SUPPORTING INFORMATION

Tables

Table S1: Physicochemical characteristics of Cu labeled, cyclodextrin-based, polymeric nanoparticles containing camptothecin. CDP-CPT was loaded with Cu the same way as CDP-CPT_3-$DOTA_1$. Only the polymer containing DOTA showed copper binding. CDP-CPT had an almost neutral surface charge while CDP-CPT_3-$DOTA_1$ was slightly more negatively charged. This is due to the presence of one excess carboxylic acid group on DOTA (Figure S1).

Parameter	**CDP polymer**	**CDP-CPT**	**Cu labeled CDP-CPT_3-$DOTA_1$**
MW, PDI	67 kDa, 2.1	67 kDa, 2.1	67 kDa, 2.1
Camptothecin	n/a	9.2% w/w	4.6% w/w
Free Camptothecin	n/a	0.01%	0.1%
Cu loading	n/a	None detected	0.41% w/w
Particle size, PDI	8 nm, 0.323	36 nm, 0.277	37 nm, 0.272
Zeta potential	n/a	- 1.81 +/- 0.79 mV	- 9.43 +/- 1.05 mV

Table S2: Membrane retention of cyclodextrin-based, polymeric nanoparticles containing camptothecin using spin filters with increasing molecular weight cut-off membranes. The parent polymer had a molecular weight of 67 kDa, a PDI of 2.1, and a particle size of 8 nm by dynamic light scattering (Table S1). After conjugation of camptothecin to the same polymer (9.2 % CPT by weight), the particle size of the resulting nanoparticles was 36 nm.

	30 kDa MWCO	50 kDa MWCO	100 kDa MWCO	300 kDa MWCO
Parent polymer	64.3%	20.9%	5.54%	< 0.1%
Camptothecin polymer nanoparticles	99%	98%	96%	4%

Methods for Fractionation Studies. The CPT conjugate of CDP was synthesized as previously described (7). A solution of parent polymer in water or IT-101 in phosphate buffered saline at the concentration of 1 mg/mL was centrifuged by using different molecular weight cut off (MWCO) Amicon® Ultra-15 Centrifugal Filter Devices (30k, 50k and 100k MWCO) and Vivaspin 20 Centrifugal Filter Devices (300k MWCO). Amicon Ultra-15 tubes were centrifuged for 25 minutes at 3,000 x g and Vivaspin 20 were centrifuged for 2 minutes at 3,000 x g. For parent polymer, filtrate and retentate were lyophilized to get the weight of parent polymer in retentate and filtrate. For IT-101, the concentration in filtrate and retentate was determined by HPLC as previously described (8). Membrane retention for each condition was calculated as

$$\text{Membrane retention} = 1 - 1/F$$

where $F = (C_0 * W_0 * \text{Recovery} - C_{r,E} * W_{r,E}) / (C_{f,E} * W_0)$, W_r = total weight of retentate before assay, W_o = weight of original starting material, W_f = weight of filtrate, C_r = retentate concentration, C_o = original starting material concentration, and C_f = filtrate concentration.

Table S3: Model parameters. The system of equations was solved with WinNonlin (Pharsight, Mountain View, CA).

Name	Description (units)	Determination	Value
A	Plasma concentration of low molecular component at time 0 (%ID/mL)	Fit to data	4.26
B	Plasma concentration of nanoparticles at time 0 (%ID/mL)	Fit to data	19.9
α	Rate of elimination of low molecular component from plasma (s^{-1})	Fit to data	$1.09 \cdot 10^{-3}$
β	Rate of elimination of nanoparticles from plasma (s^{-1})	Fit to data	$1.45 \cdot 10^{-5}$
P_{lmw}	Tumor apparent permeability for low molecular component (cm s^{-1})	Fit to data	$5.56 \cdot 10^{-5}$
P_{NP}	Tumor apparent permeability for nanoparticles (cm s^{-1})	Fit to data	$3.11 \cdot 10^{-7}$
k_{IS}	Uptake of nanoparticles by tumor cells (s^{-1})	Fit to data	$3.08 \cdot 10^{-5}$
V_P	Plasma volume mouse (mL)	Estimated from (1)	1.50
V_{tv}	Tumor vascular volume (mL)	Estimated from (2), 0.3 mL tumor	0.045
V_I	Tumor interstitial volume (mL)	Estimated from (2, 3), 0.3 mL tumor	0.105
V_C	Tumor cellular volume (mL)	Estimated from (2, 3), 0.3 mL tumor	0.150
S_V	Surface area of tumor vasculature (cm^2)	Calculated for cylindrical vessels with 30µM diameter	$\frac{2 \cdot V_{tv}}{0.0015}$
$HCRT$	Hematocrit	Estimated from (1)	33%

