Master's Thesis of Chemistry

Simple and Rapid Sandwich Immunoassay Platform by Plasmonic Copper Polyhedral Particle

Plasmonic Copper Polyhedral Particle에 의한 간단하고 신속한 샌드위치 면역분석 플랫폼

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Abstract

Over the years there has been an ever-growing demand for a rapid and simple immunoassay system years. To acknowledge this need several colorimetric immunosensors which utilize plasmon nanoparticles have been proposed in recent years. However, there are issues which must be addressed, such as the time required for the catalytic reaction, and the possibility of being affected by the external environment. To overcome the temporal limitations, homogeneous-plasmonic copper polyhedral nano-shell were grown on gold nanoparticle probes. These nanoparticles scatter in such an efficient manner that light can be photographed and quantified by mobile phones. By using this method we provide a highly sensitive and quick detectable immunoassay system.

Initially, the prostate specific antigen was chosen as the standard target, and its measurement had a 4-digit dynamic range with a total assay time of 15 minutes and a lower detection limit of 50 pg/mL (1.5 pM, 6 attomole). Furthermore, we have developed a method to simultaneously identify and quantify two inflammatory biomarkers (C - reactive protein and Procalcitonin) by dots pattern in one sample. This method suggests the possibility of providing a platform that can detect and quantify multiple biomarkers in an efficient manner

Keyword: Immunoassay, Gold nanoparticles, Copper nano-shell, Microarray dot patterning, Simultaneous detection, Inflammatory markers
Student Number: 2019-20165

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1. Introduction

Noble metal nanoparticles exhibit unique optical properties different from bulk metals due to its nanometer-sized effect, namely Localized Surface Plasmon Resonance (LSPR), which absorb and scatter specific wavelengths and are visible to the naked eye in the visible light region. This phenomenon had been utilized for coloring stained glass or glass tableware since ancient times when there was no accurate physical understanding. LSPR changes depending on its atom composition, size, or shape of the particle, and can absorb and scatter light with wavelengths from visible to the near / mid-infrared region.[1,2] In recent years, various attempts have been made to apply this optical characteristic to biosensors,[3–6] optical sensors,[7,8] or energy conversion of solar cells.[9–11]

Lateral flow Immunoassay (LFIA) is an example that is widely used in practical applications for biosensors utilizing Plasmon nanoparticles.[12,13] Gold nanoparticles modified with antibodies and samples containing antigens were dropped onto a nitrocellulose membrane. The modified gold nanoparticles then traveled upward by capillary flow to a second group of immobilized antibodies, and a sandwich complex is formed between the immobilized antibodies and the modified gold nanoparticles to form a converged gold complex, which can be determined by the naked eye. Although this LFIA method is simple and short (about 15 minutes), its sensitivity limit is lower than that of conventional immunoassay because of an increase in the number of ineffective analyte molecules bound to gold nanoparticle surface. In addition, problems remain regarding quantitative

analysis and reproducibility due to the non-uniformity of the components and flow uncertainty.

In order to solve these problems, several immunosensors utilizing Plasmon nanoparticles have been proposed.[1418] One of these methods is changing the shape of nanoparticles by etching to convert the change in color tone of LSPR into the amount of target antigen.[18] In this method, a sandwich-type immunoassay was constructed and 3,3′,5,5′-Tetramethylbenzidine (TMB) is oxidized by an enzymatic reaction by Horse Radish Peroxidase (HRP) conjugated antibody. When gold nanorods coexist here, gold is reduced from the surface and the aspect ratio of the nanorods becomes smaller. Since the wavelength absorbed by the LSPR changes depending on the aspect ratio of the gold nanorod, the color tone of the solution changes. The amount of the target antigen is proportional to the amount of HRP conjugated antibody, the change in color tone can be converted into the amount of antigen and quantified. This method has a large change in color tone as compared with the single color tone dye used in conventional ELISA, and the amount can be visually estimated by a color sample. It can be easily performed by only adding gold nanorods to a conventional assay system. However, this method has problems such as the time required for catalytic reaction by enzymes, or the morphological changes of nanoparticles may be greatly affected by the external environment such as temperature, pH, and interfering compounds derived from living organisms.

