**A THESIS FOR THE DEGREE OF MASTER OF SCIENCE**

# Study on system parameters of gold nanoparticle aggregation-based visible detection system

금나노 입자의 응집을 이용한 시각적 검출 기작 방법의 주요 특성에 대한 분석

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# Abstract

There has been a lot of research pursuing an easy, rapid and sensitive detection method for pathogenic microorganisms, proteins and chemicals. However, many methods have their own problems such as complicated sample preparation, low sensitivity, and complex procedure often demanding trained experts or sophisticated instruments. In the preceding studies, a rapid and simple detection method using gold nanoparticle (AuNP) was developed. This method has two steps for detection. First, the target reacts with switchable linker (SL). Second, After SL reacting with the target, add streptavidin coated AuNP (stAuNP) to the mixture. By biotin-streptavidin binding force, biotins on the SL connect with the streptavidin on the stAuNPs. The aspect of aggregation is observed differently by the concentration of SL. In the low concentration of SL, the number of SL is not enough for forming aggregation after reacting with the targets. In the high concentration, the number of remaining SL after reacting with target is too many. These many SL covers the stAuNPs, so they cannot exhibit their bridging ability for making aggregation. If this quantitative relationship is appropriate for making aggregation, we can observed with naked eye. At this time, the key factors of the former detection system were studied for

giving background information on further improvement of the system. The effect of the size of AuNPs on the range exhibiting a visible color change (REVC) and reaction time was investigated. The bigger the size of AuNPs REVC region moved to high concentration of SL and took more time for making enough aggregation. Furthermore, how the number of biotins on the SL and activity of antibody affect the REVC and aggregation appearance was studied. The more biotins binds on the single SL, the less SL is needed for making same amount of aggregation. Lastly, the impact of the environment (pH and salts existence) on the stability of the system was evaluated. 1M NaCl doesn't affect the stability of the system. In the pH 6-8, the detection system works well. However, in the pH 5 and pH 9, the time for making enough aggregation took much longer than the control. In the real application, the conditions are very different according to what kinds of foods or ingredients make use of. Thus, the result of this study could be used as valuable references for improving and applying this detection system.

Keywords: biosensor, size-growth of gold nanoparticles, aggregation, biotinylation, visible detection

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# I. Introduction

There had always been great needs for rapid and accurate detection methods for food. As food system has many safety risk factors by microbial, chemical and physical causes, a rapid detection system can benefit various fields of food industry. Pathogenic microorganisms have made a lot of trouble directly connected to lives of people. According to the Center for Disease Control and Prevention, each year 1 in 6 Americans get sick due to food poisoning associated with notorious pathogens such as *Escherichia coli* O157:H7, *Salmonella* typhimurium, *Salmonella* enteritidis, and *Listeria* monocytogenes. These pathogens have also been responsible for most cases of hospitalization and deaths arising from foodborne illness (1). Also, many allergens are a big problem. This type of allergy, which affects approximately 1% of the population worldwide, persists in 80% of sensitized individuals, and for many of them, contact with even very small amounts of the allergens is sufficient to elicit mild reactions (e.g., anaphylaxis) (2-3). Moreover, melamine was also a big problem about adulteration of foods (4).

Nowadays, colorimetric detection methods which can verify result with naked-eye got interest due to its simplicity (5-7) and these methods did not require sophisticated detection equipment or techniques (8-9). Among these

visible detection principles, nanoparticle (NP)-based indicating methods have been widely used (10-13) in recent years.

Among metal nanoparticles, gold nanoparticles specially have been received remarkable attention because of their unique and interesting properties. Using these properties they can be used as potential technological application (14-15). Bulk size of gold has literally golden color caused by a reduction of reflectivity (16). When the particles of gold are small enough, their color becomes ruby red due to their strong absorption of green light at about 520 nm, corresponding to the frequency at which a plasmon resonance occurs with the AuNPs. AuNPs exhibit several interesting changes in their physical and chemical properties, especially their distance-dependent optical property. AuNP solutions show a particular color due to the collective oscillations of the surface electrons induced by visible light of suitable wavelength, which is highly dependent on the interparticle distance (17, 18). The key to the AuNPs-based colorimetric sensing platform is the control of the colloidal AuNP dispersion and aggregation stages with a biological process (or sometimes a chemical process) of interest.

The aggregation of stable colloidal AuNPs has been interested to produce visible signal due to such advantages as fast, simple and easy to develop detection system (19). For this reason, previous studies for naked-

eye AuNP based detection system, signal-based amplification strategy introducing switchable linker (SL) to mediate aggregation of AuNPs. The SL is an element allowing multiple bindings, which can be selectively enabled or disabled. When the mechanism for producing indication signal is independent from that for target recognition, it affords an opportunity to amplify the signal during its transmission. This transducing the signal transmitted from target recognition into visible indication based on aggregation of AuNPs was accomplished. Thus, In addition to being highly sensitive, this strategy allows designing detection systems regardless of any limiting target characteristics.

Here, an enhancing background knowledge for this system through controlling size of particle size, the amount of biotins binding on the single antibody and environment tolerance about pH and salts conditions was reported. The aggregation region of the larger particle was different from that of the smaller particle. Gold nanoparticles were made by kinetically controlled seeded growth synthesis of citrate-stabilized gold nanoparticles (20). Using biotinylated BSA (Bovine Serum Albumin) as SL, 3 different sizes of AuNPs (519.5 nm, 524 nm and 533 nm) were tested. The different size of AuNPs showed different colors one another, which is already discussed. REVC region moved to the high concentration of the SL as the

particle size grows. The time for making enough aggregation was also taken long because of the number of enough aggregation took long time for bigger size of AuNPs. Moreover, the amount of biotin of the antibody and its activity have a critical impact on the detection system. The number of biotins was very critical for making enough aggregation. This properties can be adjusted to the target.

# II. Materials and methods

## 2.1 Materials

Gold(Ⅲ) chloride trihydrate (HAuCl4·3H2O), bovine serum albumin (BSA), Polyclonal biotin-conjugated BSA and streptavidin (from Streptomyces avidinii) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tri-sodium citrate was purchased from Yakuri pure chemical Co., Ltd. (Osaka, Japan). Tetraborate pH standard solution was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Phosphate buffered saline (PBS) was purchased from GIBCO (Gaithersburg, MD, USA). Pearl™ antibody clean up kit and HOOK™ were purchased from G-biosciences (St. Louis, MO, USA). *Salmonella* Typhimurium strain ATCC 19585 was thankfully provided by Food Safety Engineering Laboratory in Seoul National University. Pierce™ *Salmonella* Antibody, Biotin conjugate PA1-79022 and Pierce™ *Salmonella* Antibody PA1-7244 were purchased from Thermofisher (Austin, TX, US). Tryptic soy broth (TSB; MB Cell, Seoul, Korea). Lysine desoxycholate agar (XLD; OXOID CM0469, Basingstroke, UK ) were used for this experiment.

## 2.2 Instrumentation

Extinction measurements were performed on a UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). The pH of all the buffer solutions was examined with a pH Benchtop Meter PP-15 (Satorious, Bohemia, NY, USA). Transmission electron microscopic (TEM) pictures were obtained on a JEM1010 80 kV transmission electron microscope (JEOL, Tokyo, Japan).