Methods for Determining Tumor Vascular Permeability. Microvascular permeability of solutes is often described by the Kedem-Katchalsky equation, originally devised to describe the transport of a solvent and a solute across a membrane (33).

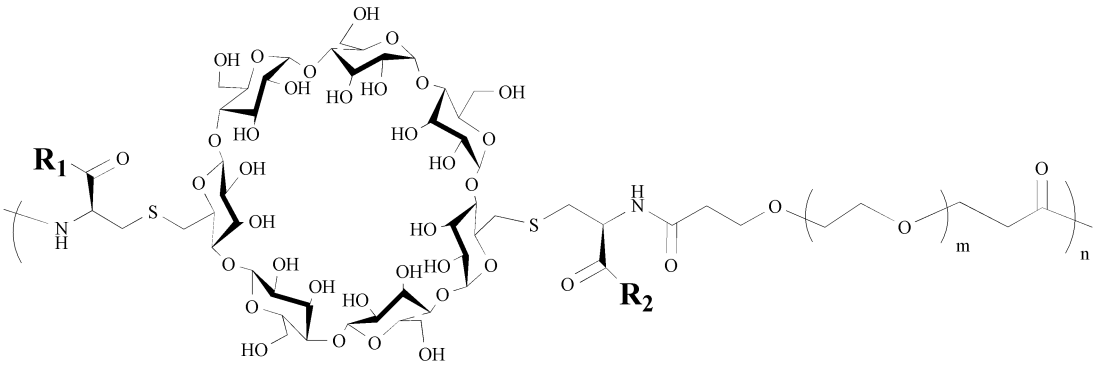
$$J_S = J_V\left(1-\sigma_f\right)\overline{C}_S + P\,S_V\,\Delta C \qquad (1)$$

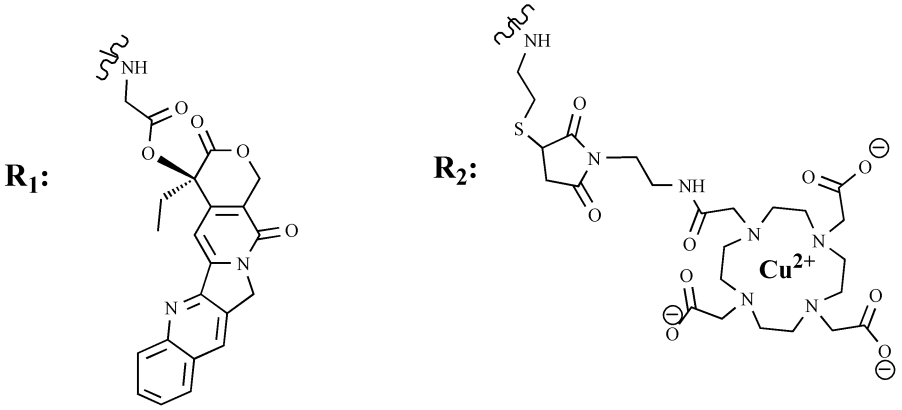
The first term in Equation 1 describes solute transport due to convection, where J_V is the solvent flow, $\overline{C}_S$ is the average concentration in the vessel wall, and σ_f is the reflection coefficient. The second term describes solute transport due to diffusion, where P is the microvascular permeability, S_V the endothelial surface area and ΔC the concentration difference across the vessel wall. The relative contributions of convection and diffusion to solute transport in our tumor model are unknown and may depend on various factors such as tumor size, tumor type, lymphatic drainage and interstitial pressure (34). We therefore ignored the convective term and introduced an apparent permeability $\left(P_{app}\right)$ to reflect the fact that there may be an unknown influence of convection:

$$J_S = \left(P_{app}\,S_V\,\Delta C\right)_{J_V \neq 0} \qquad (2)$$

This approach is supported by studies by Yuan et al. (10) as well as Dreher et al. (11), who were able to successfully describe the extravascular accumulation of fluorescently labeled macromolecules of varying molecular weight in tumors using in vivo microscopic imaging techniques.

