Supplementary information

Enhancing Nanoparticle-Based Visible Detection by Controlling the Extent of Aggregation

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Material and methods

Preparation of colloidal streptavidin-coated gold nanoparticles.

Colloidal gold nanoparticles (AuNPs) were prepared following the well-established citrate reduction method[1]. The average particle size (~13 nm) and its size distribution were measured by dynamic laser scattering (DLS) (Fig. S1). The particle morphology was verified by transmission electron microscopy (TEM, JEM1010, JEOL, Japan) and UV-Vis spectra (UV-1700 PC, Shimadzu), whose peak (λ_{max}) appeared at 520 nm. The concentration of AuNPs in solution was estimated based on the absorbance value (0.43 @ 520 nm at 1/10 dilution) as ~7×10^{12} particles/mL[2, 3]. The AuNPs prepared were functionalized with streptavidin via the well-known electrostatic adsorption procedure[4].

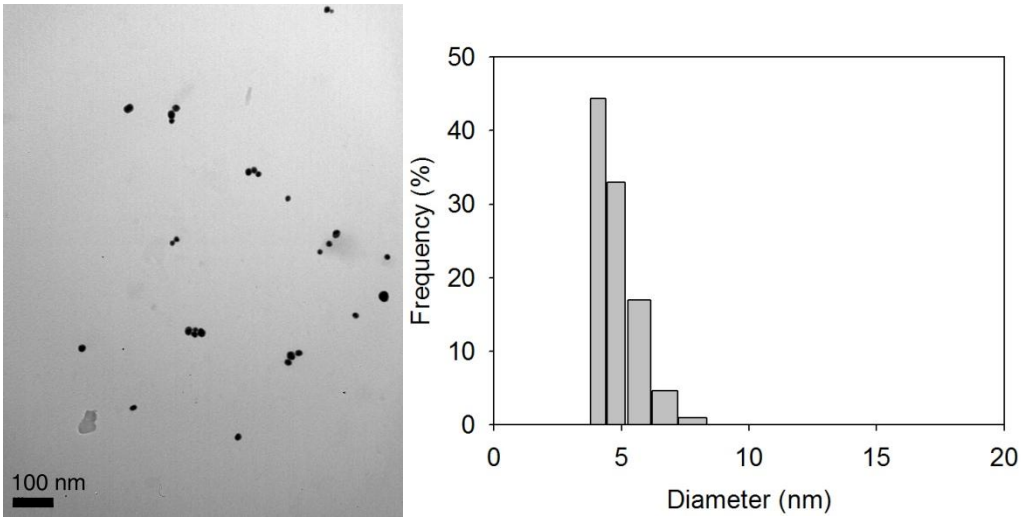

Figure S1. TEM image of AuNPs prepared for streptavidin coating (left) and its mono-disperse size distribution (right).

When AuNPs were coated using several molecules of streptavidin, λ_{max} shifted to appear at 531 nm [5]. However, when the amount of streptavidin used is insufficient to cover entire surface

of the AuNPs, a molecule of streptavidin on the surface will conjugate citrate-stabilized AuNPs (Fig S2a). Thus, when less than 40 μg of streptavidin prepared in 400 μL of borate buffer (pH 7.5) was applied to 600 μL of citrate stabilized AuNPs, the streptavidin helped aggregate AuNPs, as determined from the shift in λ_{max} to higher than 531 nm (Fig. S2b).

While citrate-stabilized AuNPs can experience non-crosslinked aggregation when electrostatic, steric or electrosteric interaction are destabilized. Streptavidin-coated AuNPs (stAuNPs) are highly stable against salt or other ions in solution [6]. Thus, 600 uL of AuNPs samples to which more than 10 μg of streptavidin was applied were stable; they did not undergo non-crosslinked aggregation even when high concentration of sodium chloride (1 M) was added (Fig. S2c and d). Although insufficient amount of streptavidin (10~30 μg) causes the aggregation of AuNPs (600 μL of citrate-stabilized AuNPs), streptavidin covers outer surface of AuNPs adequately to prevent non-crosslinked aggregation. This observation supports our hypothesis that more than certain minimum amount of streptavidin is needed to functionalize a given amount of AuNPs to prevent them from crosslinking via electrostatic adsorption. In our case, at least 40 μg of streptavidin was needed to coat 600 μL of citrate-stabilized AuNPs.