## 2.3 Synthesis of raw gold nanoparticles

Aqueous gold nanoparticles of 13 nm diameter were synthesized via previous study and colloidal gold was synthesized by reduction of a HAuCl4·3H2O aqueous solution by sodium citrate at ebullition as previously reported. Deionized water (DI water) was used in all the experimental steps. In a Erlenmeyer flask equipped with a hot plate, 1 mL of 10 mM HAuCl4·3H2O and 9 mL of DI water was brought to a rolling boil. One mL of 1% (w/v) citrate sodium was added to 10 mL boiling solution of 1 mM HAuCl4·3H2O. The solution turned dark brown within 10 s; the final color change to burgundy occurred 50 s later. The solution was cooled down to the

room temperature and a red solution of gold nanoparticles was obtained. The resulting solution of colloidal particles was characterized by an absorbance is 0.42 and wavelength is 520±0.5 nm and fixed this condition. Transmission electron microscopy (TEM) indicated a particle size of 13 nm ± 3 nm.

## 2.4 Kinetically controlled seeded growth synthesis of citrate-stabilized AuNPs.

A solution of 2.2 mM sodium citrate in diluted water (150 mL) was heated with a heating mantle in a 250 mL three-necked round-bottomed flask for 15 min under vigorous stirring. A condenser was utilized to prevent the evaporation of the solvent. After boiling had commenced, 1 mL of HAuCl4 (25 mM) was injected. The color of the solution changed from yellow to bluish gray and then to soft pink in 10 min. The resulting particles (～10 nm, ～$3 \times 10^{12}$ NPs/mL) are coated with negatively charged citrate ions and hence are well suspended in $H_2O$. Immediately after the synthesis of the Au seeds and in the same vessel, the reaction was cooled until the temperature of the solution reached 90°C. Then, 1 mL of sodium citrate (60 mM) and 1 mL of a $HAuCl_4$ solution (25 mM) were sequentially injected (time delay ～2 min). After 30 min, aliquots of 2 mL were extracted for further characterization by

transmission electron microscopy (TEM) and UV-vis spectroscopy. By repeating this process (sequential addition of 1 mL of 60 mM sodium citrate and 1 mL of 25 mM $HAuCl_4$), up to 14 generations of gold particles of progressively larger sizes were grown. The concentration of each generation of NPs was approximately the same as the original seed particles ($\sim 3\times10^{12}$ NPs/mL).

## 2.5 Concept of detection principle

One way to implement SL for achieving detection is by designing an entity that can perform two functions, conjugate two labelled AuNPs and bind to the target selectively, and creating a condition at which only one of the two functions is switched on (Fig. 1). This can be accomplished using a specific conjugation (e.g., biotin-streptavidin, immunoreaction) in conjunction with a receptor molecule such as antibody, aptamer, or oligonucleotides. The conditions for signal amplification or switching functions are readily achieved, because the size of the receptor molecules is vastly different from that of AuNPs. For the SL designed to function as intended, the assay should be performed in two sequential steps. First mix SL with the sample to avail the function of SL to bind to the target for target

recognition and then add labelled AuNPs to avail the function of SL to mediate the aggregation of AuNPs for producing indication signal – a visible color change. However, when our SL binds to the target, it can no longer conjugate two AuNPs

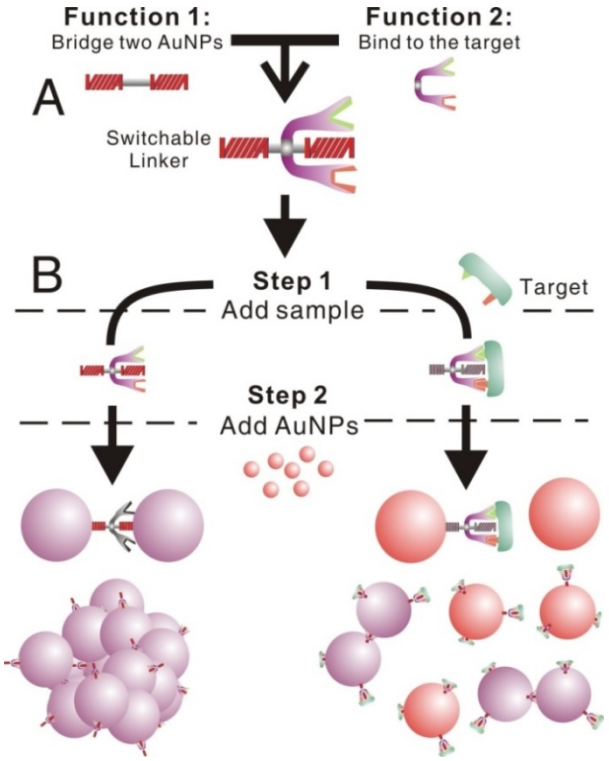



Fig. 1. Schematic representation of a design of switchable linker (SL), and whose role in two-step detection assay. (A) An SL with the design to perform two functions: conjugate two labeled gold nanoparticles (AuNPs) and bind to the target selectively. But only one of those two functions is enabled according to switching conditions such as the relative size of AuNPs and hindrance of binding sites of target to that for AuNPs. (B) Two-step assay for SL to function as intended. The sample was first reacted with the SL (Step 1) for determining one of two functions and then with labeled AuNPs (Step 2) for signal generation.

Thus, the amount of SL that can mediate aggregation of AuNPs determines

the extent of aggregation and the accompanying color change. Hence, signal production indicates change in the SL's ability to mediate AuNPs aggregation, which correlates to the amount of target in the sample.

## 2.6 Preparation of streptavidin coated gold nanoparticles

Streptavidin-coated gold nanoparticles (stAuNPs) were prepared according to modifications of study. Six hundreds µLµL of colloidal gold nanoparticles (wavelength: 520±0.5 nm abs.: 0.417) were added 400 µLµL of borate buffer (pH 7.4) and 200 µL of streptavidin (0.2 mg/mL in borate buffer (pH 7.4)). The solution of mixture was incubated at room temperature and centrifuged at 9,358×g (10,000 rpm) for 30 min several round to remove extra streptavidin. Streptavidin can be capped on the anionic-charged gold nanoparticles by strong electrostatic adsorption.

## 2.7 Detection of *Salmonella* Typhimurium via immunoreaction

Colloidal nanoparticle was verified by attaching the prepared stAuNPs on *Salmonella* typhimurium antibody (ThermoFisher PA1-73022). Excess b-

Abs (at 100 μg/100 μL) was promptly applied to E.coli cells prepared in PBS at various concentrations (from about $2\times10^2$ to $2\times10^{11}$ CFU/mL), which was verified via plate culture method using XLD (Xylose Lysine Deoxycolate) agar (Difco). 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

## 2.8 Purification of antibody

Purified antibody of melamine was prepared according to antibody cleaning up by using Pearl™ antibody clean up kit. Antibodies are routinely stored in buffers containing bovine serum albumin that act as stabilizers during storage. The presence of the protein stabilizers do interfere with antibody labeling and conjugation with biotin. 0.5 mLmL of melamine antibodies

were got through the clean-up column and centrifuged at 1000×g for 2 min. IgG purification resin and IgG isolation buffer were centrifuged at 4000×g for 1 min. Added 500 µL desalted antibody solution to the column and incubated for 5 min at room temperature. Purified IgG was collected after centrifuging at 4000×g for 1 min.

