Electronic Supplementary Information

Cholesterol-Driven Guest Exchange Tunes Plasmon Damping in Single β-Cyclodextrin-Modified Gold Nanorods

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EXPERIMENTAL METHODS

Host–Guest Complex Inclusion of SH-βCD and MB. SH-βCD-functionalized nanoparticles (AuNRs@βCD) were mixed with 8.688 μL of a 1 mM methylene blue (MB) solution to achieve a 1:1 host–guest molar ratio. The mixture was incubated at room temperature for 2 h without agitation to allow host–guest inclusion. The resulting AuNRs@βCD/MB complexes were collected by centrifugation and washed twice to remove excess, non-encapsulated MB. For guest exchange, the AuNRs@βCD/MB dispersion was subsequently treated with 8.688 μL of a 1 mM cholesterol (Chol) solution to achieve a 1:1 guest-exchange ratio. The mixture was incubated under identical conditions, followed by centrifugation and washing to remove unbound Chol, yielding AuNRs@βCD/Chol.

Sample Preparation for Single Particle Microscopy and Spectroscopy. Glass slides (76 × 26 × 1 mm, MARIENFELD) and cover glasses (22 × 22 mm or 24 × 50 mm, BRAND) were ultrasonically cleaned in ethanol, deionized (DI) water, and isopropyl alcohol for 15 min each. For single-particle measurements, an AuNR stock solution (6.94×10^{12} NRs/mL) was diluted with DI water to minimize interparticle localized surface plasmon resonance (LSPR) coupling and subsequently sonicated for 15 min at room temperature to prevent aggregation. The diluted AuNR dispersion was deposited onto a pre-cleaned glass slide and dried under ambient conditions. An oxygen plasma cleaner (PDC-32G-2, Harrick Plasma, USA) was then used to remove the residual CTAB layer surrounding the AuNRs. DI water was added as the surrounding medium, and a cover glass was placed on top of the slide to form a sealed liquid cell.

Single Particle Microscopy and Spectroscopy. For single-particle characterization, sequential guest exchange was performed *in situ* by introducing each solution into the gap between the glass slide and cover glass (a sealed liquid cell), with real-time measurements conducted after each step. To functionalize the immobilized AuNRs, 100 μL of an 8.688 μM SH-βCD solution was introduced into the gap to form AuNRs@βCD. After characterization of the SH-βCD-functionalized AuNRs, 100 μL of an 8.688 μM MB solution was introduced into the same gap to induce host–guest inclusion, yielding AuNRs@βCD/MB. After each measurement step, the solution in the liquid cell was exchanged by introducing fresh solvent while simultaneously removing the old solution from the opposite side using absorbent paper. This procedure ensured the removal of unbound molecules prior to the introduction of the next component. Subsequently, 100 μL of an 8.688 μM cholesterol solution (in ethanol) was introduced to trigger guest exchange, resulting in AuNRs@βCD/MB/Chol. Additional aliquots of the respective solutions were added only when necessary to improve imaging quality and signal stability.

UV-Vis Absorption Spectroscopy Study. UV–Vis absorption spectroscopy was performed using the same MB-to-nanoparticle ratio as in the single-particle experiments, but at higher absolute concentrations to improve ensemble signal intensity. The samples were prepared by diluting the MB solution to 10 μM and the synthesized AuNR@βCD dispersion to 3.91 μg/mL, while maintaining the same molar ratio used throughout the study. A tungsten lamp was used as the light source, and spectra were recorded using a 1 cm path-length quartz cuvette. Baseline correction was performed using a water-filled cuvette prior to measurement. Spectra were collected over the wavelength range of 400–800 nm.

Photoluminescence Study. Photoluminescence (PL) measurements were conducted using samples prepared at concentrations comparable to those used in the UV–Vis absorption studies. Steady-state fluorescence spectra were recorded using a time-correlated single-photon counting (TCSPC) system equipped with a diode laser. The excitation wavelength was selected near the absorption maximum of each sample, as determined from the UV–Vis spectra. For fluorescence lifetime measurements, a 375 nm pulsed diode laser was used as the excitation source. All fluorescence lifetimes were measured using a picosecond pulsed diode laser with a pulse period of 500 ns and an average excitation power of 5 mW.