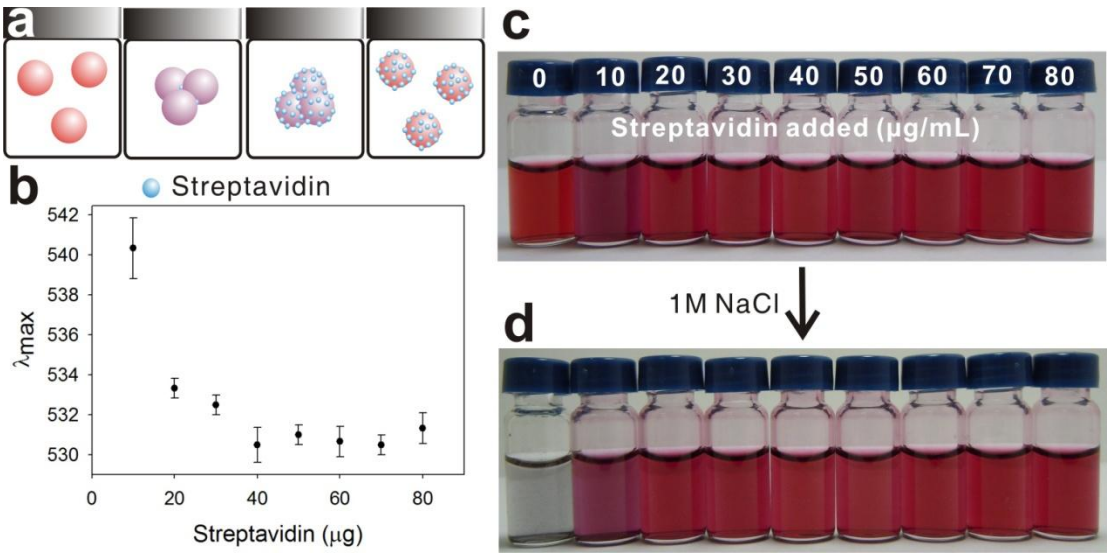

Figure S2. The effect of amount of streptavidin added on the stability of citrate-stabilized AuNPs (approximately 4.2×10^{12} particles). (a) Schematic representation of the amount of streptavidin used to coat the AuNPs (increasing from left to right) and the extent of crosslinking aggregation via electrostatic absorption. (b) Shift of λ_{max} due to possible aggregation by adding different amounts of streptavidin (0 to~80 ug/600 μL of AuNPs in 1 mL of sample). (c) Change in color of colloidal AuNPs by adding streptavidin. (d) Verifying the effect of streptavidin addition in preventing non-crosslinking aggregation of AuNPs by adding 1M of sodium chloride to c.

The effectiveness of streptavidin coating was tested by adding 100 μL (5 μg/mL) of biotinylated BSA (bBSA, Sigma) to 200 μL of stAuNPs prepared with different amounts of streptavidin (30~80 μg/600 μL of AuNPs in 1 mL). Since bBSA was labelled with 8 to 16 moles of biotin per mole of BSA (according to Sigma), a molecule of bBSA can bridge two of stAuNPs. However, some molecules of streptavidin, which are not absorbed on the AuNPs, remain in the system screening some bBSAs from crosslinking with stAuNPs. Therefore, stAuNPs did not aggregate in the samples prepared with more than 50 μg of streptavidin (Fig. S3). Even the extent of aggregation in the samples prepared with 30 and 40 μg of streptavidin (Fig. S3a) was not as large as that was induced after removing the surplus streptavidin by decanting the

supernatant following centrifugation and restoring in PBS (Fig. S3c). For the experiments reported, we prepared stAuNPs using 40 μg of streptavidin per 600 μL of citrate-stabilized AuNPs and restored in BSA dissolved in PBS (0.1% w/v) after washing to have three different concentrations that yielded absorption values of 0.21, 0.43 and 0.86 at 531 nm, respectively .

