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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Sunlight-assisted route to antimicrobial plasmonic aminoclay catalysts

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Experimental

1) Materials and reagents

All experiments were carried out using Ultrapure Millipore water polished to a resistivity of 18.2 MΩ cm. Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$; 99.995% trace metals basis), (3-aminopropyl)trimethoxysilane (APTMS; 99%), silver(I) nitrate (99.9999% trace metals basis), gold(III) chloride hydrate ($HAuCl_4 \cdot xH_2O$; 99.999% trace metals basis), 4-nitrophenol (4-NP; ≥99%), and sodium borohydride ($NaBH_4$; granular, 99.99% trace metals basis) were purchased from Sigma-Aldrich (St. Louis, MO). *Escherichia coli* (ATCC® 25922™; FDA strain Seattle 1946) were sourced from the American Type Culture Collection (ATCC; Manassas, VA) and maintained as indicated by the supplier.

2) Characterization techniques

The absorption properties of aqueous 10-fold diluted suspensions of the (bi)metallic aminoclay hybrids were measured in disposable PMMA cuvettes using a Cary Bio 50 UV-Vis spectrophotometer. Catalytic data were acquired using semi-micro 1.4 mL Spectrosil® quartz optical cuvettes from Starna Cells Inc. (Atascadero, CA). X-ray diffraction patterns were collected at 25 °C on a Rigaku PXRD system using Cu Kα radiation (λ = 1.54059 Å) at 40 kV and 44 mA with a spectral range of 2θ from 2 to 80°. Samples were drop-cast onto pre-cleaned glass slides and allowed to air dry at room temperature prior to data collection. Transmission

electron microscopy (TEM) studies were performed on carbon-coated copper grids (01822-F, support films, ultrathin carbon type-A, 400-mesh copper grids from Ted Pella, Inc.) using an FEI Tecnai (F30 G2, Twin) microscope operated at a 300 keV accelerating electron voltage. Samples were drop-casted onto the grid and allowed to dry before imaging.

EDX analyses were performed in STEM mode at 300 keV. The as-synthesized AC@BMNP samples were drop-casted onto carbon-coated grids (the same type of grids employed for our TEM imaging) and allowed to air dry. EDX spectra were collected on the areas of interest for at least 2 min to ensure adequate counts. In order to generate BMNP compositions, EDX spectra from at least 5 different areas of particles were analyzed for the Ag/Au ratio using only the L lines of Ag and Au. The Ag/Au ratios for each spectrum were calculated using TEM Imaging and Analysis (TIA), software provided by FEI, and the resultant values were averaged together to generate the %Ag plot provided in Fig. 6A. The TIA software was prompted to produce %Au values in an atomic % basis rather than a weight % basis. The composite elemental mapping images were generated in ImageJ, a public domain, open source, Java-based image processing program developed at the National Institutes of Health (NIH).

3) Aminoclay synthesis and exfoliation

The aminoclay (AC) was prepared according to previously reported literature.[S1,S2] In a typical procedure, 1.35 g of (3-aminopropyl)trimethoxysilane (APTMS) was added dropwise to 0.846 g of magnesium chloride hexahydrate in 20 g of ethanol at room temperature. Immediately upon addition of APTES, a small amount of white turbid material was observed. After the addition was complete, the sample was allowed to stir overnight. The obtained white material was then separated by centrifugation (8,000 rpm for 15 min) and the supernatant discarded. The remaining white sediment was re-dispersed in ethanol (35 mL) by manual shaking and vortexing. This solution was again centrifuged at 5,000 rpm for 5 min and the ethanol supernatant discarded, resulting in an opaque white gel. The gel was transferred to a PTFE septum-capped vial and dried under vacuum overnight on a Schlenk line while heating the sample at 40 °C. After drying, a solid white hard (stone-like) material was obtained. The solid product was gently ground using an agate mortar and pestle to yield (1.16 g; yield of 83%) a fine, free-flowing white powder suitable for exfoliation. Exfoliation was achieved by dispersing 100 mg of as-prepared AC into

10 mL of Millipore water under vigorous agitation with a vortex mixer for one minute followed by low-power ultrasonic bath (Branson 3510) treatment for 3 min to result in a clear dispersion that displayed Tyndall scattering under laser pointer illumination.