Molecular Modeling Study of Host-Guest Inclusion. To elucidate the molecular-level interactions between the host (βCD) and the guest molecules (MB and Chol), molecular dynamics (MD) simulations were performed using the MMFF94x force field. The host molecules were protonated under ambient conditions (pH 7.4, 300 K) to appropriately model their solution-state configuration. Under the conditions described above, βCD was treated as a neutral host molecule, as its hydroxyl groups ($pK_a \gg 7$) remain protonated. The thiol group of SH-βCD was also modeled in its neutral (–SH) form, as shown in Fig. 2. The guest molecules were prepared through energy minimization and system equilibration, during which unbound or non-interacting species were removed to improve the accuracy of the host–guest interaction analysis. Furthermore, the AuNR surface was not explicitly included in the simulation. Therefore, Au–S bond formation and direct guest–Au surface interactions were not modeled within the MMFF94x framework. We emphasize that the purpose of the simulations was to compare the relative binding preferences of the β-CD cavity toward MB versus Chol, which represents the key driving force for guest exchange.

SUPPLEMENTARY TABLE

	τ_1 (ns)	τ_2 (ns)	τ_{total} (ns)
AuNRs@βCD/MB	0.453(± 0.002)	3.036(± 0.518)	3.489(± 0.519)
AuNRs@βCD/MB + Chol	0.459(± 0.004)	2.659(± 0.149)	3.119(± 0.151)

Table S1. Comparison of fluorescence lifetimes obtained from PL measurements reveals that the total fluorescence lifetime (τ_{total}) decreases from 3.489(± 0.519) ns to 3.119(± 0.151) ns upon Chol addition.