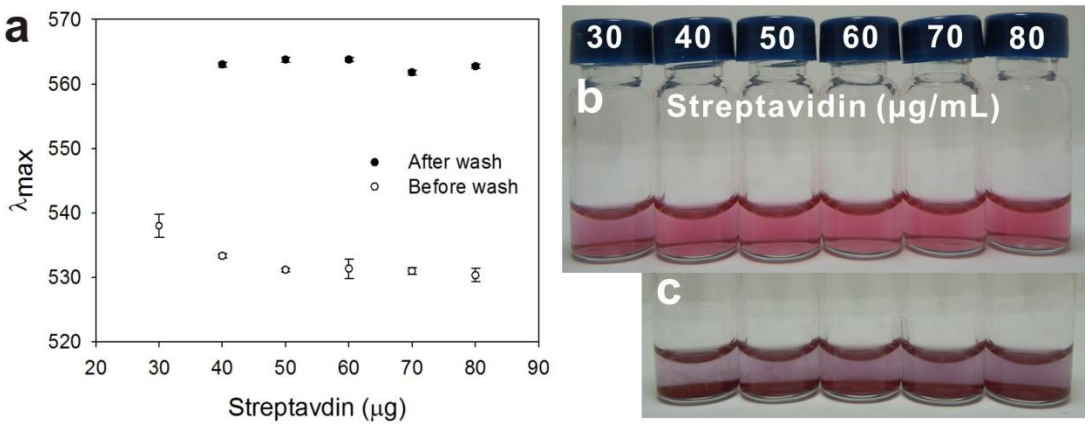

Figure S3. Effect of free streptavidin on the aggregation of stAuNPs induced by adding bBSA as the linker. (a) Shifting of λ_{max}, which indicates possible aggregation of stAuNPs, in stAuNPs solution prepared with different amounts of streptavidin. (b) samples before washing-off the unbound streptavidin show little or no aggregation. (c) color change in samples after washing-off the unbound streptavidin indicates aggregation is occurring.

stAuNPs are highly stable, and even can be stored in dehydrated form by lyophilization without losing its activity for weeks (Fig S4).

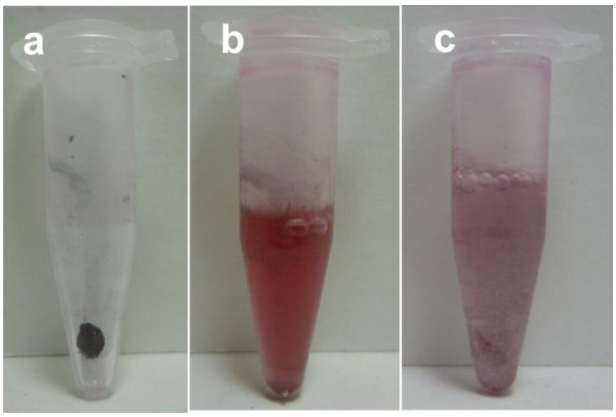

Figure S4. Stable dehydrated form of stAuNPs. (a) Dehydrated form of stAuNPs (600 μL, absorption: 0.43 @531) via lyophilization. (b) stAuNPs restored by adding 600 μL of distilled water to a. (c) Streptavidin activity in restored stAuNPs was verified by adding 1 μg of biotinlyated BSA in100 μL of b, which caused aggregation.

The extent of stAuNPs aggregation: as a function of time and the amount of bBSA, the linker.

When the aggregation of a given amount of stAuNPs is facilitated by adding bBSA, the extent of aggregation increases with time at a rate depending on the amount of bBSA added (Fig. S5). Accordingly, the color of the colloidal stAuNPs changes from red to purple with time; but after a prolonged duration as the aggregated particles settle down, the color disappears and the solution become clear (Fig. S5a). As the extent of aggregation increases, the peaks of UV-Vis spectra shift to higher wavelengths and the absorption value at 700 nm increases, (Fig. S5b). However, there is a range of bBSA concentration, which leads to an appropriate extent of aggregation producing visually distinguishable color change. When experiments are performed without disturbing the sample, as we had done for tests reported in this manuscript, the color change becomes noticeable within 30 min of reaction time, with the rate of color change significantly slowing down after about two hours. However, when the samples were agitated during the reaction time, the color change is perceptible within 10 min.