Figures





R_1:R_2 = 3:1

Figure S1: Schematic representation of the structure of copper labeled CDP-CPT_3-$DOTA_1$. R_1 indicates camptothecin; R_2 indicates DOTA loaded with copper; m indicates number of ethylene glycol repeating units (average m = 77 for PEG with M_w 3400); n indicates number of repeating units (average n = 14 ± 4 for parent polymer).

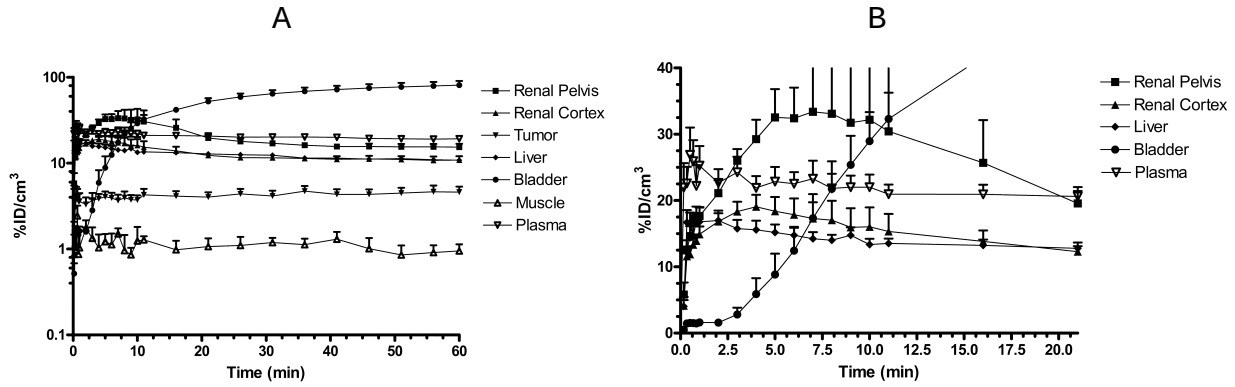


Figure S2: Average time-activity curves for the first 60 min (A) or the first 20 min (B) after i.v. injection of ^{64}Cu-labeled IT-101 in tumor bearing mice. Error bars indicate SEM.

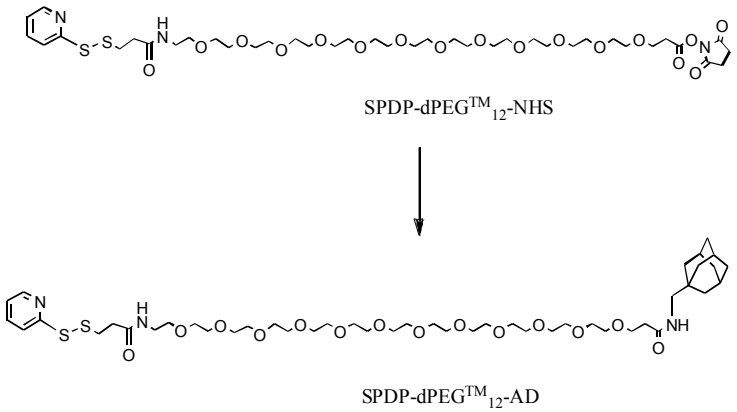
Creation of stain for IT-101

Materials

mPEG-thiol (MW 1000 g/mol) was purchased from Laysan Bio. SPDP-dPEG$^{TM}_{12}$-NHS (MW 912.08 g/mol) ester was purchased from Quanta biodesign. 5 nm spherical gold nanoparticles were obtained from Nanopartz.