SUPPLEMENTARY FIGURES

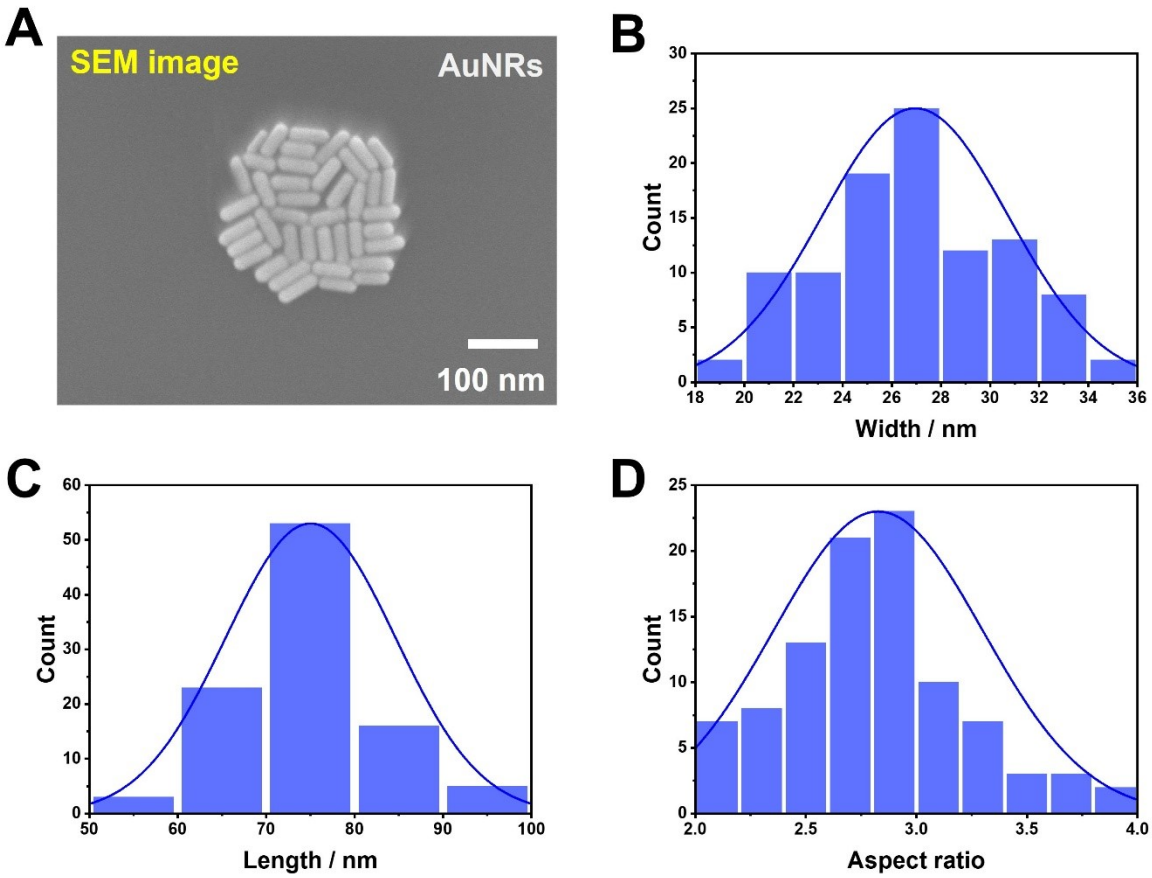

Fig. S1 (A) SEM image of the bare AuNRs used in this study. Histograms showing the distributions of **(B)** width, **(C)** length, and **(D)** aspect ratio (AR) of individual AuNRs.

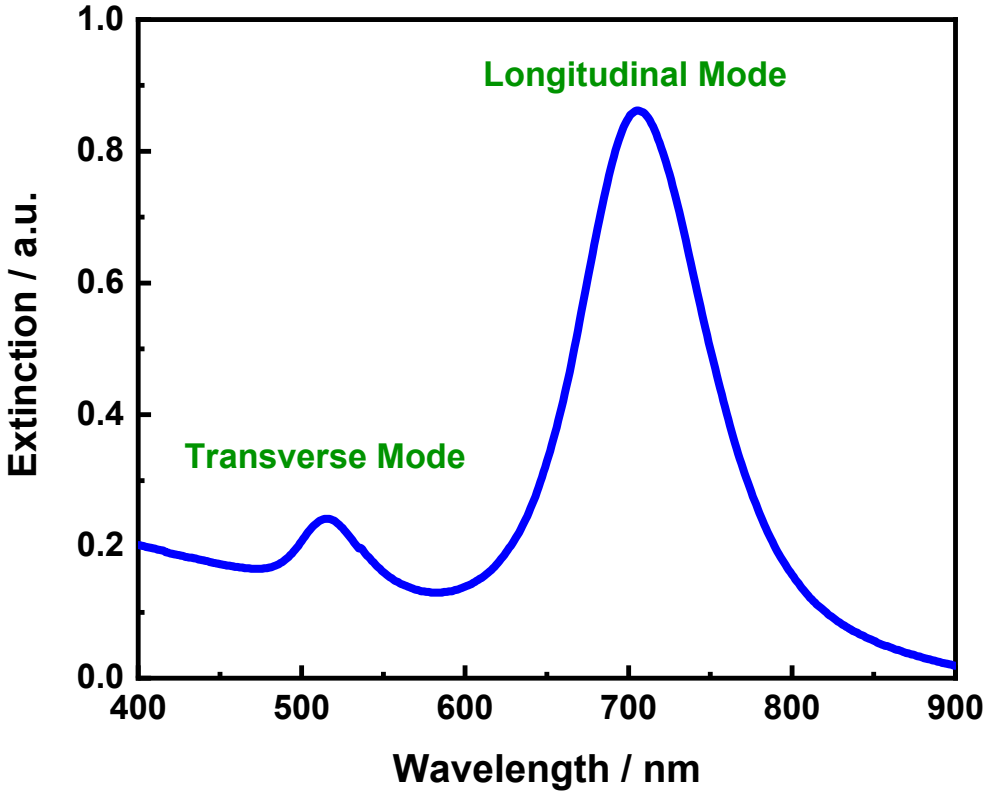

Fig. S2 The UV-Vis extinction spectrum of AuNRs dispersed in distilled water exhibited two LSPR peaks: a transverse peak at approximately 514 nm and a longitudinal peak at around 714 nm.