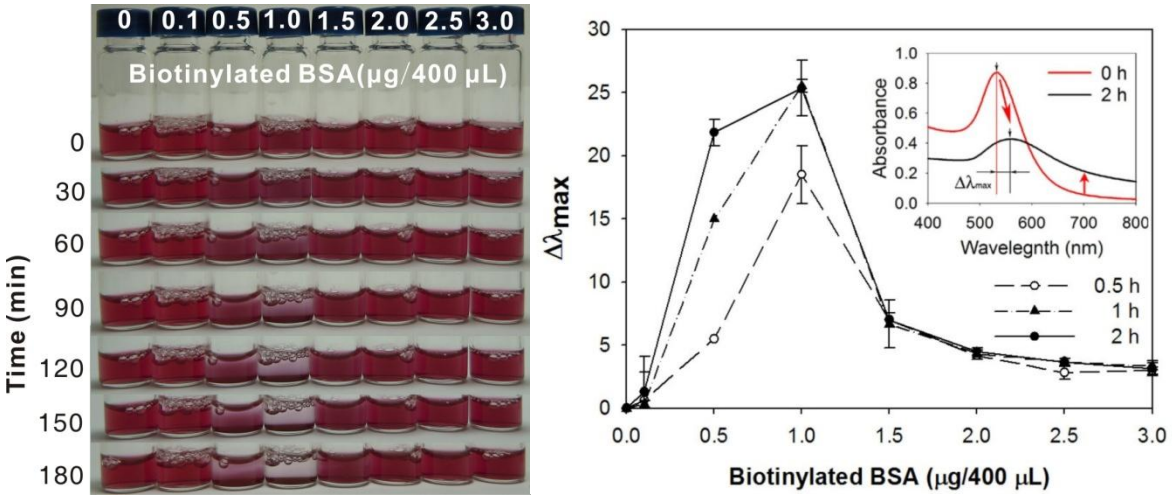

Figure S5. (a) Color of the colloidal suspensions of AuNPs with different amounts of bBSA (0 to 3 ug/400 uL) as a function of time (b) Shift in the peak wavelength ($\Delta\lambda_{max}$) of UV-Vis spectra of colloidal AuNPs with different amounts of bBSA (0 to 3 ug/400 uL) as a function of time. (Inset) UV-Vis spectra at the beginning (red line) and after 2 h (black line) since adding stAuNPs. The red arrows indicate a peak shift at ~550 nm and an increase in the absorbance at 700 nm as the extent of aggregation increased with time.

Quantitative relationship between stAuNPs and bBSA on proper extent of aggregation for producing visible color change.

For a given amount of functionalized AuNPs in a certain overall volume, the concentration of linker determines the extent of aggregation. Thus, the range of linker concentration exhibiting visible change (REVC) varies with the amount of stAuNPs and bBSA in the system (Fig. S6). The larger amount of NPs present, the larger amount of bBSA is needed to sufficiently aggregate NPs and produce a visible change at low end of REVC. At the high end of REVC, the color change does not happen because stAuNPs are completely covered by bBSA. Therefore, the amount of bBSA needed at the high end of REVC is a lot more than what is necessary at the low end Therefore, the span of REVC widen with increasing concentration of stAuNPs as shown in

Fig S6. Therefore, when using low concentration of stAuNPs, at the low end of REVC, the amount of linker required for producing a large extent of aggregation is small. Thus, relatively speaking, the system is more sensitive – aggregation is caused or prevented with only a small change in linker concentration – when it contains lower concentration of AuNPs.

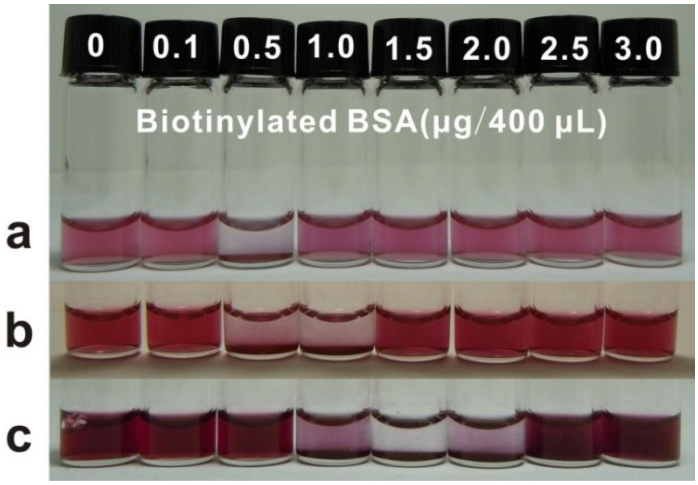

Figure S6. The span of REVC varies with the concentration of stAuNPs. Test systems a, b, and c were by 1x, 2x, and 4x concentration, prepared to yield absorption values, at 1/10 dilution, of 0.21, 0.43 and 0.86 (@531±0.5 nm), respectively.

Determination of reaction time for target streptavidin to crosslink with bBSA.