Preparation of SPDP-dPEG$^{TM}_{12}$-AD (see Scheme S1)

To a solution of SPDP-dPEG$^{TM}_{12}$-NHS (100 mg, 0.11 mmol) in 3 mL of anhydrous dichloromethane was added adamantanemethylamine (0.11 mmol, 21 μL). The solution was stirred for 16 hours at room temperature, after which the solvent was removed and the product dried under vacuuo. (MALDI-TOF) $[M+Na]^+$ 984.08, $[M+K]^+$ 1000.16



Scheme S1: Synthesis of SPDP-dPEG$^{TM}_{12}$-AD

PEGylation of 5 nm gold nanoparticles with mPEG-thiol (Au_5-PEG)

To a pre-sonicated 1 mL solution of 5 nm gold nanoparticles (10^{13} particles per mL) was added mPEG-thiol (28 µg, 28 nmol). The solution was vortexed for 30 minutes and diluted to 4 mL with deionized water. The resulting 4 mL solution was diafiltered twice with a 10 kDa membrane (Millipore) and finally resuspended to 1 mL.

PEGylation of 5 nm gold nanoparticles with SPDP-dPEG$^{TM}_{12}$-AD (Au_5-PEG-AD_{25})

To a pre-sonicated 1 mL solution of 5 nm gold nanoparticles (10^{13} particles per mL) was added mPEG-thiol (21 µg, 21 nmol) and SPDP-dPEG$^{TM}_{12}$-AD (8 µg, 7 nmol). The solution was vortexed for 30 minutes and diluted to 4 mL with deionized water. The resulting 4 mL solution was diafiltered twice with a 10 kDa membrane (Millipore) and finally resuspended to 1 mL.

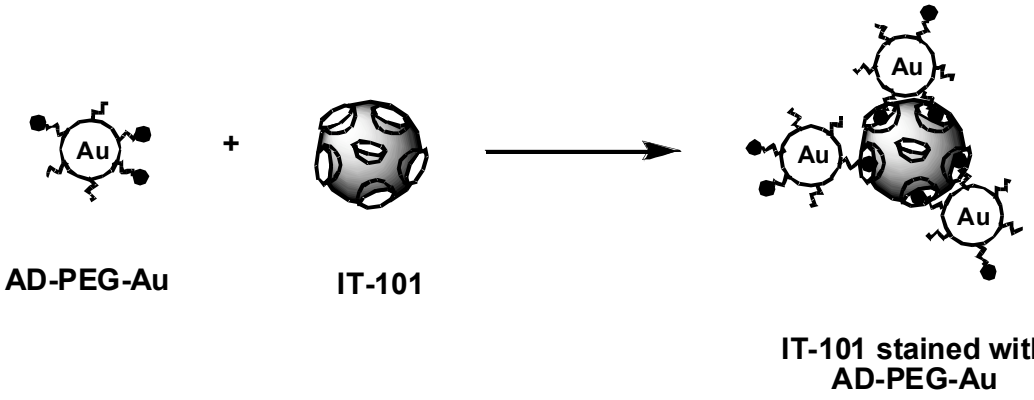
PEGylation of 5 nm gold nanoparticles with SPDP-dPEG$^{TM}_{12}$-AD (Au_5-PEG-AD_{50})

To a pre-sonicated 1 mL solution of 5 nm gold nanoparticles (10^{13} particles per mL) was added mPEG-thiol (14 µg, 14 nmol) and SPDP-dPEG$^{TM}_{12}$-AD (16 µg, 14 nmol). The solution was

vortexed for 30 minutes and diluted to 4 mL with deionized water. The resulting 4 mL solution was diafiltered twice with a 10 kDa membrane (Millipore) and finally resuspended to 1 mL.

IT-101 Staining with PEGylated 5 nm gold nanoparticles

A schematic reprensentation of the interactions between the Au-PEG-AD particles and IT-101 is shown in Schematic S2.



Scheme S2: Representation of the staining process of IT-101 by AD-PEG-Au particles.

In Vitro Transfection of IT-101

$5*10^4$ mouse neuroblastoma cells (Neuro2A; N2A) were cultured on 625 mm^2 cover-slips pre-deposited in a 6-well plate for two days at 37 °C and 5 % CO_2 in complete growth medium (DMEM medium supplemented with 10 % fetal bovine serum (FBS), 100 units/ml penicillin, and 100 units/ml streptomycin). On the day of transfection, the cells received transfection of 30 µg polymer/ml of IT-101 in complete growth medium for 7 hours at 37 °C and 5 % CO_2.