4) Preparation of AC@MNP and AC@BMNP nanohybrids

Metal nanoparticle (MNP) decorated aminoclay hybrid materials were prepared by combining 1 mL of 10 mM aqueous metal salt (*i.e.*, $AgNO_3$ or $HAuCl_4$ to yield AC@Ag or AC@Au, respectively), 1 mL of exfoliated AC (10 mg mL^{-1}), and 5 mL of Millipore H_2O. These mixtures were vortexed for 1–2 min and then treated for 1 h under unfiltered 450 W Xe arc lamp (Ushio) irradition. The bimetallic metal nanoparticle (BMNP) decorated AC hybrids (denoted AC@Ag_xAu_y where x and y designate the relative molar amount of the corresponding metal employed; *i.e.*, $y = 1–x$ where $0 \leq x \leq 1$) were prepared in similar fashion by combining the appropriate ratio of the two metal salts. For instance, 0.1 mL of aqueous $HAuCl_4$ (10 mM) and 0.9 mL of aqueous $AgNO_3$ (10 mM) were combined with 1 mL of AC (10 mg mL^{-1}) and 5 mL of H_2O to give the resulting AC@$Ag_{0.9}Au_{0.1}$ (10% Au) hybrid. The remaining Ag:Au (x:y) ratios were prepared similarly in 10% increments from AC@$Ag_{0.8}Au_{0.2}$ (20% Au) to AC@$Ag_{0.1}Au_{0.9}$ (90% Au).

5) Catalytic activity measurements

To assess the catalytic activity of the AC@MNP and AC@BMNP hybrids toward 4-nitrophenol (4-NP) reduction, 300 μL of freshly prepared 0.1 M aqueous $NaBH_4$ was added to 700 μL of 0.2 mM aqueous 4-NP solution (which had been previously degassed for 20 min) in a semi-micro (1.4 mL) quartz cuvette, to yield a deep yellow solution indicative of the formation of the 4-nitrophenolate ion (4-NPO; see Fig. S4). To initiate the reaction, 30 μL of an aminoclay hybrid (diluted 10-fold from the as-prepared material) was added to the above mixture and gently homogenized (by slow manual inversion four times) to avoid bubble generation. The reduction of 4-NPO was monitored colorimetrically at room temperature, collecting spectra every 15 seconds in the scanning range of 200–800 nm at a scan speed of 24,000 nm per minute. Control experiments were also conducted in the absence of AC@Ag_xAu_y addition as well as in the presence of naked (unmodified) exfoliated AC. In each case, the decrease in the absorbance intensity of 4-NPO was quantitatively monitored at 401 nm every second using a Cary Bio 50

UV-Vis spectrophotometer and apparent rate constants for the conversion were calculated from the linear correlation of $\ln(A_t/A_0)$ versus time plots. To test the AC@x catalysts over multiple cycles, an aliquot of fresh 4-NP was added to the cuvette once the previous reaction had gone to completion. This process was repeated 4–5 times, however, no supplemental $NaBH_4$ was added with each cycle.

We note that the analytical molar concentration of total metal (M = Ag+Au) used in these catalytic tests was held constant at 4.16×10^{-6} M. This results in slightly different loadings on a mass basis for the various metals, as follows:

AC@Ag: 449 ng mL^{-1} Ag (*i.e.*, 449 parts-per-billion, ppb)

AC@Au: 820 ng mL^{-1} Au

AC@$Ag_{0.5}Au_{0.5}$: 634 ng mL^{-1} Ag+Au

6) Antimicrobial assays

The antimicrobial efficacy of the AC@Ag_xAu_y hybrids was evaluated against *Escherichia coli* ATCC 25922, a standard strain of the commonly occurring pathogen. Mid log-phase cultures of the bacterium were prepared by inoculating in tryptic soy broth (TSB) and culturing overnight at 37 °C. The turbidity of the suspension was assayed using a spectrophotometer (Thermo Scientific, Spectronic 20D+, Model 333183) and a turbidometrically standard "0.5 McFarland" solution, which corresponds to a bacterial load of ~1.0×10^8 colony forming units (CFU) of *E. coli* per mL of suspension.[S3] This suspension was serially diluted in TSB to obtain a suspension containing ~1×10^6 CFU mL^{-1} of *E. coli*. To this, an equal amount of suspension containing the prospective antimicrobial agent was added, such that the antimicrobial agent acted on approximately 5×10^5 CFU mL^{-1} of bacteria. Each candidate AC@Ag_xAu_y aminoclay hybrid was prepared in the manner described in Section 4), but scaled-up to thrice the volume used earlier. These nanohybrid materials were then lyophilized for 48 h to obtain freeze-dried solids which were stored at room temperature in the dark until needed. Each hybrid material (~30 mg of AC) was dissolved in 2.1 mL of sterile Millipore water. Prior to use, the stock solution was diluted 5-fold by adding 100 μL of the stock to 400 μL of sterile Millipore water. The final concentration of the prospective antimicrobial agent in the assay was 1.43 mg mL^{-1}. Also