## 2.9 Biotinylation of antibody

Biotinylation resin kit was ready to warm to room temperature. Equilibrate the resin with 15 mL 1X PBS and pipetted the purified antibody solution onto the center of the column. Added 200 µL NHS-dPEG$_4$-biotin agent with PBS to the column. Once the flow has stopped, it was incubated for 30 min at room temperature. The biotinylated antibody was collected after adding 3 mL elution buffer to the column. Biotinylated antibody was stored in the buffer at 4°C.

# III. RESULTS AND DISCUSSION

## 3.1 Principle for the two step-based detection of streptavidin

For the SL designed to function as intended, the assay should be performed in two sequential steps. First mix SL with the sample to avail the function of SL to bind to the target for target recognition and then add labelled AuNPs to avail the function of SL to mediate the aggregation of AuNPs for producing indication signal – a visible color change. However, when our SL binds to the target, it can no longer conjugate two AuNPs (Fig. 1B). Thus, the amount of SL that can mediate aggregation of AuNPs determines the extent of aggregation and the accompanying color change. Hence, signal production indicates change in the SL's ability to mediate AuNP aggregation, which correlates to the amount of target in the sample. To validate our strategy, a system for detecting streptavidin using the well-known streptavidin–biotin interaction was desigend. Biotinylated bovine serum albumin (bBSA) labeled with 8–16 biotin molecules per molecule for conjugation with two or more streptavidin molecules was used as a linker to bridge streptavidin-coated AuNPs (stAuNPs, average diameter ~13 nm). Because streptavidin is a tetramer, a streptavidin molecule can conjugate with at least two bBSA molecules. Thus, bBSA can be used as an SL to

detect streptavidin by aggregation stAuNPs amplifying the recognition signal. Streptavidin in the sample is able to crosslink with bBSA to form a linker complex, which can bridge stAuNPs. However, one unit of the linker complex could not bind a third stAuNPs unless it grew very large, which requires large amounts of bBSA and streptavidin. Therefore, the presence of target streptavidin disables the ability of some individual bBSA molecules to bridge stAuNPs, decreasing the amount of linker capable of crosslinking with stAuNPs. The extent to which SL can conjugate AuNPs is equivalent to the effective amount of linker (EAL) available to bridge labeled AuNPs in the system. Thus, in a target-free system (control), the EAL includes all of the SL. Certain concentration of labeled NPs aggregate to varying extents depending on the linker concentration and time. When the linker concentration is less than a minimum level, aggregation is negligible (Region 1, Fig. 2b). In contrast, when it exceeds a certain maximum, the linker occupies all available binding sites on the labeled AuNPs, preventing aggregation (Region 3, Fig. 2b). When streptavidin coated spherical AuNPs were prepared and simply mixed with biotinlyated BSA, which was labeled with 8 to 16 mole of biotin per a mole of BSA, as the control system, the aggregation of colloidal AuNPs at given concentration occurs at 0.5 μg of biotinlyated BSA exhibiting distinguishable change in color of system.However, when the linker concentration was between those of

Regions 1 and 3, AuNPs agglomerate sufficiently, exhibiting a color change (Region 2, Fig. 2b). In this study, the linker concentration range exhibiting a visible color change (REVC) for the control system was from 0.5 to 1.0 μg/400 mL of bBSA (Fig. 2b). The presence of target molecules should lower the EAL because some of the SL binds to the target and becomes unavailable; thus, additional SL is required to aggregation AuNPs to the same extent as in the control system, and as a result, the REVC will shift to higher SL concentrations. Indeed, as the amount of target streptavidin was increased, the REVC shifted to higher bBSA concentrations (Fig. 2b). The transmission electron microscopy (TEM) images revealed those of 1-3 regions (Fig. 2c).

