Supporting Information

The Role of Protein Characteristics in the Formation and Fluorescence of Au Nanoclusters

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Figure S1 shows the UV-visible absorption spectra of Au nanoclusters from trypsin and lysozyme, where the protein to Au ratio was kept the same. Even thought larger sized clusters were observed from the transmission electron microscopy images (Figure 1d and f), the typical 520 nm absorption of large Au nanoparticles was barely seen.

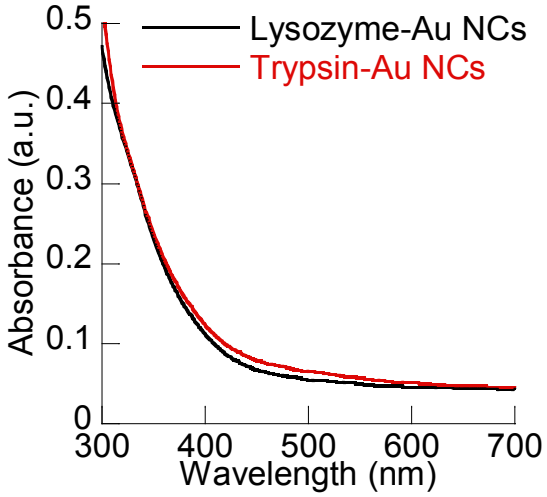

Figure S1. UV-vis spectra of protein-Au nanoclusters generated from trypsin and lysozyme.

Figure S2 shows the UV-visible absorption spectra of Au nanoclusters from pepsin, where the protein to Au ratio was kept 0.04 to 1, the same as other proteins. The typical 520 nm absorption band of Au nanoparticles was clearly observed, indicating the formation of large Au nanoparticles. This observation was also consistent with dark red color (Figure S2b) and the loss of fluorescence of the pepsin-Au solution.

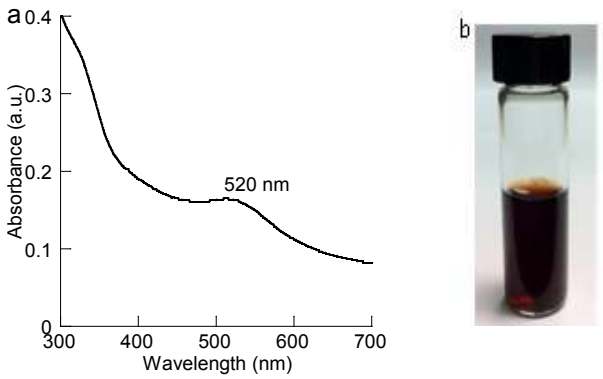

Figure S2. (a) a UV-visible spectrum of pepsin-Au nanostructures, (b) a photo image of pepsin-Au nanoparticles in solution.