included amongst the samples tested were a *true* control (with no candidate antimicrobial agent present) as well as a control containing only virgin aminoclay (*i.e.*, with no MNPs attached to it).

For completeness, the estimated amounts of Ag in the actual antimicrobial assays were as follows:

AC@Ag: 154 μg mL^{-1} Ag

AC@$Ag_{0.9}Au_{0.1}$: 139 μg mL^{-1} Ag

AC@$Ag_{0.8}Au_{0.2}$: 123 μg mL^{-1} Ag

AC@$Ag_{0.5}Au_{0.5}$: 77.0 μg mL^{-1} Ag

AC@$Ag_{0.3}Au_{0.7}$: 46.2 μg mL^{-1} Ag

AC@$Ag_{0.1}Au_{0.9}$: 15.4 μg mL^{-1} Ag

The mixtures of candidate antimicrobial agents and bacteria were incubated at 37 °C on a nutating mixer (Benchmark Scientific Inc., Serial-1105122) to allow for good mixing at low-shear. After fixed durations (2 and 5 h), 100 μL aliquots of the mixtures were withdrawn and assayed for the number of bacteria remaining alive at that point by serial dilution in PBS and plating 100 μL of the dilute suspension(s) on a tryptic soy agar plate. The number of colonies observed after 24 h yields the number of living bacteria in that (diluted) aliquot, which in turn allows us to estimate the number of living bacteria in the suspension being tested. All these measurements were conducted in triplicate and the mean values and standard deviations are reported in Fig. 7.