Since streptavidin is a tetramer, several molecules of streptavidin possibly present in the sample can crosslink with bBSAs to compose a complex, which may still bridge stAuNPs. Thus, the number of linkers available to bridge stAuNPs (nLK) should decrease as the extent of aggregation between bBSAs and target streptavidin increases. Since an increase in the extent of aggregation is a function of time, nLK should also decrease with reaction time. When the aggregation of stAuNPs was facilitated in a series of tests over varying reaction times, the REVC appeared at higher concentration of bBSA at longer reaction times in the presence of a given amount of streptavidin (5 ug) (Fig. S7). Although the REVC shifted to higher concentrations of bBSA in proportion to longer reaction times, longer than 60 min was ineffective on the shift of REVC. From the observation, even large amount of streptavidin in the sample can achieve the

maximum extent of aggregation to reduce nLK within 60 min. Therefore, we set the reaction time for the sample and SL as 60 min.

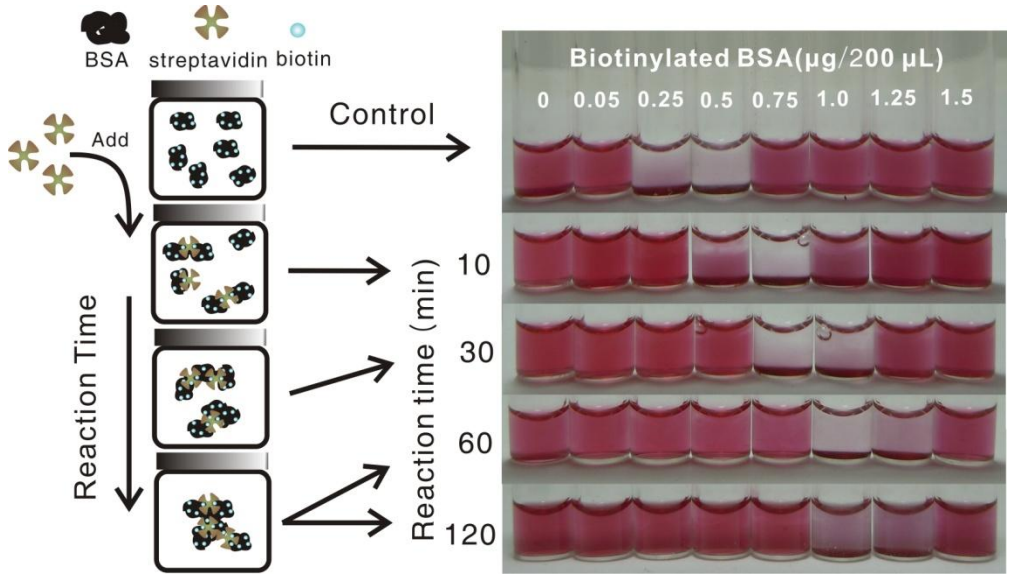

Figure S7. Shift in REVC with increasing reaction time between bBSA and the presence of 5 ug of target streptavidin for 120 min since adding 100 uL of stAuNPs (absorption: 0.43 @ 531±0.5 nm) in 200 uL total test system volume. As the reaction time progresses, REVC appeared at higher concentration of bBSA, which indicates nLK reduced according to increased extent of aggregation between bBSA and the target streptavidin.

Confirmation of the specific attachment of AuNPs via immunoreaction influencing the color of the colloidal system

The effect of covering bacteria cell surface with AuNPs, a feature of aggregation, on the color of colloidal nanoparticle was verified by attaching the prepared stAuNPs on *Escherichia coli* cell surface using biotinylated polyclonal anti-*E.coli* antibody (b-Ab, GTX40640, Genetex). Excess b-Abs (at 100 μg/100 μL) was promptly applied to *E.coli* cells prepared in PBS at various concentrations (from about 2×10^{2} to 2×10^{11} CFU/mL), which was verified via plate culture method using Petrifilm™ selective count plate (3M). After 30 min of reaction time under shaking for b-Abs to bind antigens on the cell surface, any unbound b-Abs still present were