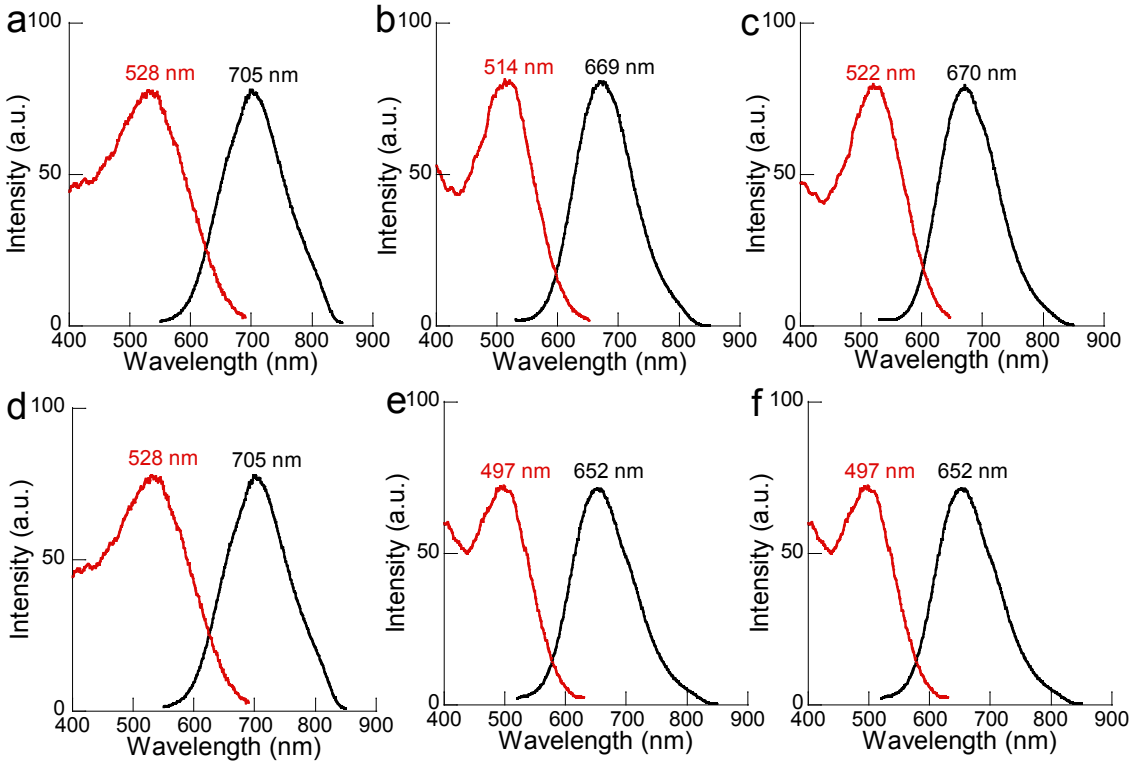

Figure S3. Fluorescent emission and excitation plots of protein –Au nanoclusters generated with the same amine group to Au ratios: (a) BSA, (b) trypsin, and (c) lysozyme; and protein-Au nanoclusters generated with the same tyrosine/tryptophan to Au ratios: (d) BSA, (e) trypsin, and (f) lysozyme.

Figure S3 shows the fluorescent emission/excitation plots of Au nanoclusters generated using the same ratios of amine to Au and trypsin/tryptophan to Au. When the amine functional groups and Au ratios (4:1) were kept the same, the protein-Au nanoclusters showed exact the same fluorescent emission and excitation behaviors as the nanoclusters generated with excess proteins shown in Figure 2. When the amine to Au ratios were kept the same, the proteins to Au ratios turned out to be 0.04 to 1 for BSA, 0.2 to 1 for both trypsin and lysozyme. The ratios for trypsin and lysozyme are much higher than that of the proteins needed to stabilize Au nanoclusters, such as 0.13 to 1 for trypsin and 0.18 to 1 for lysozyme. In contrast, when keeping the ratios of

tyrosine/tryptophan, the reducing power, to Au the same (1:1), we observed the same fluorescent emission and excitation behaviors for BSA protein, but both the fluorescent emission/excitation spectra of trypsin and lysozyme-Au nanoclusters exhibited blue shifts, as shown in Figure S4. This observation was mainly because the lower contents of amine groups in trypsin and lysozyme. At the 1 to 1 reducing power to Au ratio, the proteins to Au ratios were 0.04 to1 for BSA, about 0.06 to 1 for trypsin, and 0.1 to 1 for lysozyme. The ratios of trypsin and lysozyme were lower than the proteins needed to stabilize Au nanoclusters, such as 0.13 to 1 for trypsin and 0.18 to 1 for lysozyme. Therefore a larger blue shift than the excess protein reactions but smaller blue shift than that with the same protein to Au ratios.