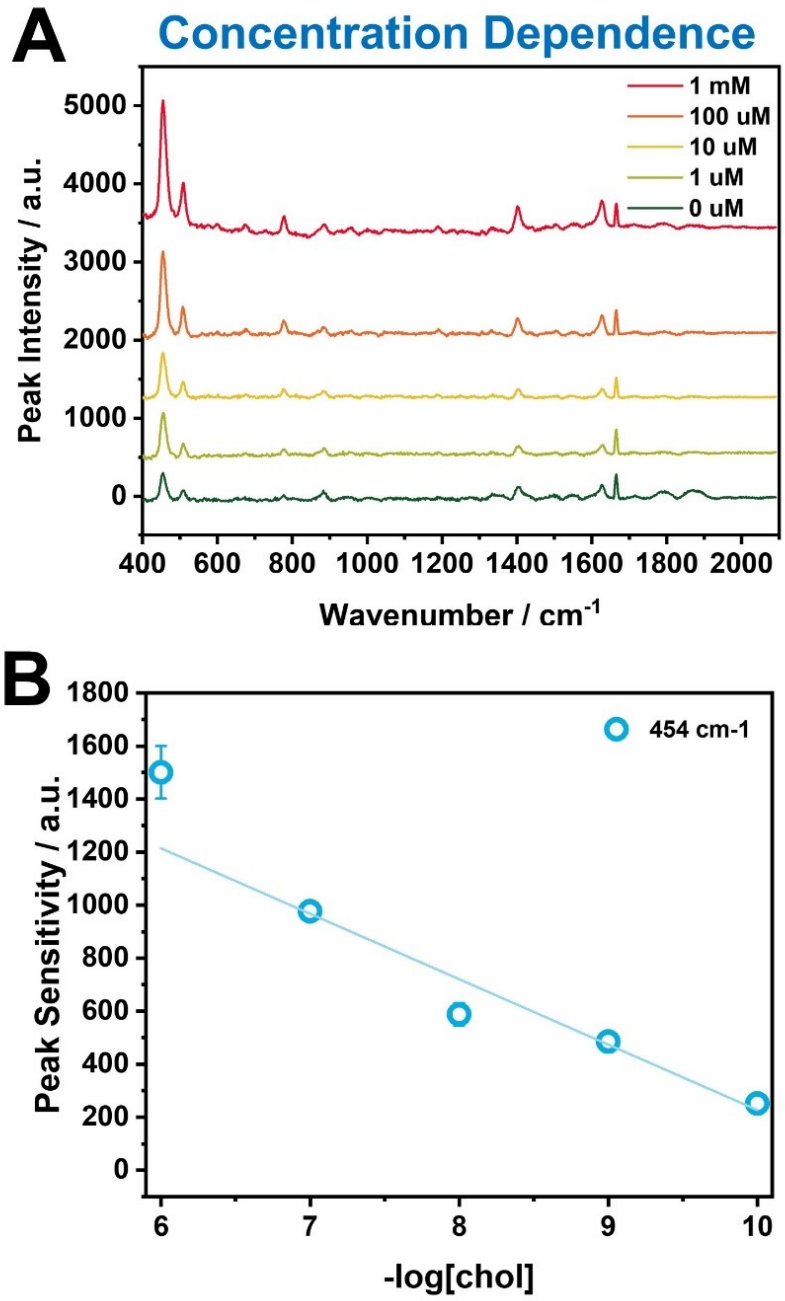

Fig. S3 Concentration-dependent SERS response during Chol-induced guest exchange. **(A)** SERS spectra of AuNRs@SH-βCD/MB recorded after incubation with increasing Chol concentrations (0 μM to 1 mM). The Raman intensity, particularly at 454 cm^{-1}, increases systematically with increasing Chol concentration. **(B)** Corresponding plot of the Raman peak intensity at 454 cm^{-1} as a function of −log[Chol], showing a clear concentration-dependent trend. Error bars represent the standard deviation obtained from multiple measurements.