removed by several rounds of washing process. Washing process was performed by removing the supernatant following light centrifugation to sink down the cells. Bacteria cells, whose antigens fully bound b-Abs, were restored in filtered PBS at initial concentrations, which were also verified using Petrifilm. 200 μL of stAuNPs (abs: 0.43 @531) was added to 200 μL of the prepared cell solution, whose concentration was diluted by one-half via mixing 100 μL of PBS and 100 μL of the prepared cell solution. Even when 10^{11} CFU cells were present to attach stAuNPs on the cell surface, visible color change did not occur (Fig. S8a). Since it is possible that the ratio of attached stAuNPs was too low to produce visible change in color of the overall system, the experiment was repeated at half-scale, but with the same cell load. The effect of attachment on the color of system was verified by comparing the test performed with b-Abs applied cell that with naked cell. When stAuNPs were attached, the color of system became lighter and purplish as shown in Fig. S8b. However, such difference can hardly found when using low cell concentration ($< 10^6$ CFU/ml). As expected, when both tests with cells with and without stAuNPs attached via b-Abs promptly bound to antigens, were settled down by light centrifugation, the color of supernatant in both systems are hardly distinguishable (Fig. S8c). The attachment of stAuNPs on the cell surface was verified by examining the color of the centrifuged sediment. Color of sediment should be dark red due to the agglomeration of AuNPs being attached to bacteria cell by light centrifugation, by which unbound AuNPs could not sink, while it was sufficient to sink the bacteria cell (Fig S8c and d). By suspending the sediments obtained in PBS, the color of the suspension exhibits light red, not purple, enough to be distinguished with naked eye (Fig. S8e). If the change in surface plasmon resonance can be induced according to the agglomeration of AuNPs via attaching on bacteria cell surface, the color of re-suspended solution should be purplish that could not be observed in the performed test. Consequently, specific

attachment of AuNPs on bacterial cell surface is hardly expected to induce visually distinguishable change in color of overall colloidal system.

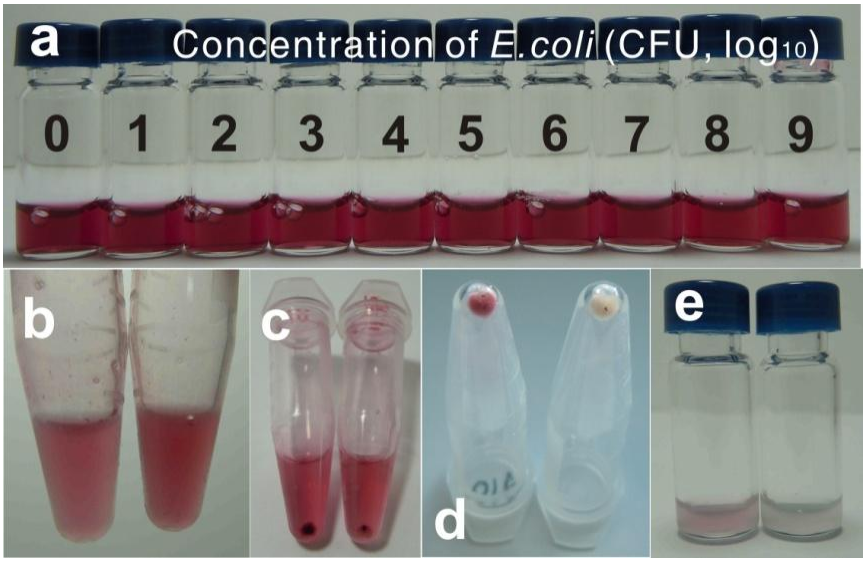

Figure S8. Confirmation and the effect of AuNPs attachment on cell surface on the color of colloidal system. (a) A series of tests containing increasing amount of *E.coli* from 0 to 10^9 CFU (colony forming unit), whose antigens readily bind biotinlayted antibody (b-Ab), were performed with the same concentration of stAuNPs (200 uL of stAuNPs in 400 ul of total volume). (b) Amplified effect of attachment on the color of systems by applying one half amount of AuNPs to b-Ab pretreated bacteria sample (left) and naked bacteria sample (right) both at 10^9 CFU. (c) Centrifuged samples for verifying the attachment of stAuNPs on cell surface with b-Ab pretreated bacteria sample (left) and with naked bacteria sample (right). Distinguishable red color of the sediment in b-Ab pretreated bacteria sample confirms the attachment of stAuNPs. However, the color of the supernatants in both the samples is indistinguishable revealing that the portion of attached stAuNPs is too small to produce a visible change, even at a high cell load (10^9 CFU in 400 μL). (d) The color of sediments after removing the unbound stAuNPs. (e) The color of re-suspended sediments in 200 μL of PBS.

References

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