A biomolecules detection system using a copper polyhedral nanoshell formation has been recently proposed.[19] Antibodies conjugated gold

nanoparticles are used on the detection side, and polyhedral copper nanoshells are grown on the gold nanoparticles as a seed. The grown copper nanoparticles are as large as about 1 μm and scatter visible light very strongly, which can be detected with the naked eye. Detecting a target DNA hybridized between a complementary probe immobilized on solid phase and a detection probe labeled with gold nanoparticles, and an inactivated whole virus directly fixed on a slide glass with antibody conjugated gold nanoparticles were reported in the above paper. The image of developed copper nanoparticles accumulation can be taken with a smartphone under moderate room light on a black background. This method does not use an enzyme-catalyzed reaction, and the growth of copper nanoshells is very specific to the seed gold nanoparticles and grows into a uniform morphology, which could overcome the above problems.

In this thesis, the above method was further extended to construct a sandwich-type immunoassay system and applied a copper nano-shell formation to improve its detection capability. It was verified that this method has a practically sufficient measurement range and detection sensitivity, and that it is applicable to clinical specimens. Furthermore, a method that can simultaneously identify and quantify two inflammatory biomarkers by a dot pattern at a significantly reduced time has been developed. We show the experimental results in detail, and discuss the possibility that this method could be a platform for simultaneous detection of multiple biomolecules.

2. Experimental Section

2.1. Reagents and Materials

HAuCl4·3H2O, CTAB, L-ascorbic acid, AgNO3, CTAC, Bovine Serum Albumin (BSA), Casein Sodium, CuCl2, (+)-Sodium ascorbate, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), Tween 20, 50% Glycerol, Acetone, (3-aminopropyl)-triehoxysilane (APTES) were purchased from Sigma-Aldrich (St. Louis, MO, USA). NaBH4 was purchased from DAEJUNG Chemicals & Metals Co., Ltd (Siheung-si, Korea). NaBr was purchased from Samchun Chemicals Co., Ltd (Seoul, Korea). Polyethylenimine, Branched, Mw 1,200 (bPEI 1200) was purchased from Polyscience, Inc. (Warrington, PA, USA). Fetal Bovne Serum, certified, heat inactivated, United States was purchased from Thermo Fischer scientific (Waltham, MA USA).

Microscope slides was purchased from Paul Marienfeld GmbH & Co.KG (Lauda-Konigshofen, Germany). A press-to-seal silicon isolator (24-2mm diameter x 0.8mm depth) was purchased from Grace Bio-Labs (Emkay Drive Bend, Oregon, USA).

Anti-PSA No.38 and Anti-PSA No.64 were purchased from Mikuri Immunological Lab.Co. (Osaka, Japan). Anti-CRP C5 and 135 monoclonal antibody, Anti-calcitonin 16B5, Anti-procalcitonin 42 monoclonal antibody, Anti-procalcitonin polyclonal antibody, human C-reactive protein and Prostate specific antigen were purchased from HyTest Ltd (Turku, Finland). Recombinant human procalcitonin was purchased from RayBiotech Life

(Peachtree Corners, GA).

2.2. Gold nano-spheres

All citrate-capped gold nanospheres (20, 40, 60, 80 nm diameter) were obtained from TANAKA KIKINZOKU KOGYO K.K.,

2.3. Synthesis of 48 nm gold nanocube

The gold nanocube was synthesized by slightly modifying the method described in the previous report.[20] First, 25 μL of 10 mM $HAuCl_4$ was added to 975 μL of 100 mM CTAB and mixed, and then 60 μL of 10 mM $NaBH_4$ was dissolved in ice chilled water and was quickly added. The mixture was vigorously stirred with a vortex for 2 minutes. This gave 1-2 nm seed particles. The resulting seed particles were stored at 30 ° C for 4 hours and then left to react with excess $NaBH_4$.