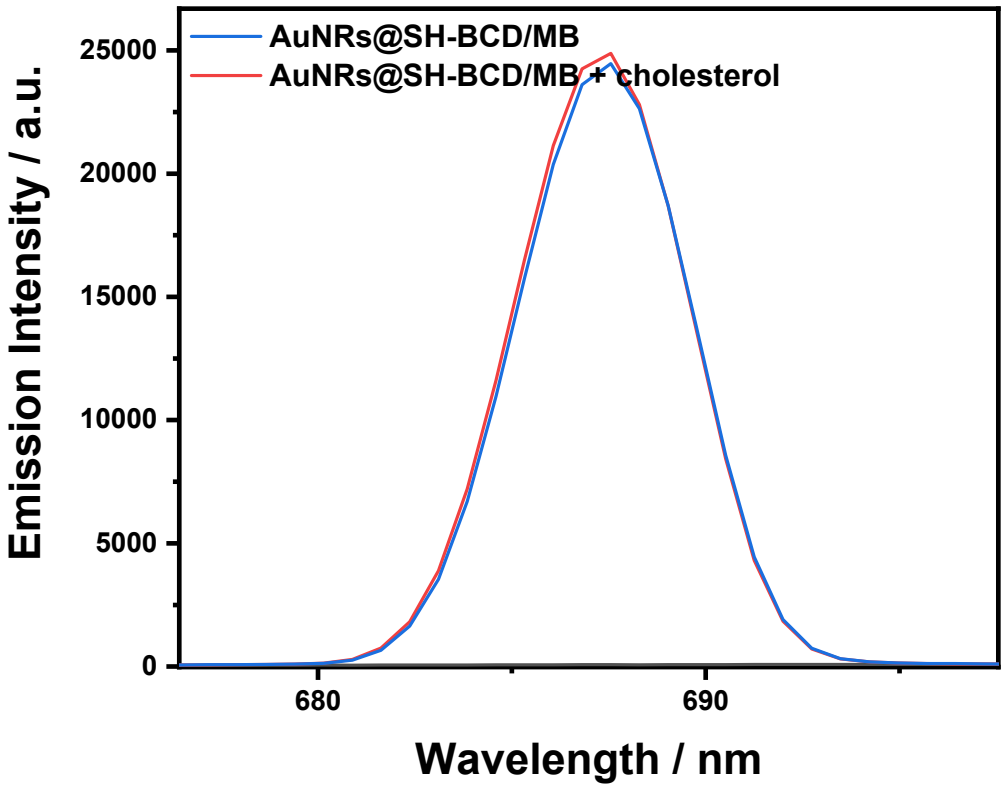

Fig. S4 Comparison of PL spectra before and after Chol-induced guest exchange.

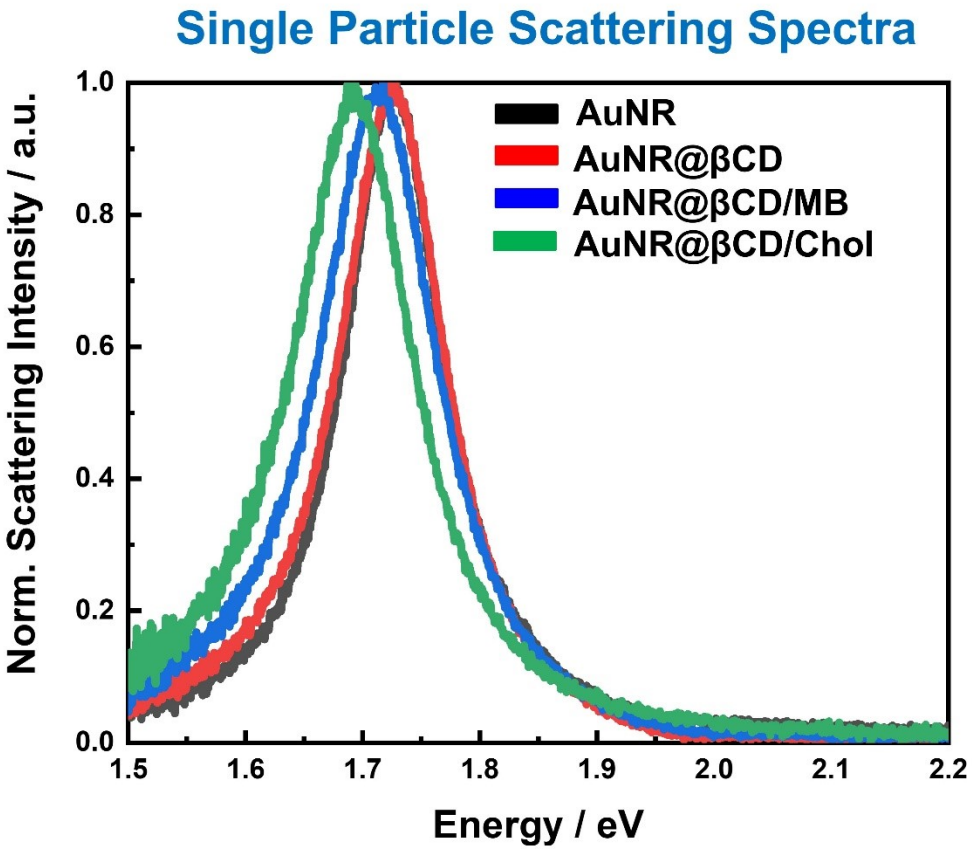

Fig. S5 Single-particle LSPR scattering spectra of an AuNR showing sequential adsorption of βCD, encapsulation of MB, and subsequent replacement of MB with Chol on the same particle.