In Vitro Intracellular Staining of IT-101 with Au-PEG-AD

After rinsing cells with PBS to remove any free IT-101, fixation with 4 % paraformaldehyde (in PBS) at room temperature for 15 minutes and permeabilization with acetone at -20 °C for 10 minutes followed. To detect any intracellular IT-101, cells received staining by 5 nm gold nanoparticles covalently modified with (polyethylene glycol) PEG moieties, whose extremities contain 0 %, 25 %, or 50 % of adamantane for the specific interaction with the exposed cyclodextrin cups on IT-101. Staining with Au-PEG-AD particles (in 1x PBS) proceeded at room temperature for 2 hours. With PBS washes to remove any free Au-PEG-AD, cell mounting on glass slides with Mowiol 4-88 took place to allow direct visualization under a Zeiss LSM 510 Inverted confocal scanning microscope with a 40 x objective and double optical zoom. (Camptothecin, the active ingredient of IT-101: excitation: 370 nm, emission: 440 nm; Au-PEG-AD particles: excitation: 488 nm, emission: 510 nm)

Nature of IT-101 Staining by Au-PEG-AD

To elucidate the nature of staining, the transfection of N2A cells on cover-slips proceeded with the presence of pure cyclodextrin polymer (CDP) for 7 hours. As another separate control, the same *in vitro* transfection and intracellular staining experiments were repeated with N2A cells on cover-slips and transfected with pure camptothecin (CPT). To prevent cytotoxic effects, the transfection with naked camptothecin lasted for 90 minutes. N2A cells transfected with CDP and CPT received subsequent staining by Au-PEG-AD, as described above.

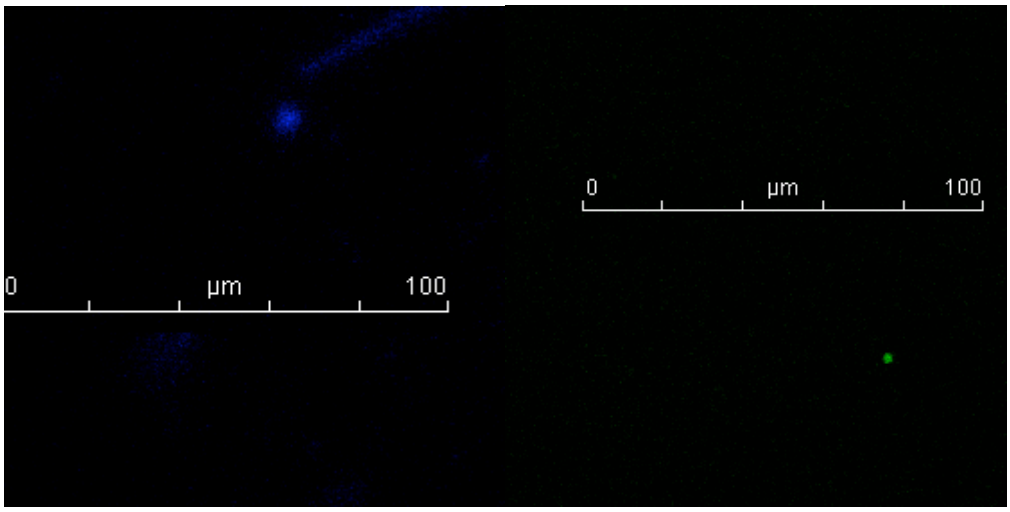


Figure S3: Confocal micrographs showing the fluorescence of IT-101 and AD-PEG-Au. Left: IT-101 excitation: 370 nm; emission: 440 nm (for CPT). Right: PEGylated gold nanoparticles with 50 % adamantane: excitation: 488 nm; emission: 507 nm. Both IT-101 and nanoparticles appear as bright dots.