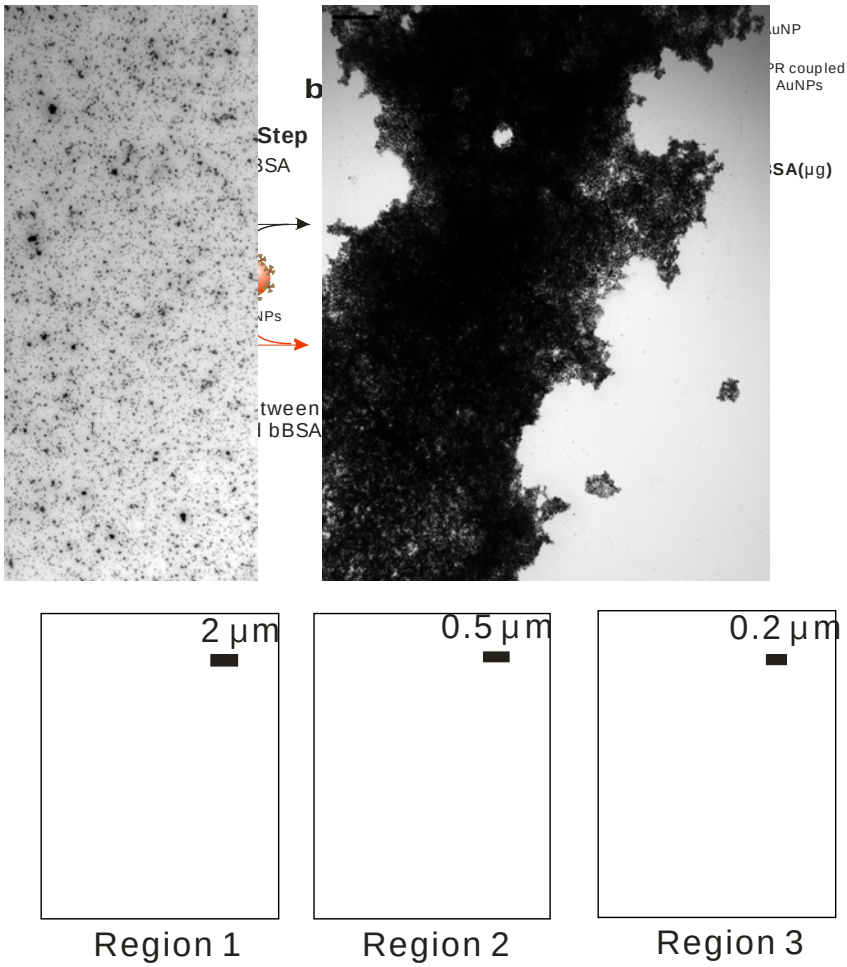


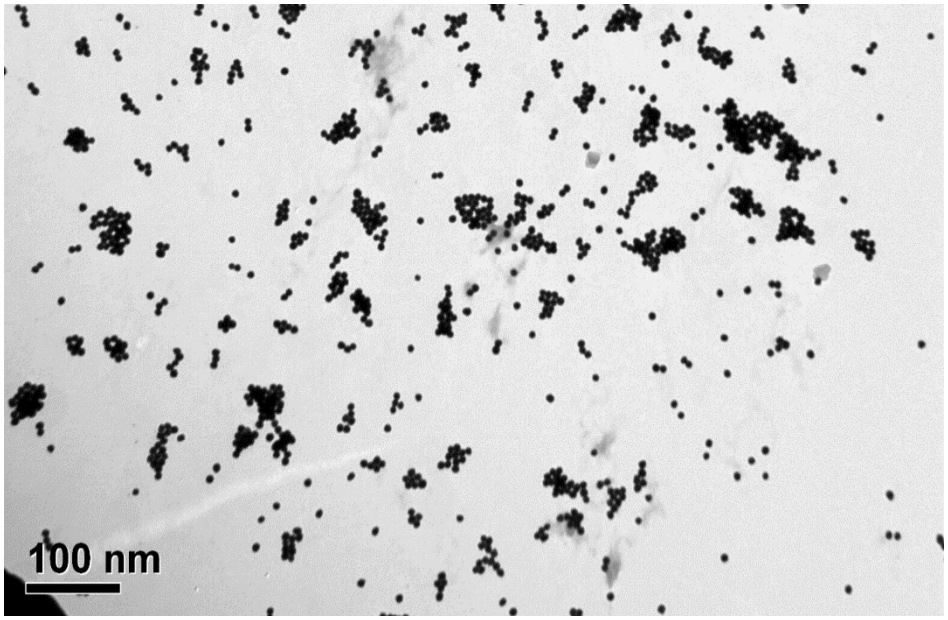

Fig. 2. Validation of the proposed assay by streptavidin detection using biotinylated BSA (bBSA) as an SL to aggregation streptavidin-coated AuNPs (stAuNPs). **(a)** Schematic representation of the method; two-step **(b)** Region 1: The lack of linkers for conjugating labeled NPs results aggregation insufficient to induce a visible change. Region 2: Sufficient amount of linker cause a visible color change. Region

3: Excess linker unable to induce visible change. Target streptavidin shifted the range of bBSA concentration exhibiting a visible change (REVC). **(C)** TEM images of the AuNPs in region 1-3

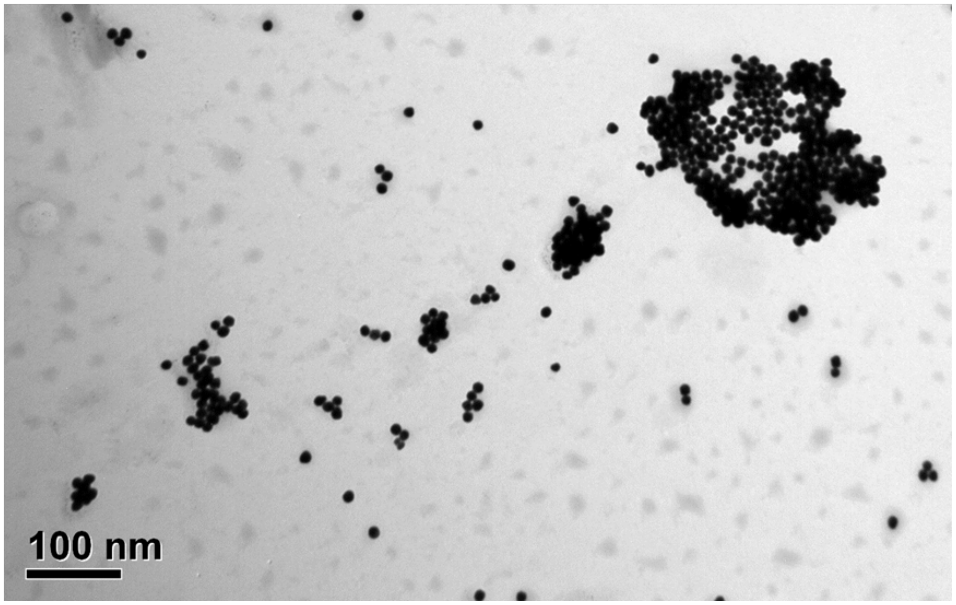
## 3.2 Effect of size of AuNPs in the detection system

To validate size effect of AuNPs, the particle size of 13± 1.2 nm, 26.1±2.8 nm and 43±2.4 nm were selected. After growing of AuNPs, the size of AuNPs can be confirmed by spectrophotometer and Transmission Electron Microscopy (TEM) (Fig. 3). Spectrum $\lambda_{max}$ of the particle gives the indirect values of particle size. Therefore, $\lambda_{max}$ of each particle was measured to confirm the size of particle. At this time the values of $\lambda_{max}$ for each particle are 519 nm, 524.5 nm and 533.5 nm respectively. Bigger size particles are made by kinetically controlled seeded growth synthesis method. Absorbance of all particles was set as around 4. If the particle size grow up, the number of particles are expected to be diminished remarkably. This is based on the Ostwald ripening effect. As this result, REVC region was moved to the lower linker concentration (Fig. 4). However, the larger the particle size decreases the time which takes for making enough amount of aggregation for REVC.

(a)




(b)



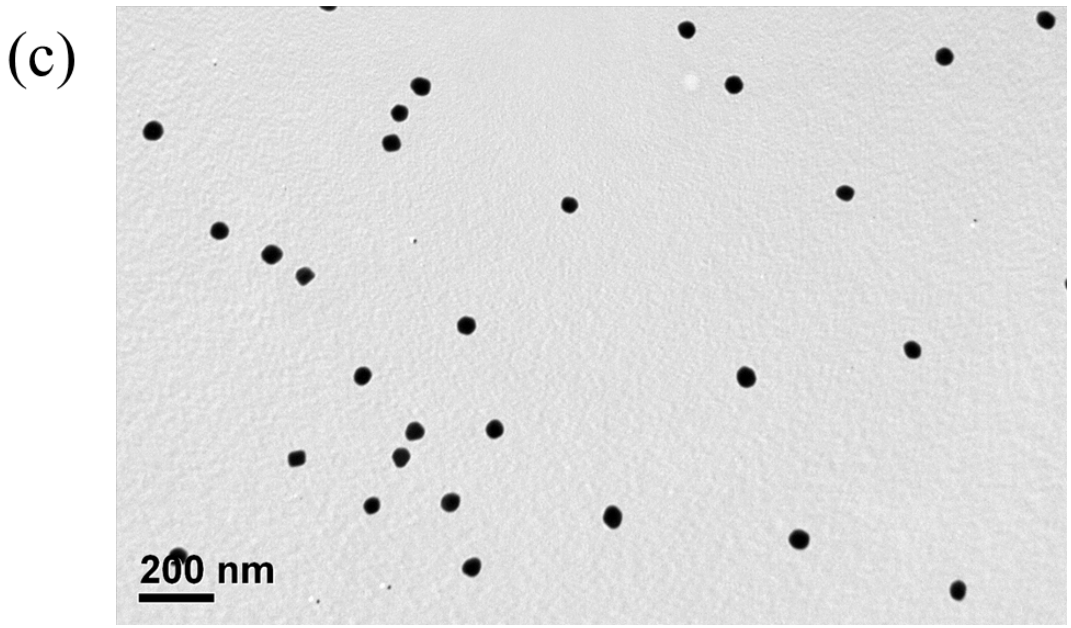



Fig. 3. The Transmission Electron Microscopy (TEM) image of each AuNPs. The particle size is (a) 13 nm, (b) 26 nm and (c) 43 nm respectively.