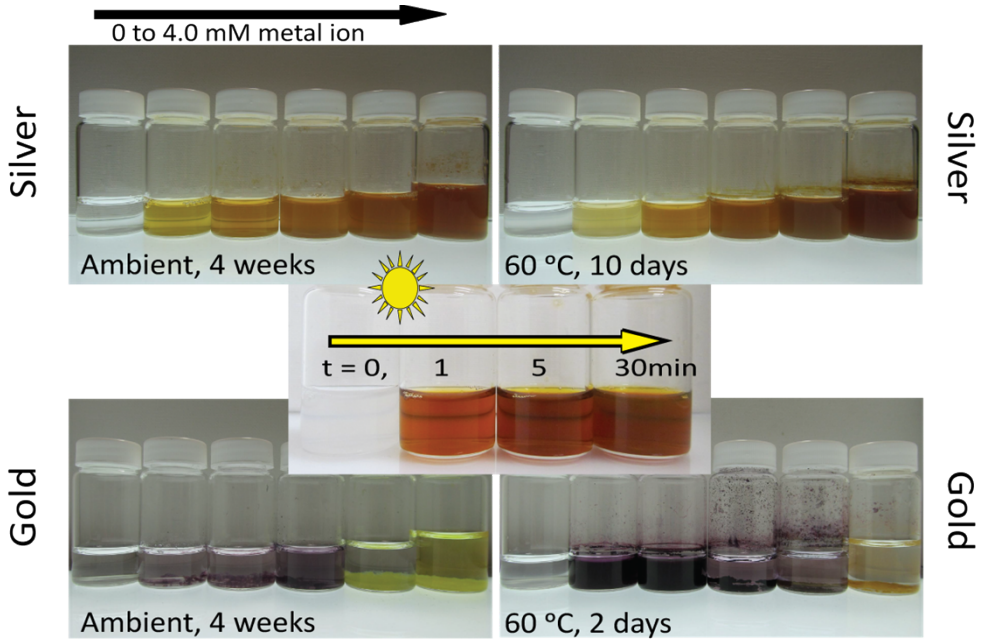

Fig. S1 Directly treating aqueous aminoclay suspensions with metal (M = Ag, Au) salts can generate metal nanoparticle (MNP) decorated aminoclay hybrids, but this either requires several weeks at room temperature (left column; the concentration of the Ag or Au salt mixed with the AC solution increases from left to right) or several days at 40 to 90 °C (right column), yielding heterogeneous NP populations and pronounced agglomeration and precipitation, particularly in the case of Au. On the other hand, photoreduction proved to be a facile, expedient, and less energy-intensive route to stable AC@MNP. In fact, exposure to natural (unfocused) sunlight is sufficient for generating stable plasmonic nanoclays within a few minutes, as illustrated in the central series of images.

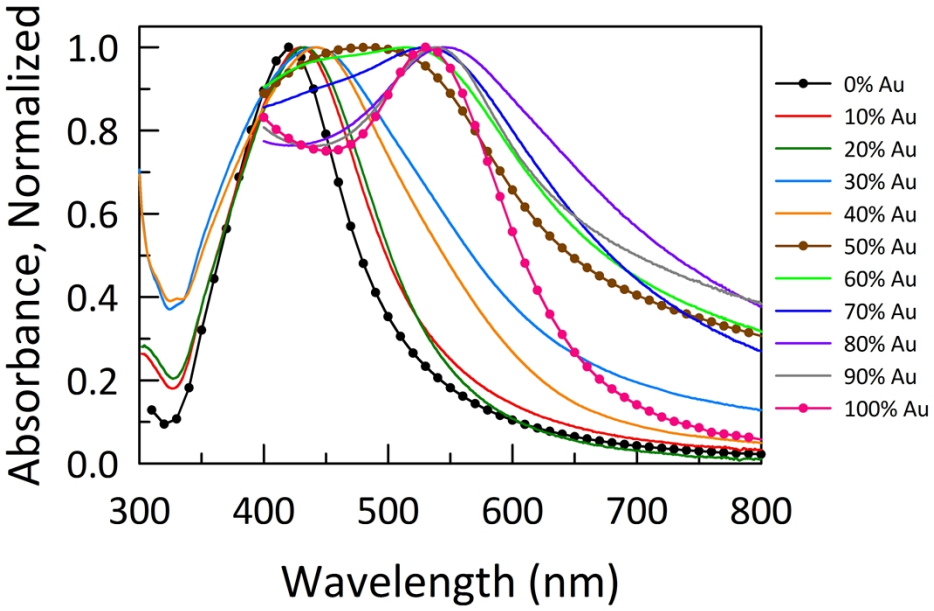

Fig. S2 Normalized absorbance spectra of AC@Ag_xAu_y hybrids generated by controlling the initial Ag:Au ratio.