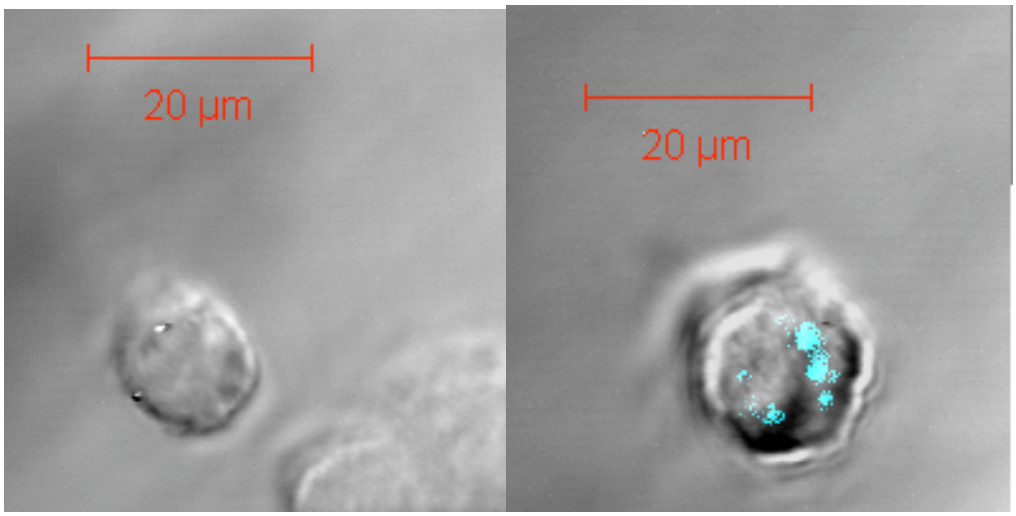


Figure S4: *In Vitro* transfection of cells with IT-101 without staining by Au-PEG-AD particles Left: Untransfected N2A cells showed no obvious fluorescence signal. Right: Transfection with IT-101 led to cellular uptake by N2A cells, shown by punctuate, blue dots inside the cell.