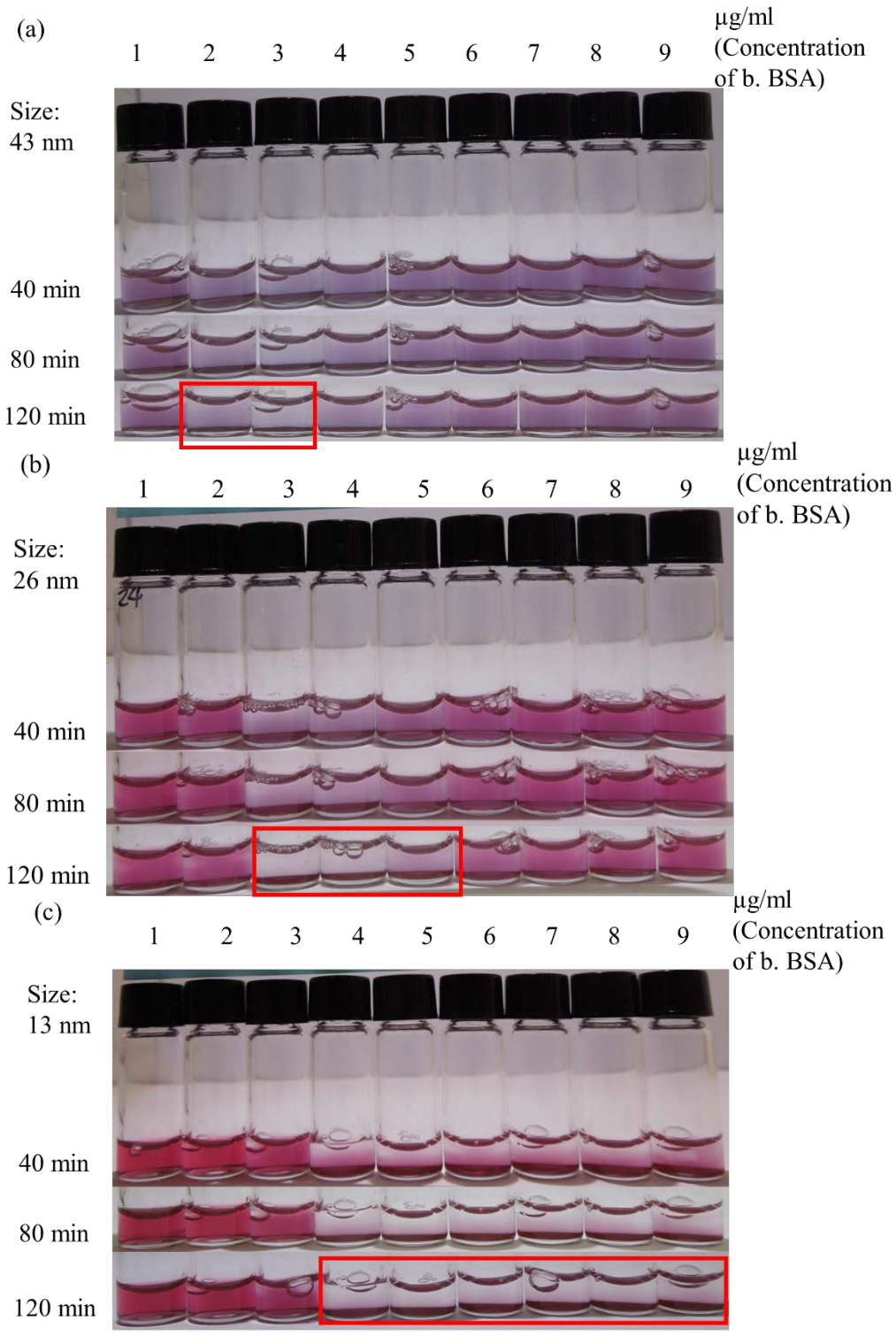

(a)
1 2 3 4 5 6 7 8 9
µg/ml (Concentration of b. BSA)
Size: 43 nm
40 min
80 min
120 min
(b)
1 2 3 4 5 6 7 8 9
µg/ml (Concentration of b. BSA)
Size: 26 nm
40 min
80 min
120 min
(c)
1 2 3 4 5 6 7 8 9
µg/ml (Concentration of b. BSA)
Size: 13 nm
40 min
80 min
120 min

Fig. 4. Shift change according to the particle size of the AuNP in time sequence. (a) 533 nm (b) 524 nm (c) 519.5 nm. Biotinylated BSA was used as SL in this experiment. When the particle size is getting bigger, it takes more time to make enough aggregation for giving signal and REVC shows low concentration region.

## 3.3 Effect of biotinylation of the linker in the detection system

Biotins are very important key factor of the SL. The noncovalent but exceptionally strong interaction of streptavidin-biotin was used in this system. According to researchers discovered that, streptavidin, a 60-kDa nonglycosylated protein with a near-neutral isoelectric point, exhibits less nonspecific binding than avidin. Both avidin and streptavidin bind four biotin equivalents per molecule with high affinity (Ka is about $10^{14}$ $M^{-1}$) and low reversibility, thus permitting numerous combinations of avidin, biotin, and antibody If all conditions are same, it is the number of biotins on the antibody that decides the amount of binding of the system.

### 3.3.1 Biotinylation of the antibody

Labeling antibodies by covalent coupling of a biotinyl group is simple and, usually, does not have any adverse effect on the antibody (21). Most biotinylations are performed using a succinimidyl ester of biotin. The reagent reacts with primary amines of the lysine residues or the amino terminus on the antibody to form amide bonds. In this study, biotinylated antibody was made with NHS-dPEG$_4$-Biotin and Nickel Chelating Resin support. The

reason for choosing NHS-dPEG$_4$-Biotin was it has long arm, so that could give more chance for accounting targets in the system.

### 3.3.2 Check the activity of the antibody after biotinylation

After biotinylation of the antibody, the activity of the antibody had to be checked before use. As a SL, it had to be attached to the target before binding with stAuNPs. Therefore, it in no use if the antibody lost their activity during the biotinylation process. Indirect ELISA test was used for this confirmation. 4 different concentration of biotinylated antibodies were compared (0, 10, 30 and 50 μg/mL). As a result, there was no signal at the 0 μg/mL of antibody which was set as control. By the way, in the concentration of 10, 30 and 50 μg/mL, the activity was shown with linearity (Fig. 5). After this comparison, additional experiment for measuring the antibodies which was biotinylated by the manufacturing company (Thermo fischer) against the antibodies which was originally manufactured as normal antibody and biotinylated with biotinylation kit. This was also compared with indirect ELISA method. Antibody biotinylated using kit had more activity than the antibody in every concentration. This result showed the

method which is used for the biotinylation of the antibody in this experiment, didn’t affect to the activity of the antibody.