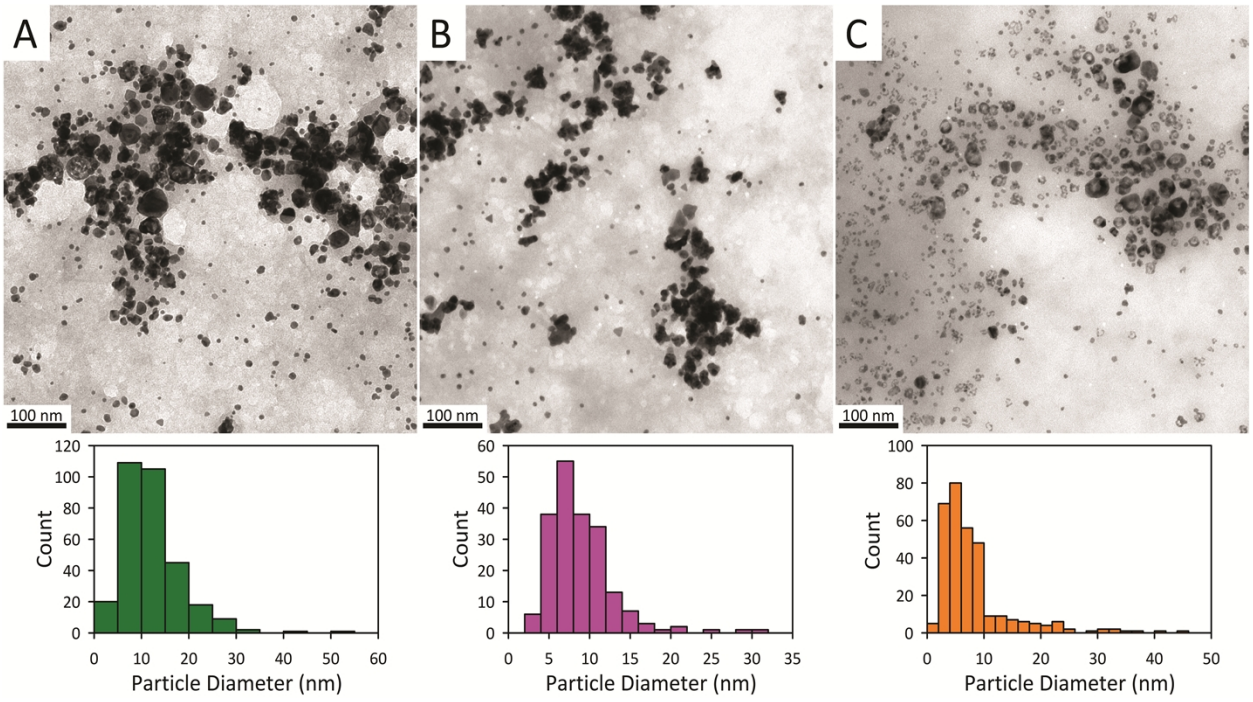

Fig. S3 Representative TEM images of AC@Ag_xAu_y hybrids. (A) AC@$Ag_{0.3}Au_{0.7}$, (B) AC@$Ag_{0.5}Au_{0.5}$ and (C) AC@$Ag_{0.1}Au_{0.9}$ hybrids and their corresponding size distributions.