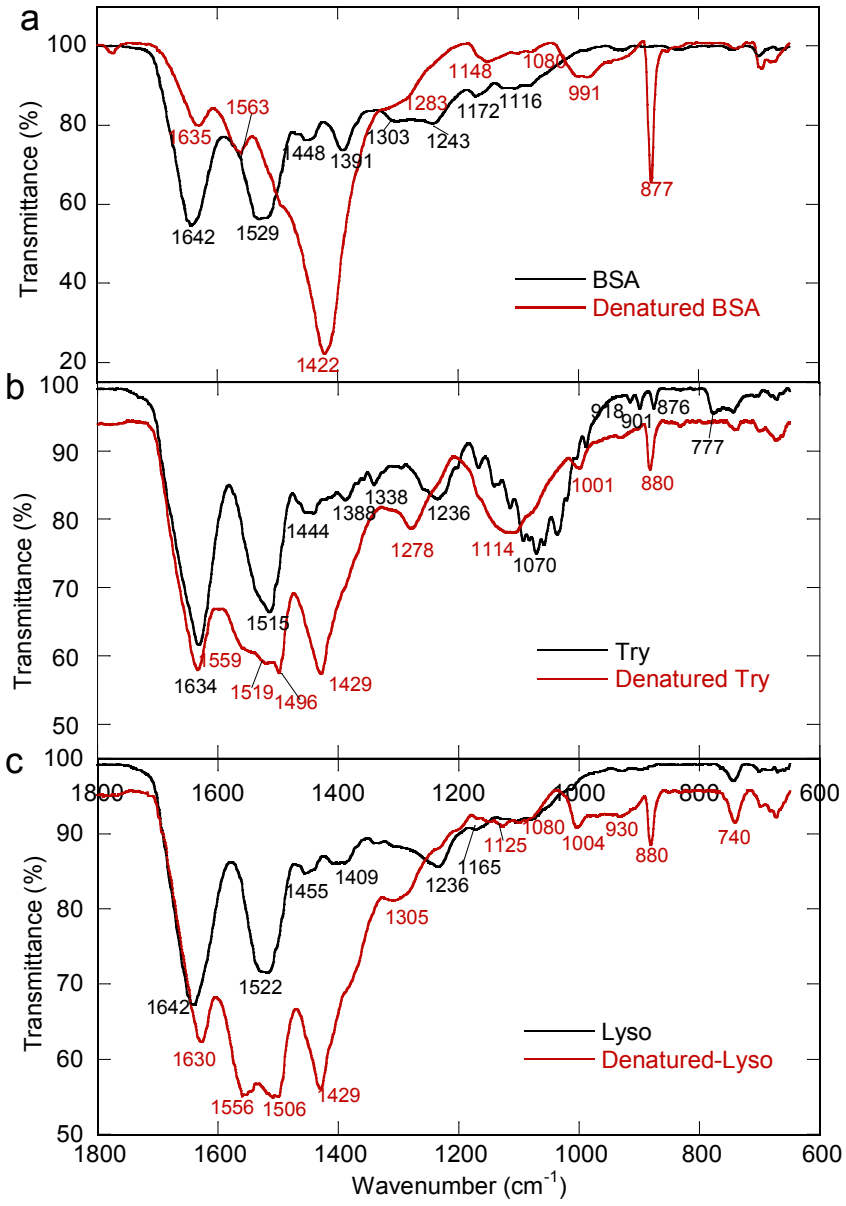

Figure S4. FTIR spectra of proteins and denatured proteins in basic environments: (a) BSA, (b) trypsin, and (c) lysozyme.

Compared with the IR spectra of native proteins, the IR spectra of the denatured proteins mainly showed signal reduction in the amide I and amide II bands. Evident signals of hydrophobic chains also appeared, such as -CH_2 bending and rocking bands at 1422 and 877 cm^{-1} for BSA and 1429 and 880 cm^{-1} for trypsin and lysozyme. Another notable feature was the band shift in amide III regions (1129-1301 cm^{-1}) including -C-N stretching and N-H bending, such as 1243 t0 1308 cm^{-1} for BSA, 1236 to 1278 cm^{-1} for trypsin, and 1236 to 1305 cm^{-1} for lysozyme. Compared to BSA and lysozyme, the IR spectrum of the native trypsin showed the signature peak of tyrosine at 1515 cm^{-1} and a clear shift from 1007 cm^{-1} (phenol-OH) to 1114 cm^{-1} (phenol-O^-) after denature under basic environment along with the Tyr-O^- (stretching vibration of -C-C) at the 1559 cm^{-1} and Try-O^- (-C-H in plane bending) at 1496 cm^{-1}. In contrast, lysozyme exhibited the CH and NH bending of the indole ring at 1506 cm^{-1} along with the out plane mode of indole mode at 740 cm^{-1}.

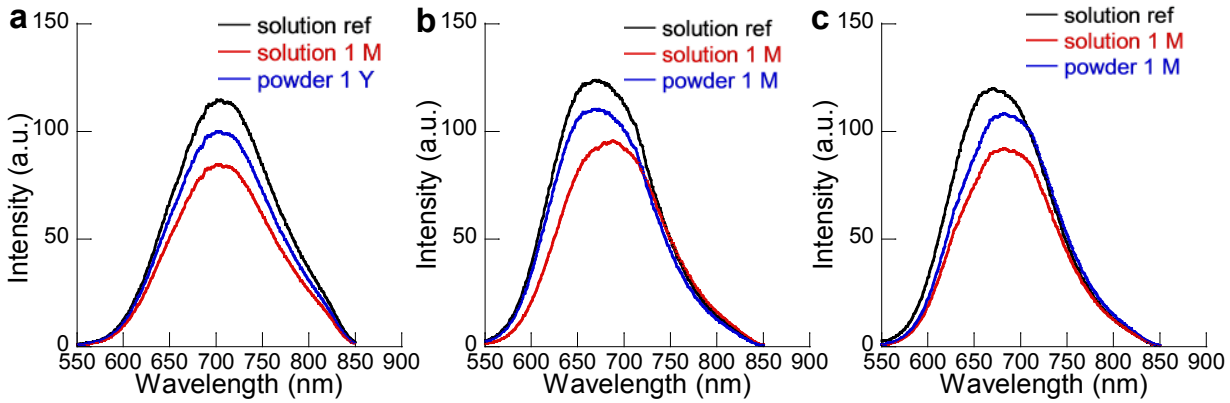

Figure S5. Fluorescent emission plots of protein-Au nanoclusters in solution and powder forms (a) BSA-Au nanoclusters, (b) trypsin-Au nanoclusters, and (c) lysozyme-Au nanoclusters.

