Supporting Information

Nano–Cell Interactions of Non-Cationic Bionanomaterials

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Supplementary Materials and Methods

Synthesis of citrate-capped gold nanoparticles (AuNPs). AuNPs of ~20 nm in diameter were prepared by an established seed-mediated growth method. In brief, 150 mL of 2.2 mM anhydrous sodium citrate (Alfa Aesar) was brought to boil in a three-necked round bottom flask with rapid stirring, followed by injection of 1 mL of 25 mM gold (III) chloride trihydrate ($HAuCl_4 \cdot 3\ H_2O$, Sigma). After becoming orange red in about 15 min, the solution was cooled down to 90˚C and maintained at this temperature. Next, 1 mL of 25 mM Au^{3+} was injected twice at 30-min intervals. The solution was diluted by replacing 55 mL of the solution with 53 mL of Nanopure water (Thermo Scientific) and 2 mL of 60 mM sodium citrate. Addition of 1 mL of the 25 mM Au^{3+} solution was repeated 2 times every 30 min. The solution was used for subsequent NP growth. The process of dilution, addition of sodium citrate solution and three doses of Au^{3+} solution was repeated until the AuNPs measure ~20 nm in diameter, as evidenced by their surface plasmon resonance peak at 521 nm.[1]

Preparation of polyethylene glycol-coated AuNPs (PEG-AuNPs). An aqueous suspension of citrate-capped AuNPs was mixed with HS-PEG_{5000}-OCH_3 (JenKem Technology) at a total PEG concentration at 20 PEG molecules per nm^2 of NP surface to be coated. The PEGylation reaction typically lasted for 1 h with sonication, followed by purification by 5 rounds of centrifugation at 13,000 rpm for 15 min and resuspension in deionized water.

Preparation of dodecyl$_{4\%}$-PEG-AuNPs.[2] To prepare HS-PEG_{5000}-dodecyl, the carboxyl group of the bifunctional PEG linker, HS-PEG_{5000}-COOH (JenKem Technology), was activated by EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) (Sigma)/NHS (N-hydroxysuccinimide) (Sigma) chemistry. Briefly, HS-PEG_{5000}-COOH (0.1 μmol), EDC (0.5 μmol), NHS (0.5 μmol) and trimethylamine (0.5 μmol) (TAE) (Sigma) was dissolved in 1 mL of freshly distilled

dichloromethane (DCM) and vortexed for 2 h. Next, 0.5 μmol of dodecylamine (H_2N-$C_{12}H_{25}$) (Sigma), initially dissolved in 0.5 mL of DCM, was added in excess to the 0.1 μmol of activated PEG linker, followed by vortexing overnight. The reaction mixture was added dropwise into cold diethyl ether and then centrifuged at 3,000 × g for 3 min. The precipitated PEG product was washed with cold diethyl ether for 5 more times with sonication and briefly dried. Finally, the product was dialyzed against methanol for 1 wk, against 0.1% acetic acid in water for 1 d, and against deionized water for 1 more d. Successful conjugation of dodecylamine to the bifunctional PEG linker was confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) (Bruker). To prepare dodecyl-terminated PEG-AuNPs (dodecyl$_{4\%}$-PEG-AuNPs), an aqueous suspension of AuNPs was mixed with 4 mol% of HS-PEG_{5000}-dodecyl and 96 mol% of HS-PEG_{5000}-OCH_3 (JenKem Technology) by keeping the total PEG concentration at 20 PEG molecules per nm^2 of AuNP surface to be coated. The reaction lasted for 1 h with sonication, followed by purification by 5 rounds of centrifugation at 13,000 rpm for 15 min and resuspension in deionized water.

Preparation of T30-AuNPs.[3] Thiolated DNA oligonucleotides with 30 repeating thymidines (T30-SH) were synthesized by an automated synthesizer (Oligo 800 Azco Biotech) using standard solid-phase synthesis and reagents. All oligonucleotides were purified using a high-performance liquid chromatography instrument (Agilent 1260) with a Microsorb C18 column (Varian). To synthesize T30-AuNPs, an aqueous suspension of AuNPs was mixed with T30-SH (at a molar ratio of 1:4000) and vortexed for several seconds. Sodium chloride (Sigma) solution was sequentially added to the NP solution at time intervals of 30 min up to a final concentration of 0.5 M to achieve a dense coverage of with DNA oligonucleotides on the NP surface. The solution was centrifuged for three times to remove excess unreacted DNA oligonucleotides.