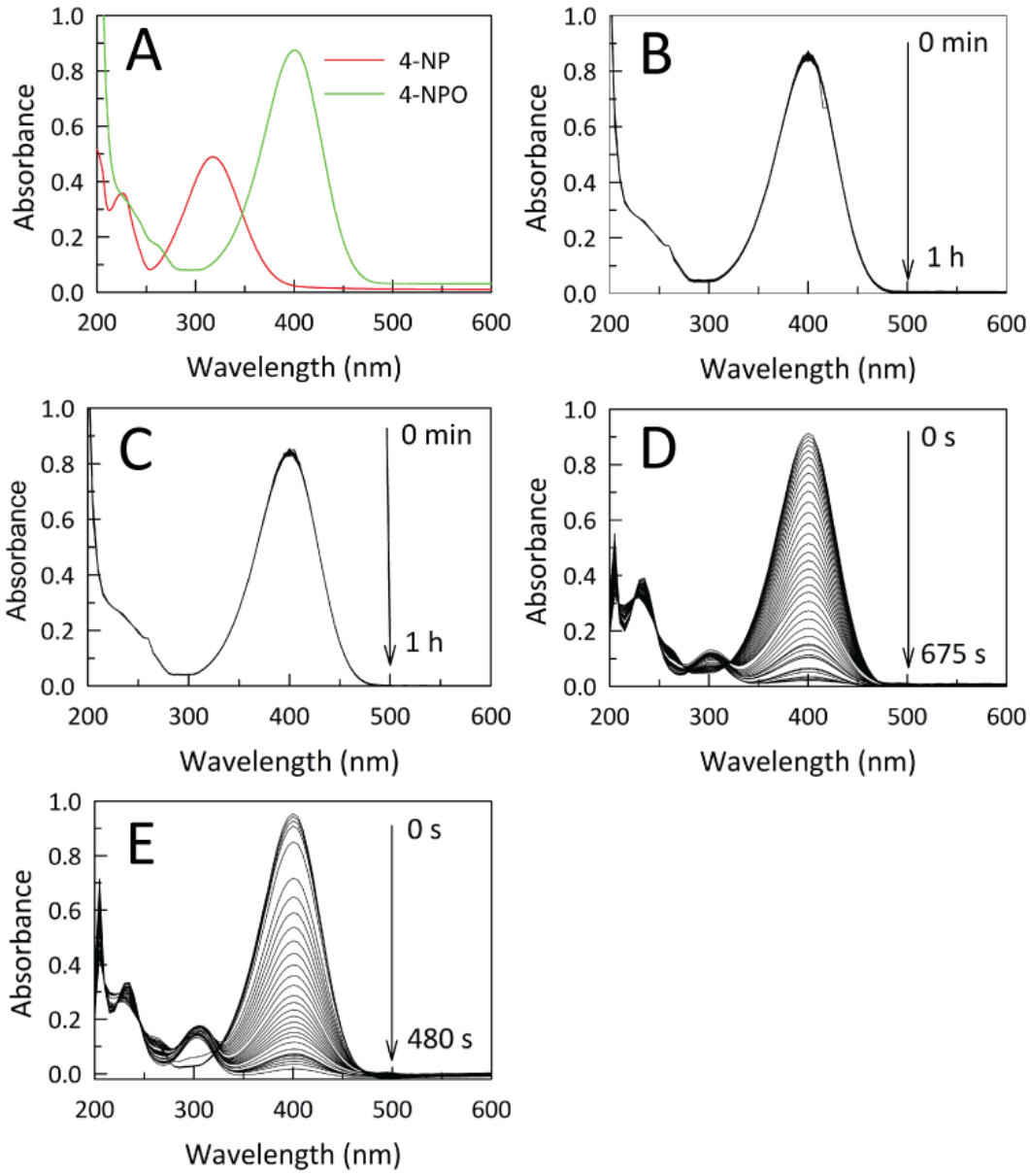

Fig. S4 UV-Vis absorbance spectra of the 4-NP reduction by $NaBH_4$ in the presence or absence of catalyst. (A) UV-Vis absorbance spectra of 4-nitrophenol (4-NP) and 4-nitrophenolate (4-NPO). (B) UV-Vis absorbance spectra of the 4-NP reduction by $NaBH_4$ in the absence of catalyst. UV-Vis absorption spectra of 4-NP reduction by $NaBH_4$ in the presence of (C) naked AC, (D) AC@Ag, and (E) AC@Au hybrids.