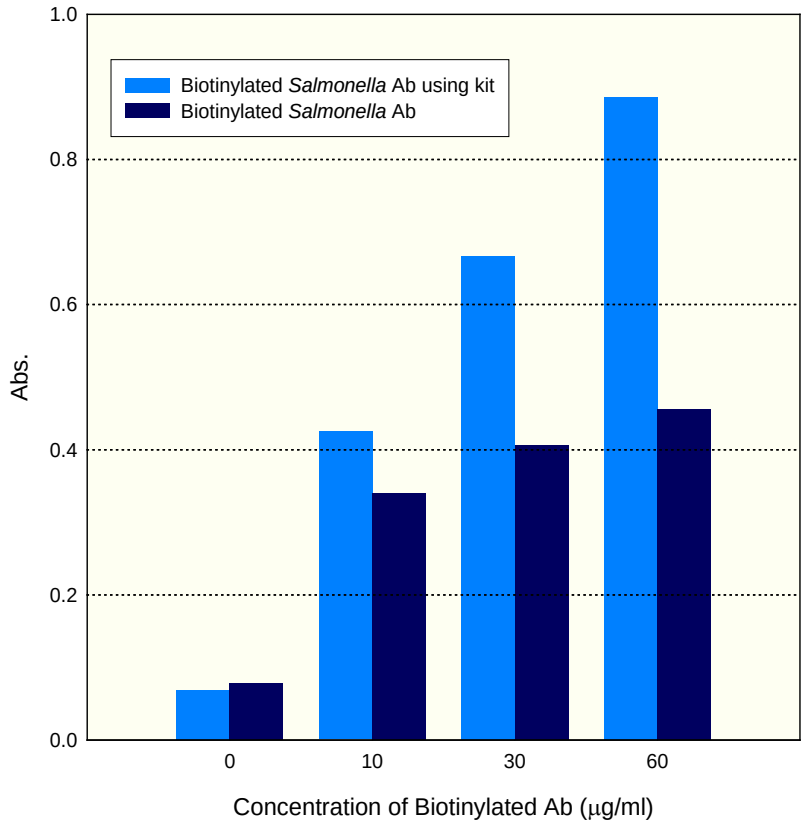



Fig. 5. Comparing absorbance (at 450 nm) of originally biotinylated *Salmonella* antibody (dark blue) and biotinylated using NHS-dPEG$_4$-Biotin with chelating resin (light blue) with indirect ELISA test. This shows biotinylated *Salmonella* antibody with NHS-dPEG$_4$-Biotin is more active than originally biotinylated one.

### 3.3.3 The number of biotins on the antibody and its effect on the detection system

The number of biotins is very critical for this detection system. This system is based on the streptavidin-biotin affinity for making aggregation. In the same concentration, more number of biotins gives more chance for binding with stAuNPs (Fig. 6). So it needs less SL for making same scale of aggregation for the detection, if the antibody has more biotins on it. According to the Thermo-Fischer company, originally biotinylated antibody has 8-16 biotins per one antibody. Moreover, as the information which is given by the biotinylation kit, it was calculated as 3-6 biotins. Normal IgG antibody has 150 kDa molecular weight and 4 mg of antibody was diluted to the 1 mL. After calculating to molarity, 26.67 mM was obtained. By the information which was given by the company. Molarity of the biotins which put in to the biotinylation was about 0.16 M. Finally, calculate all the factors and errors, it can be ensured that the number of biotins which binds to single IgG antibody is 3-6. Therefore, the antibody which was biotinylated by NHS-dPEG4-Biotin has less biotins than the originally biotinylated antibody. In the control experiment with these two antibodies (Fig. 7). It showed the REVC region was very different. The REVC region of the originally biotinylated antibody appeared at the low concentration of SL. On the other

hand, the REVC region of the biotinylated NHS-dPEG4-Biotin showed up at the high concentration of SL.

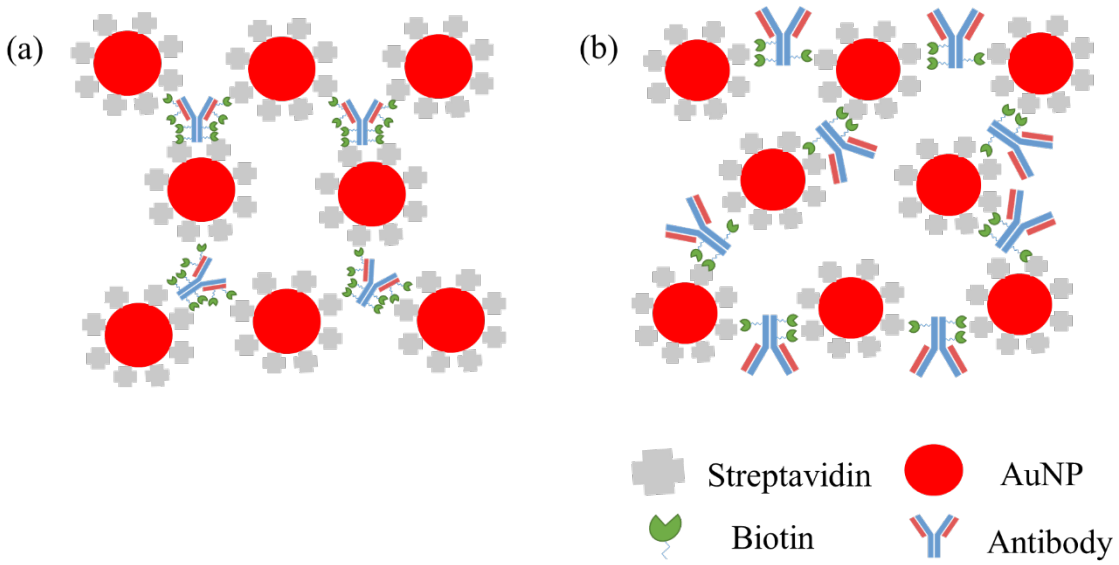



Fig. 6. Scheme of making aggregation in the presence of same concentration of stAuNP. It needs more biotinylated antibody for making same scale of aggregation if the number of biotins are less. (a) The number of biotins per one antibody is 6-12 (b) the number of biotins per one antibody is 2-4.

10 20 30 40 50 µg/ml (Concentration of b. Ab)

(a)

10 20 30 40 50 µg/ml (Concentration of b. Ab)

(b)

Fig. 7. Shift change of the control with *salmonella* antibody, when tests performed using biotinylated antibody as switchable linker in 400 μL total sample volume with a fixed stAuNPs concentration (abs. 0.4, λmax: 520). (a) has 8-16 biotins per single antibody, (b) has biotins per single antibody. It shows REVC is moved to the higher concentration of linker region.

### 3.3.4 Effect of the antibody activity and amount of biotinylation to the detection system

For the detection of the *Salmonella* Typhimurium, used same detection system performed before as control. *Salmonella* Typhimurium was incubated for 12 hours in the TSB. After incubation, it had been through serial dilution for getting $10^6$ and $10^2$ CFU $mL^{-1}$. The concentration of SL was set differently following control experiment. As a result, when used the antibody biotinylated with NHS-dPEG4-Biotin, REVC reason changed step by step as the number of *Salmonella* Typhimurium increased. However, It REVC region didn't change much with originally biotinylated antibody. It was related to the activity of the antibody, so SL didn't react with the target enough for REVC change (Fig. 8).