Preparation of PDA-AuNPs.[4] The surface of AuNPs was firstly coated with PEG by swirling. An aqueous suspension of AuNPs was swirled with HS-PEG_{1000}-OCH_3 (JenKem Technology) at a concentration of 10 PEG molecules per nm^2 of NP surface for 1–2 h, purified by 3 rounds of centrifugation at 10,000 rpm. To decorate the AuNP core with a PDA shell of ~5 nm in thickness, 5 mL of 0.5 nM PEG_{1000}-AuNPs solution was mixed with 5 mL of dopamine solution (0.1 mg/mL, buffered in 10 mM Tris at pH 8.5) under continuous sonication for 3 h at room temperature and subsequently purified by 3 rounds of centrifugation at 10,000 rpm. The surface of PDA-AuNPs were further functionalized with PEG_{1000} by incubating the reaction product with HS-PEG_{1000}-OCH_3 at a concentration of 5 PEG molecules per nm^2 (buffered in 10 mM Tris at pH 8.5) for overnight, and purified by 3 rounds of centrifugation at 10,000 rpm.

Characterization of NPs. The extinction spectrum of AuNPs was measured by an Agilent Cary 5000 UV-Vis-NIR spectrophotometer. AuNPs suspended in Nanopure water or OptiMEM (Gibco) was loaded into a quartz cuvette with a path length of 1 cm for analyzing the absorption spectrum between 400 nm and 800 nm. Concentration of NPs was determined based on the Beer-Lambert's Law and the molar extinction coefficient of AuNPs of 20 nm in diameter (5.41×10^8 $M^{-1}cm^{-1}$).[5] Hydrodynamic diameters and ζ-potentials were measured by the DelsaMax PRO light scattering analyzer (Beckman Coulter). For hydrodynamic diameters measurements, the NPs were suspended in either Nanopure water or OptiMEM. To test the stability of NPs in OptiMEM, NPs were suspended in OptiMEM and incubated at 37˚C for 24 h before measuring the hydrodynamic diameters. For ζ-potential analysis, the NPs were suspended in 1 mM KCl. Reported values represent mean ± SD from three independent measurements.

Cellular uptake. HeLa cells (human cervix adenocarcinoma, ATCC), RAW 264.7 cells (mouse macrophage, ATCC), and bEnd.3 cells (mouse brain capillary endothelial cell, ATCC) were

cultured in full Dulbecco's Modified Eagle Medium (DMEM, Gibco) [*i.e.*, DMEM supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin (Gibco)] and maintained at 37˚C and 5% CO_2. Before the uptake experiment, cells were seeded in 24-well plates and grown to ~80% of confluence. During the uptake experiments, cells were incubated with the 1 nM of NPs formulated in 0.3 mL of OptiMEM (Gibco) per well. After 24 h, the NP-containing medium was removed, and the cells were rinsed with phosphate-buffered saline (PBS) twice, followed by trypsinization for 5 min. Cell pellets were collected by centrifugation at 4,000 rpm for 5 min.

ICP-MS. Cell pellets were digested by 0.25 mL of aqua regia (3:1 volumetric ratio of concentrated HCl and HNO_3) overnight at room temperature, and further diluted by the matrix solution (2% HCl, 2% HNO_3, with 10 ppb indium as internal standard) for ICP-MS measurements (Agilent 7900, Agilent). The Au content in each resultant solution was computed based on the calibration curve derived from Au ion standard solutions of known concentrations. The number of AuNPs in each solution was calculated based on calibration curves derived from gold NP solutions of known concentrations. Reported data represent mean ± SD from 3 independent experiments. Two-tailed t-test was used for statistic test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: not significant.