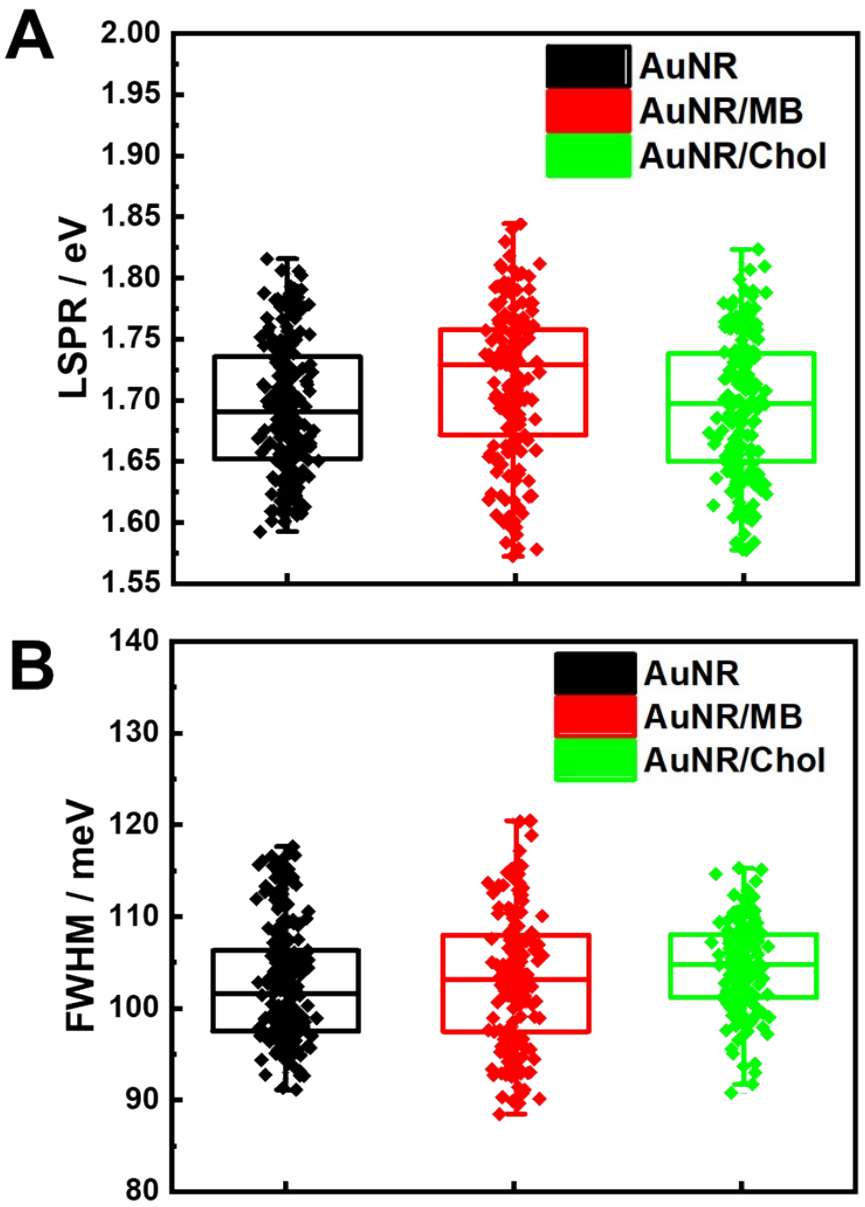

Fig. S6 β-CD-free control experiments. **(A)** Average LSPR peak energy distributions of single AuNRs at each stage: bare AuNRs, AuNRs/MB, and AuNRs/Chol. **(B)** Average LSPR linewidth distributions of single AuNRs at each stage: bare AuNRs, AuNRs/MB, and AuNRs/Chol.