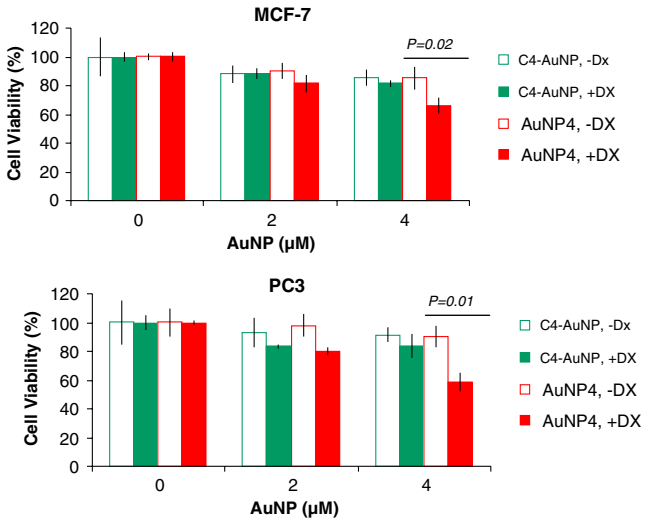

Fig. S6. The effects of C4-AuNP and AuNP 4 on sensitizing cellular response to doxorubicin (Dx). The breast cancer cell line MCF-7 and prostate cancer cell line PC3 were treated with AuNP 4 or C4-AuNP for 1 h and then exposed to 2 μM doxorubicin for another 48 h. Cell viability was measured by MTS assay.

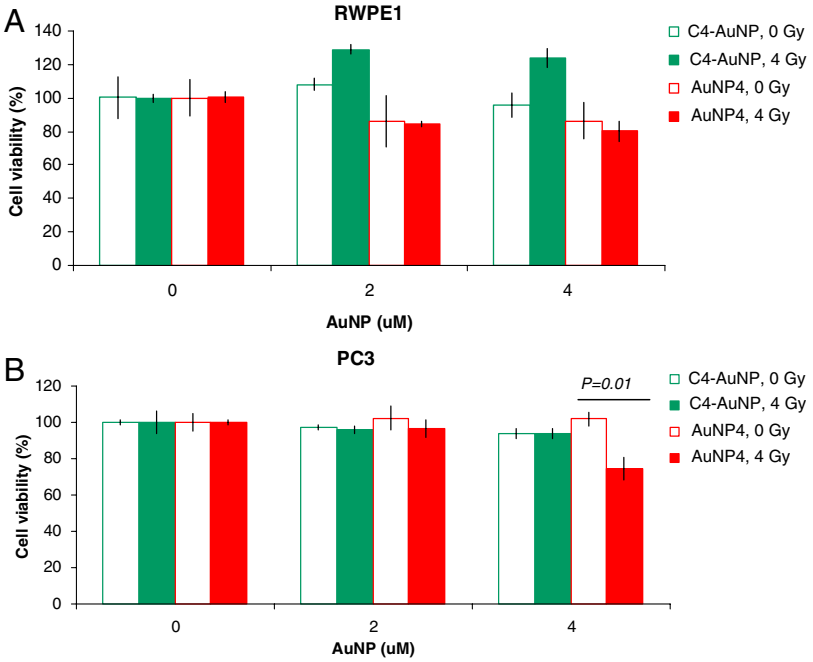

Fig. S7. Effects of AuNP 4 on cellular toxicity and sensitivity to radiation. Untransformed RWPE-1 prostate epithelial cells (*A*) or PC3 prostate cancer cells (*B*) were plated in 96-well plates one day before AuNP treatment and grown to 50% confluency. C4-AuNP or AuNP 4 was added to the wells at the indicated concentrations. After incubation (1 h) with AuNPs, cells were irradiated at 4 Gy or not irradiated and cultured for an additional 48 h. Cell viability was analyzed by MTS assay.

Table S1. Binding interactions derived from docking calculations

Compound	HBond	vdW (kcal/mol)	Coul (kcal/mol)	Emodel (kcal/mol)	CvdW (kcal/mol)
333751	4	-25.3	-13.2	-75.4	-38.6
343731	3	-24.0	-12.7	-75.5	-36.7
333761	4	-24.1	-15.5	-79.8	-38.2
117524	5	-21.6	-16.6	-76.9	-38.2
335978	3	-27.0	-10.5	-74.2	-37.5

Hbond: number of hydrogen bonds; vdW: van der Waals interaction; Coul: Coulumb interaction; CvdW: vdW + Coul