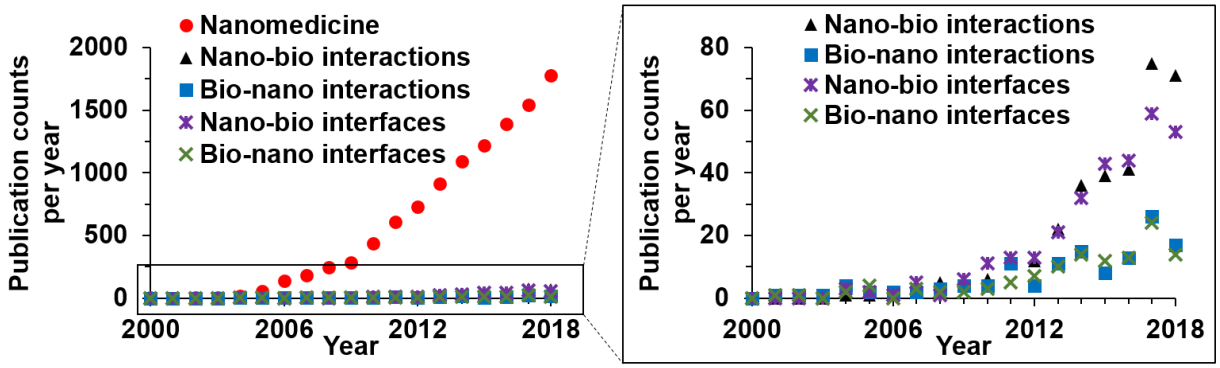


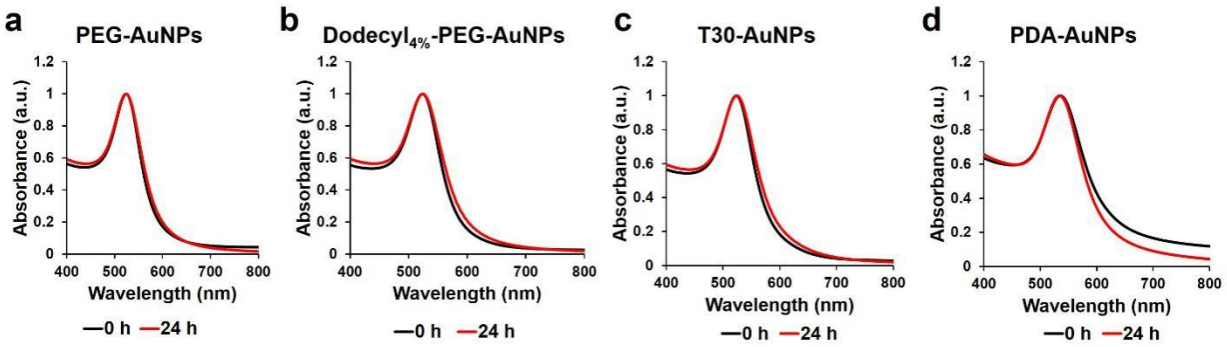
Figure S1. Count of publications per year on "nanomedicine", "nano–bio interactions", "bio–nano interactions", "nano–bio interfaces", and "bio–nano interfaces" from 2000 to 2018. The result is based on defined keyword searches in topics from the Web of Science using the Web of Science Core Collection Database. The total number of publications on "nanomedicine", "nano–bio interactions", "bio–nano interactions", "nano–bio interfaces", and "bio–nano interfaces" from 2000 to 2018 is 10655, 330, 129, 307, and 117 respectively.

Table S1. Physicochemical Parameters of Non-Cationic AuNPs and Their Stability in OptiMEM.

Sample	Zeta potential in 1 mM KCl (mV)[a]	Hydrodynamic size in water (nm)[b]	Hydrodynamic size after 24 h of incubation in OptiMEM (nm)[b]
Citrate-capped AuNPs	-39.6 ± 1.7	19.3 ± 0.1 (0.1)	N.A.
PEG-AuNPs	-10.4 ± 0.4	32.3 ± 0.5 (0.2)	29.8 ± 0.4 (0.2)
Dodecyl$_{4\%}$-PEG-AuNPs	-11.8 ± 0.7	31.0 ± 0.2 (0.2)	33.1 ± 0.5 (0.2)
T30-AuNPs	-43.7 ± 0.3	28.4 ± 0.2 (0.2)	26.4 ± 0.4 (0.2)
PDA-AuNPs	-16.1 ± 1.9	37.8 ± 0 (0.2)	42.5 ± 0.1 (0.2)

[a] Reported data represent mean ± SD from three independent measurements.

[b] Reported data represent mean ± SD from three independent measurements of Z-average sizes. Bracketed number refers to polydispersity index (PDI).



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SPR Peak (nm)		
	Duration of incubation in OptiMEM	
	0 h	24 h
Citrate-capped AuNPs	521	N.A.
PEG-AuNPs	524	525
Dodecyl$_{4\%}$-PEG-AuNPs	524	525
T30-AuNPs	524	525
PDA-AuNPs	535	533

Figure S2. UV-Vis spectra of (a) PEG-AuNPs, (b) dodecyl$_{4\%}$-PEG-AuNPs, (c) T30-AuNPs, and (d) PDA-AuNPs before and after incubation in OptiMEM at 37˚C for 24 h, denoted as "0 h" and "24 h", respectively. (e) The surface plasmon resonance (SPR) peak of the non-cationic NPs before and after incubation in OptiMEM at 37˚C for 24 h. The UV-Vis spectra of all types of NPs show a single peak without significant red-shift of the SPR peak of the AuNPs, supporting the lack of obvious NP aggregation across the 24 h incubation time window.

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