Next, to obtain 10 nm gold nanospheres, 200 μL of 200 mM CTAC, 150 μL of 100 mM ascorbic acid and 5 μL of the previously prepared seed particles, and 200 μL of 0.5 mM $HAuCl_4$ were added together and then vortexed for 30 seconds. The mixture was then left to react at room temperature for 30 minutes. The obtained 10 nm gold nanospheres were centrifuged at 12,000 g for 30 minutes, the supernatant was removed, and 1 mL of MilliQ water was added and suspended. This was repeated twice and then suspended in 50 μL of purified water. For the synthesis of gold nanocubes, 600 μL of 100 mM CTAC, 3 μL of 40 mM NaBr and 10 nm gold nanospheres 1 μL and 39 μL of 10 mM ascorbic acid were added and

mixed well. Finally, 600 μL 0.5 mM $HAuCl_4$ was added, and the solution was stirred with a vortex for 30 seconds, and left to react at room temperature for 30 minutes. The gold nanocubes obtained as described above were centrifuged at 6,000 g for 10 minutes to remove the supernatant, and then resuspended in 1 mL of purified water. This was repeated twice and finally the solution was suspended in 100 μL of purified water. The gold nanocubes obtained contained other anisotropic particles. To separate these particles, 100 mM CTAB (final concentration 5 mM) and 500 mM BDAC (final concentration 80 mM) were added and mixed to obtain a crude gold nanocube solution that was left to stand overnight at 30 °C. The gold nanocubes were associated and precipitated by inter-particle flocculation, and other particles in the supernatant. After gently removing the supernatant, pure gold nanocubes were replaced with purified water by centrifugation.

2.3. Synthesis of gold nanorod with aspect ratio of 3.5

The method described in the literature[21] was slightly modified and prepared. 500 μL of 200 mM CTAB and 500 μL of 0.5 mM HAuCl4 were mixed, and 60 μL of 10 mM $NaBH_4$ was quickly added. The mixture was dissolved in cold water and vortexed vigorously for 2 minutes. It was allowed to react at 30 degrees for 2 hours or more to prepare seed particles. 15 μL of 4 mM AgNO3, 500 μL of 200 mM CTAB, and 500 μL of $HAuCl_4$ were mixed and equilibrated at 30 °C. When 5.5 μL of 78.8 mM ascorbic acid was added and mixed, the color of the solution changed from pale yellow to clear. 1.2 μL of the previously prepared seed particles were

quickly added, stirred with a vortex for 30 seconds, and allowed to stand at 30 °C for 30 minutes.

2.4. Preparation of antibody modified gold nanoparticles

50 μL of 100 mM HEPES (pH 7.5) was mixed with 450 μL of each 100 pM gold nanoparticle solution 6.75 μg of the antibody was added, then allowed to react at room temperature for 15 minutes. After adding 10 μL of 10 mg / mL BSA, the mixture was allowed to react for 15 minutes. After centrifuging at 10,000 g for 10 minutes to remove the supernatant, it was redispersed in 500 μL of 10 mg / mL BSA and centrifugation was repeated. After removing the supernatant, the nanoparticles were suspended in 50 μL of 10 mg / mL BSA to adjust the nanoparticle concentration to 10 nM each.

2.5. Assay method

Slide glasses were washed with acetone and then immersed in 5% APTES for 15 minutes. After washing, it was immersed in 2.5% glutaraldehyde solution for 15 minutes, washed and dried. A silicon isolator (Φ2 mm, 4 x 6 wells) was crimped onto the aldehyde modified slide glass prepared above. 4 μL of a 50 μg / mL antibody solution (5% glycerol, 0.05% Tween-20 in PBS) was added dropwise to each well, and to stand overnight at 4 ° C. After washing 3 times with PBS-T, 4 μL of the blocking solution (a mixture of 0.8% BSA and 0.1% Casein sodium in PBS) was added to stand at room temperature for 2 hours. It was used for each assay after washing. 4 μL samples was added to each well to stand at room

temperature for 5 minutes. The wells were washed 3 times with PBS-T, and 4 μL of antibody-conjugated gold nanoparticles were added to react for 5 minutes. The wells were washed 3 times with PBS-T and immersed in a copper shell growth solution (mixture of 1% PEI : 0.1M CuCl2 : 0.5M Sodium L-ascorbic acid = 1 : 1 : 5) to carry out the copper shell growth reaction for 5 minutes. It was washed with purified water and dried.

2.6. Dot pattern spotting

Each antibody solution of 50 μg / mL was spotted on an aldehyde-modified slide glass by a microarrayer. After being left overnight at 4 ° C, blocking was performed.

2.7. Photo imaging

The slide glass was placed in a dark box where light was incident only from the side, and the photo was taken from above with the iPhone SE. The obtained color photos were converted to 8-bit grayscale JPEG image and Image J software was used to measure the average grayscale value of each spot.