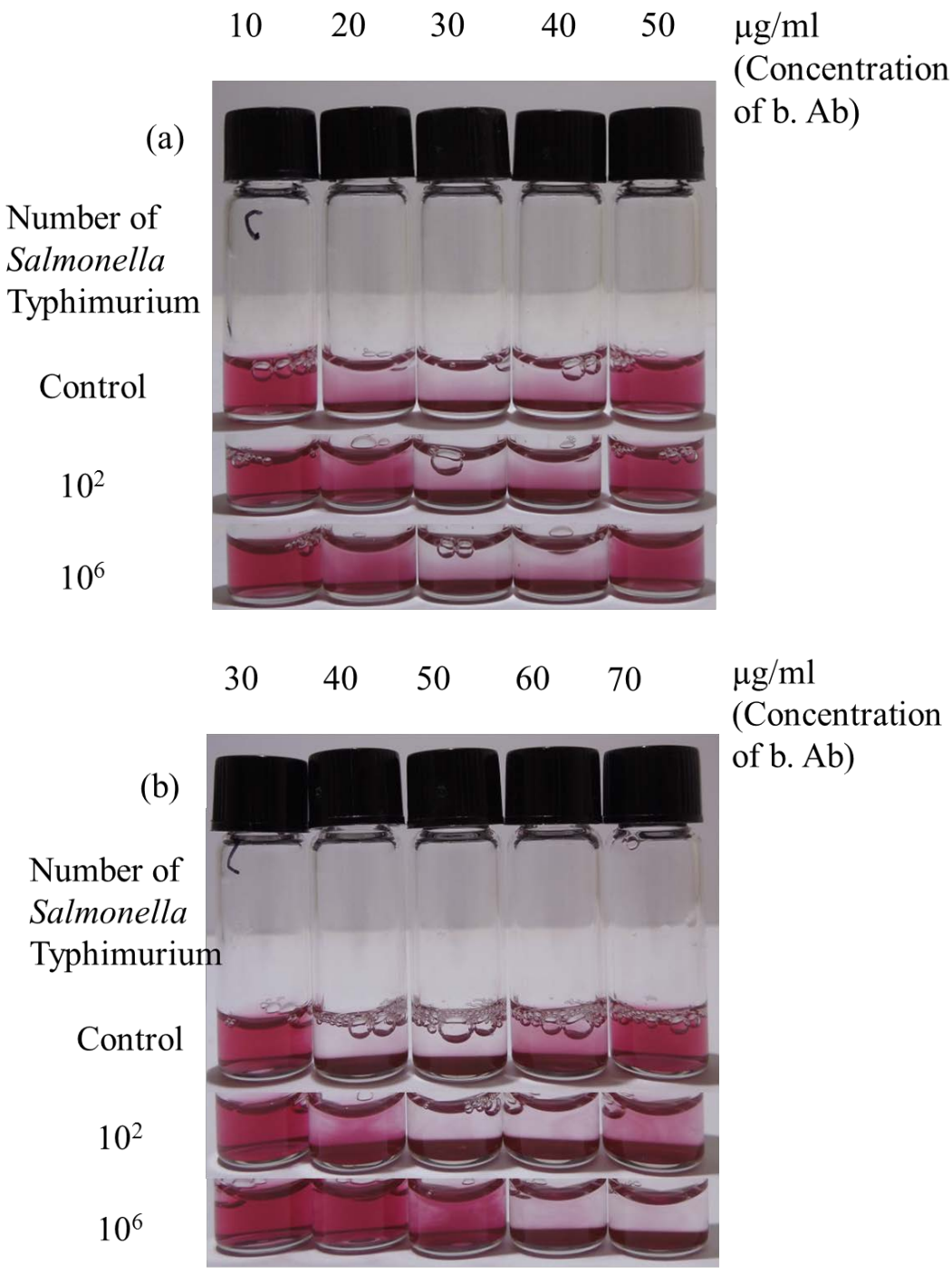

10 20 30 40 50 µg/ml (Concentration of b. Ab)
(a)
Number of *Salmonella* Typhimurium
Control
$10^2$
$10^6$
30 40 50 60 70 µg/ml (Concentration of b. Ab)
(b)
Number of *Salmonella* Typhimurium
Control
$10^2$
$10^6$

Fig.8. Shift change of detection system in the presence of *Salmonella* Typhimurium. (a) Using originally biotinylated antibody as SL (b) Using biotinylated antibody by NHS-dPEG$_4$-Biotin. Antibody which is biotinylated by NHS-dPEG$_4$-Biotin shows more distinct shift change compared to the originally biotinylated antibody.

## 3.4 Effect of salts and pH on the detection system

Not like the conditions in the buffer, real foods have many obstacles for this detection system. Foods have many kinds of salts in themselves. (22) Moreover, the pH of normal foods are various in all range, (23) but in the experiment conditions that of PBS buffer solution is 7.4. In this part, focusing on the salts and pH tolerance, tested this system in the 1M of NaCl for salts condition. The key factor of this detection system is biotin-streptavidin binding force. In the field where this system will be used, there is no way of removing salts and adjusting pH for the system. Consequently, checking for this harsh condition in necessary. Streptavidin has known for its high thermal and mechanical stability. However, in the foods, not only about temperature but also salts has big problem. (24) We tested this system in the 1M NaCl (Fig. 9). There was not any big difference between two controls. So the function of SL and binding force worked in the existence of salt. When applied to the various pH conditions (Fig. 10). It worked at too low or too high (pH 5; pH9) pH conditions, but needed more time for making enough aggregation. This harsh condition did not fully damaged to the system, but it was definitely gave more obstacles. Streptavidin or antibody could be damaged by too acidic or too basic condition.