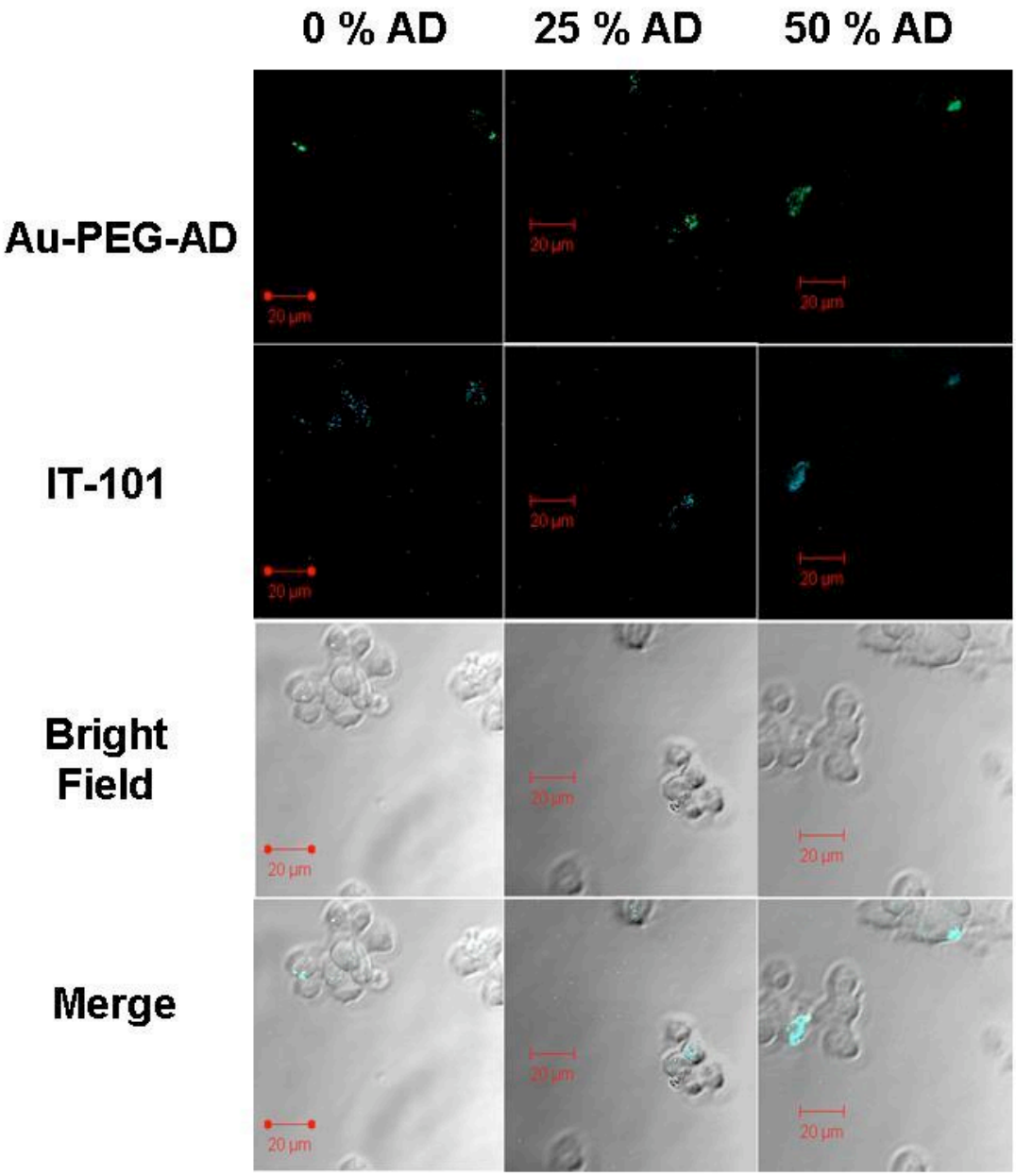


Figure S5: Effect of staining with PEGylated gold nanoparticles modified with various amounts of adamantane on N2A cells transfected by IT-101. Left column: Au-PEG without AD; Middle column: Au-PEG with 25 % AD; Right column: Au-PEG with 50 % AD. The colocalization between IT-101 and Au-PEG-AD particles increases with adamantane modification. Staining of *in vivo* tumor sections utilized PEGylated gold particles with 50 % AD.

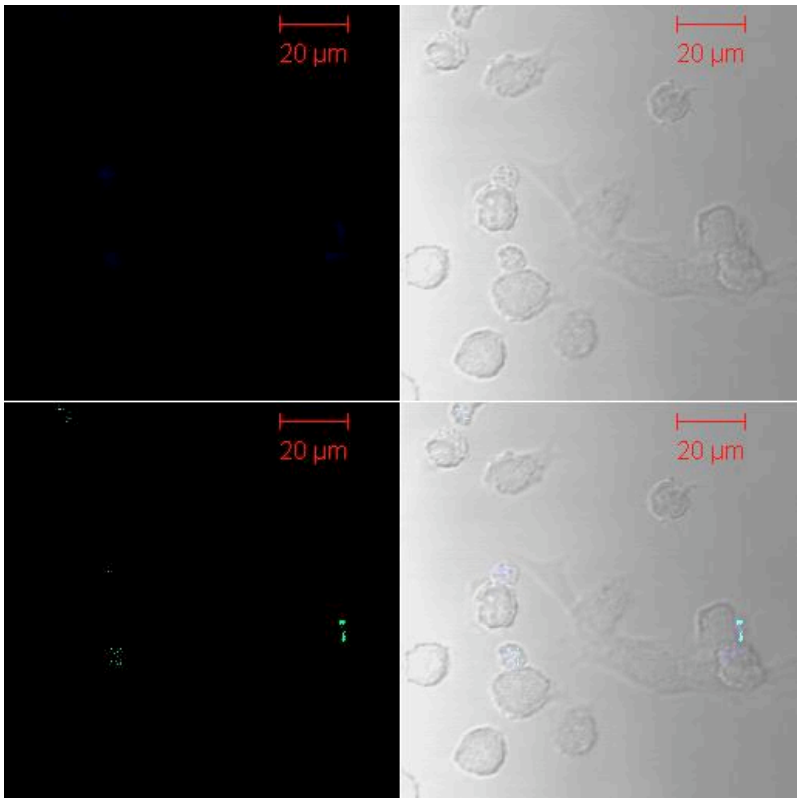


Figure S6: Nature of staining with Au-PEG-AD on cells transfected with CDP (polymer alone). Au-PEG-AD recognized CDP in the absence of CPT. No blue signals were observed due to the absence of CPT. Top left: CDP; top right: bright field; bottom left: Au-PEG-AD; bottom right: merge.

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