The long-term stability of the Au nanoclusters was studied in powder and solution forms. The Au nanoclusters generated from all of the three proteins showed a higher stability in powder form than that in solution. In addition, the protein size is important to the stability of the nanoclusters. BSA encapsulated Au nanoclusters showed great stability in both solution and powder forms; The trypsin-Au nanoclusters was stable in powder from, but some aggregation was observed in solution suggested by the red shift of the fluorescent emission; in contrast, the smallest protein, lysozyme encapsulated Au nanoclusters showed red shift in both powder and solution formed samples (Figure S5).

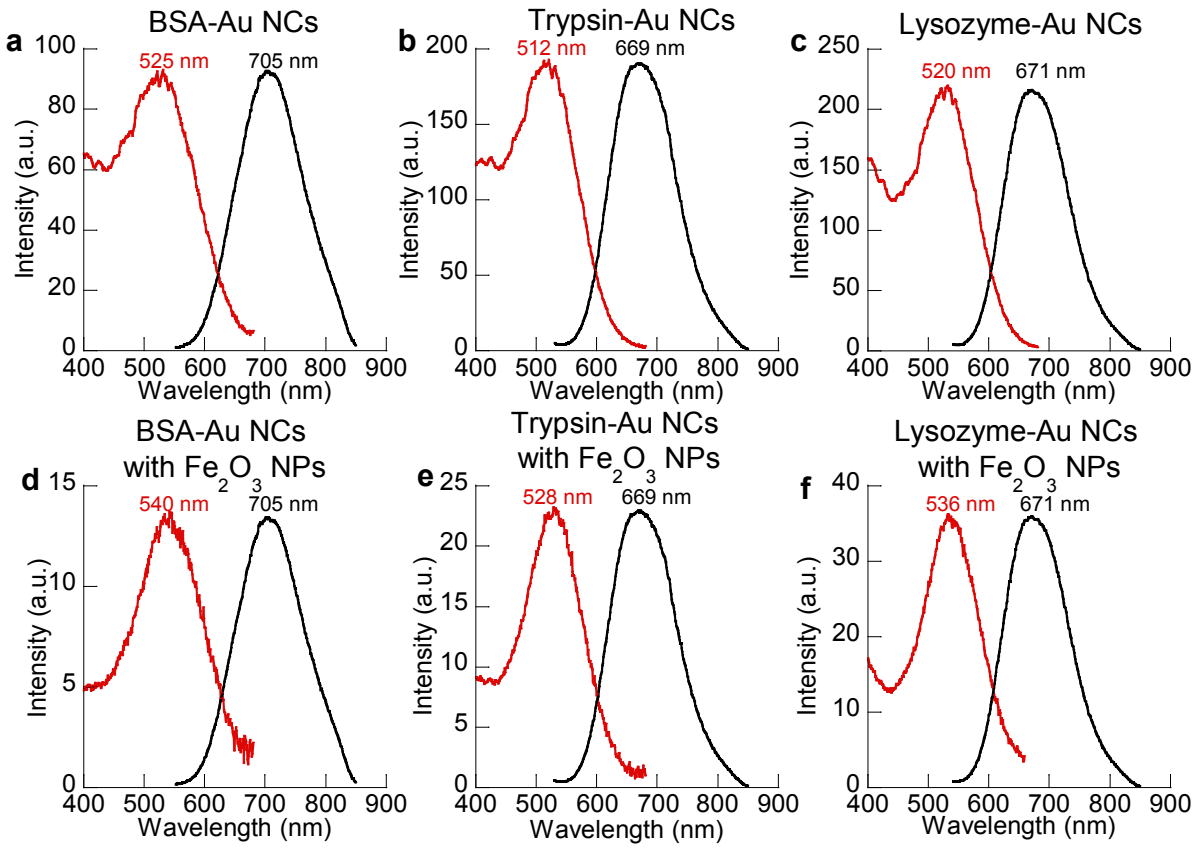

Figure S6. Fluorescent emission/excitation plots of Au nanoclusters before and after addition of iron oxide nanoparticles: (a and d) BSA-Au nanoclusters, (b and e) trypsin-Au nanoclusters, and(c and f) lysozyme-Au nanoclusters.

Figure S6 shows the fluorescent excitation/emission plots of protein-Au nanoclusters before and after the addition of iron oxide nanoparticles. For all these samples, significant intensity decrease was observed due to the absorption of iron oxide nanoparticles, but the emission peak ($\lambda_{em,\,max}$) was not affected. In contrast, the excitation peak was red shift. This observation was mainly because the iron oxide nanoparticles have absorption in the visible range, which interfered with the excitation but not the emission.