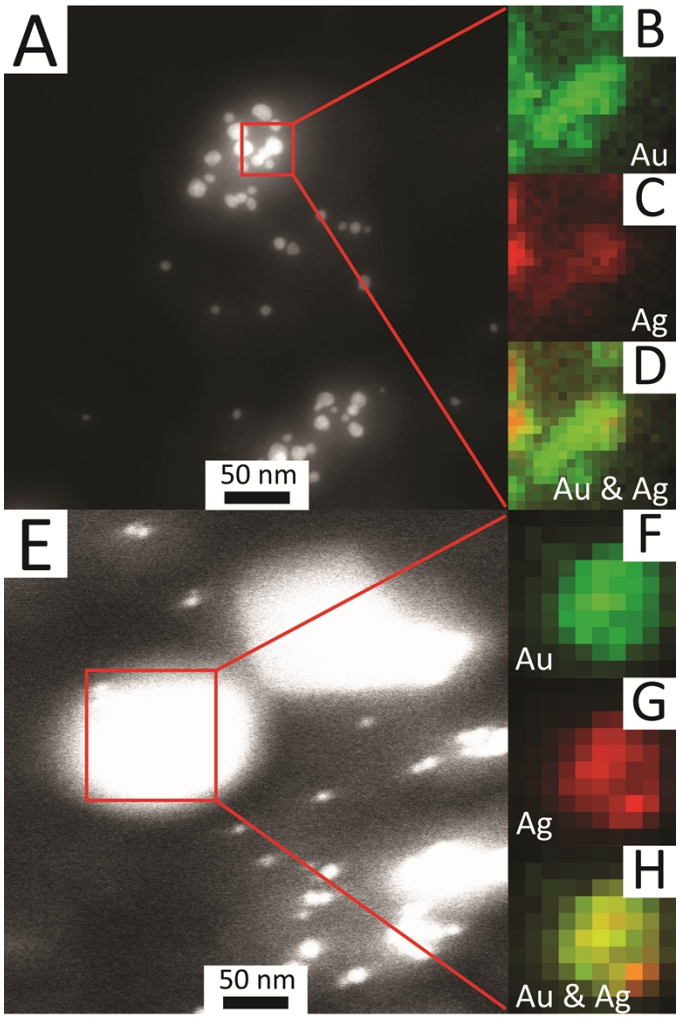

Fig. S5 STEM images of (A) several smaller nanoparticles and (E) a single larger nanoparticle, both within a AC@$Ag_{0.5}Au_{0.5}$ sample. EDX elemental mapping of Au and Ag (B–D, F–H) was performed over the areas indicated by the corresponding red boxes with Au and Ag maps displayed in green and red, respectively. Composite images (D, H) of Au and Ag from the two different areas again show that Au is generally more uniformly dispersed throughout the particles, although this is much more apparent for smaller particles than for large (>20 nm) ones. Maps B–D have a 2 nm resolution with a 5 s dwell and maps F–H have a 10 nm resolution with a 10 s dwell time.

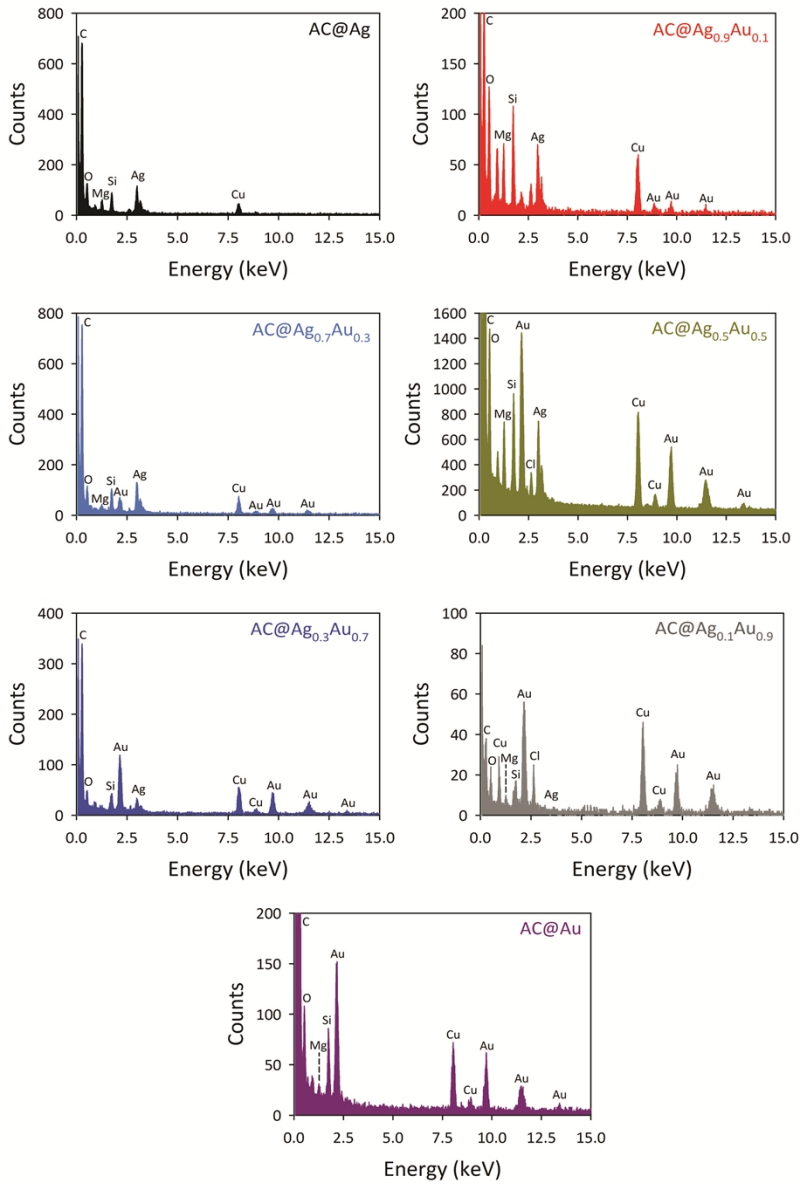

Fig. S6 Representative energy-dispersive X-ray (EDX) microanalysis of AC@Ag_xAu_y nanoclays for x = 1, 0.9, 0.7, 0.5, 0.3, 0.1, and 0. The presence of C and Cu in the EDX spectra is attributed to the carbon-coated copper grids used for the analysis, although signal from the aminopropyl group of the aminoclay is also expected to contribute to the carbon peak.

References

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