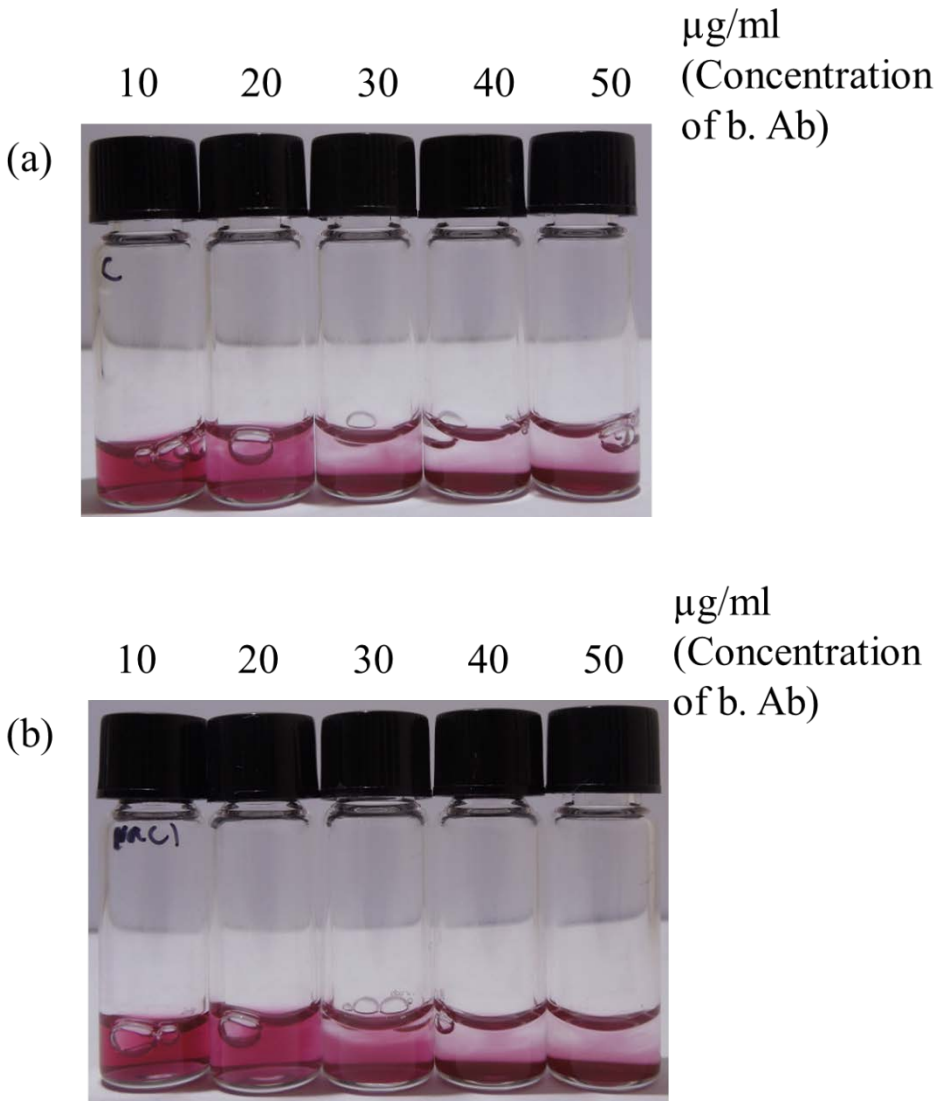



Fig. 9 Shift change of the control with *salmonella* antibody, when tests performed using biotinylated antibody as switchable linker in 400 µL total

sample volume with a fixed stAuNPs concentration (abs. 0.4, λmax: 520). (a) control (b) 100 μL of 1XPBS with 1 M NaCl.

(a)
pH: 5

30 40 50 µg/ml (Concentration of b. Ab)

(b)
pH: 6

30 40 50 µg/ml (Concentration of b. Ab)

(c)
pH: 7

30 40 50 µg/ml (Concentration of b. Ab)

(d)
pH: 8

30 40 50 µg/ml (Concentration of b. Ab)

(e)
pH: 9

30 40 50 µg/ml (Concentration of b. Ab)

Fig. 10. Shift change of the control with *salmonella* antibody, when tests performed using biotinylated antibody as switchable linker in 400 μL total sample volume with a fixed stAuNPs concentration (abs. 0.4, λmax: 520). (a) pH 5 (b) pH 6 (c) pH 7 (d) pH 8 (e) pH 9. In the condition of the pH 5 and pH 9, aggregation was not observed in the same time with other sample. It seemed to be hindered by the pH difference.

# IV. Conclusions

In summary, I gave change on the various factors which affect the function of simple colorimetric assay for visual detection. In the particle size, bigger AuNP needs more time for making aggregation and its REVC range moved to the higher concentration of SL. About the biotinylation, more biotins the antibody has easier to make aggregation because small amount of antibody could make enough aggregation. Moreover, too much biotins could affect the activity of antibody. In addition, because of stability of streptavidin in the harsh condition this system can be applied in the salts and not too high or low pH. As a result, adjusting these perimeters, sensitivity and specificity of the system could be improved. There might be more perimeters affect the aggregation of nanoparticle. Studying about these perimeters are very important for comprehending the whole system. Furthermore, by applying these factors is the key of realizing actual detection system using gold nanoparticle.

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# VI. 국문초록

오래 전부터 원하는 물질을 간편하고, 빠르게 검출 하려는 필요성이 증대되어 많은 연구가 진행되었다. 하지만 이전의 연구는 샘플의 전 처리와 검출에 오랜 시간과 복잡한 방법을 필요하다는 한계점이 있었다. 이에 대해서 선행된 이전 연구에서는 이러한 한계를 극복하기 위해 금나노 입자를 이용한 간편하며 신속한 검출방법을 개발했다. 이 방법은 두 단계로 진행되는데 첫 번째 단계는 target과 linker를 반응시킨다. 이때 linker는 금나노 입자를 응집시킬 수 있는 기능을 갖고 있으며 이 기능은 target에 의해 기능을 발휘하거나 차단될 수 있다. 두 번째 단계에서 금나노 입자와 혼합물을 반응 시킨다. 첫 번째 단계에서 반응 후 남은 linker의 수와 금 나노 입자의 수의 관계에 따라서 금 나노 입자 사이들의 응집이 유도된다. Linker의 농도가 낮은 구간에서는 응집을 유도하기에는 적은 수의 linker가 존재함으로 응집이 형성되지 않는다. Linker 농도가 높은 구간에서는 target과 반응 후에도 너무 많은 수의 linker가 존재해서 particle을 감싸버리게 되면서 linker로써의 기능을 수행하지 못하기 때문에 응집을 유도하지 못한다. 이런 현상을 이용하기 위해 센서의 구성은 linker의 양을 달리한 여러 개의 시스템으로 구성하였고, 최종적으로 target이 존재하는 양에 따라서 금나노 입자가 응집하는 시스템의 구간이 변하게 되는 것이 이 시스템의 기본 원리이다. 본 연구에서는 두 가지 단계에 대해서 개선 시킬 수 있는 방안을 찾고자 하였고, 그에 따라 첫 번째로 금 나노 입자의 크기를 변화시켜서 이에 따른 응집 정도와 응집 시간을 관찰하고자 하였다. 이 결과 입자의 크기가

커질수록 응집되는는 구간이 뒤로 이동하였고, 걸리는 시간 또한 증가하였다. linker의 역할에서 매우 중요한 역할을 하는 biotin의 수를 조절하고 이에 따라서 응집 양상에 어떤 영향을 미치는 지를 관찰하고 biotin의 수 조절 과정에서 antibody의 활성에 어떤 변화를 주는지 또한 알아보았다. 하나의 antibody에 더 많은 수의 biotin이 존재하게 되면, 좀더 적은 농도의 linker로도 응집을 유도할 수 있게 된다. 그러므로 응집 구간은 biotin이 많이 붙어 있을수록 앞으로 당겨졌다. 또한 너무 많은 수의 biotin이 영향을 항체의 활성에 영향을 줄 수도 있을 것이라는 가설을 세우고 실험결과 어느 정도 상관관계에 있다는 것을 확인하였다. 마지막으로 pH와 염과 같은 실제 식품에서의 환경에서는 응집 패턴이 어떻게 바뀌는지 알아보았다. pH 4와 pH 9의 산성과 염기성에서는 시스템이 작동하긴 했지만 응집을 만드는데 있어서 더 많은 시간이 필요했다. 그리고 일반적인 식품의 추출에 사용되는 용액의 염 농도인 1M NaCl에서는 시스템에 영향이 없다는 것을 확인하였다. 이러한 연구 결과를 바탕으로 이 검출 기술을 검출하려는 물질과 대상에 맞게 변화를 주는데 바탕이 되는 자료로 쓰일 수 있다. 기존의 시스템을 발전 시켜, 실제적으로 개발하고 적용하는 데 있어서 도움이 될 것으로 생각한다.

주요어: 바이오센서, 금 나노 입자, 응집, 바이오티닐레이션, 가시 검출