2.8. Characterization

Extinction spectra were measured by a UV-Vis spectrophotometer (HP 8453, Agilent Technologies, Inc.,). SEM images were obtained by JEM-2100 (JEOL) Images of all gold nano-particles were taken by energy-

filtering TEM (LIBRA 120, Carl Zeiss). Dark field microscope images were taken by DF microscope. (Axiovert 200M, Carl Zeiss, Germany)

3. Results and Discussion

We designed an immunocolorimetric assay and subsequent scattering signal enhancement experiment on a micro arrayed glass chip. The experimental scheme is shown in **Scheme 1**. The antibody was added to an aldehyde-modified slide glass, and left at 4° C overnight. The aldehyde group reacts with the amino group of the antibody covalent cross-linking, then the antibody was immobilized on the glass surface. Blocking was performed to prevent non-specific adsorption (0.8% BSA and 0.1% Casein sodium in PBS). A sample containing the antigen of interest was added, incubated, washed, and then reacted with the antibody conjugated gold nanoparticles to form a sandwich complex. A copper nano-shell growth reaction was carried out on the surface of gold nanoparticle core. Under ordinary circumstances, gold nanoparticles are invisible, but copper nano-shell augmented gold nanoparticles scatter light strongly and are visible to the naked eye. The results can be taken with a mobile phone camera under ambient room light on a black background.

We optimized the reaction time between the antigen and the immobilized antibody, as well as the probe concentration and sample volume. We found no significant relationship between the reaction time between the antigen and the immobilized antibody, but when the reaction time with the gold nanoparticle probe was too long, a weak signal from the negative control emerged due to the non-specific adsorptions of the probe nanoparticles. As a result of this weak unwanted signal, the signal to noise ratio (S/N) declined. A sufficient S / N could be obtained with a reaction

time of 5 minutes with the antigen and a reaction time of 5 minutes with the probe. (**Figure 1a**)

Next, the effects of probe concentration and sample volume were investigated. As shown in the **Figure 1b**, there was no significant difference when modifying the sample volume, but when the probe concentration was too high, non-specific adsorption with the solid phase increased and as a result, S / N decreased. In the subsequent experiments, the probe concentration was optimized to 200 pM and the sample volume to 4 μL. Since the growth reaction time of copper nano-shell is 5 minutes, the total assay time is completed in 15 minutes. Conventional ELISA requires about 100 μL of sample volume, and it takes several hours for the antigen to react with the immobilized antibody by diffusion in a typical 96-well ELISA plate. Our method is cost effective because the reaction volume is as small as 4 μL, the reaction time is shortened, and the amount of sample and antibody used is also reduced.

Additionally, the morphology and size effect of the core gold nanoparticles for the copper shell formation was investigated. The TEM image and UV-VIS spectrum of each gold nanoparticle used are shown in **figure S1**. The average particle sizes of the gold nanospheres were 18.4 ± 1.8, 40.2 ± 3.8, 63.4 ± 5.1, and 81.4 ± 6.0 nm, respectively. The size of gold nanocube is approximately 48.0 ± 2.6 nm, and the gold nanorod had an aspect ratio of 3.5 ± 0.4 with a longitudinal axis of 45.3 ± 3.5 nm and a transverse axis of 13.2 ± 1.2 nm.

After modifying each particle with an antibody, an antigen-antibody reaction was carried out by the method described above, and detection was

performed using a copper shell. (**Figure 2**) The 20 nm gold nanosphere seemed to have the best sensitivity. This is considered to be because the small particles can move faster by diffusion than the large particles within the identical time, and it is possible to make more contact with the antigen captured by the immobilized antibody. Therefore, a greater number of gold nanoparticles seeds in same surface area remain before copper shell growth. As can be seen in the DFM image, the density of copper shell positively relates to the antigen concentration. (**Figure 2**)

To further investigate the relationship between Cu particle density and target molecule concentration, we counted the number of Cu particles at each antigen concentration using dark-field microscopy (**Figure S2**) and showed that a doubling of particle number corresponded to an approximately 10-fold increase in antigen concentration. As antigen concentration was linearly correlated with image color intensity, the method of photographing the coloration of Cu nanoshells with a cell phone camera provided a logarithmic relationship for the target concentration, thus delaying color saturation and consequently broadening the dynamic range in the visible region.

The SEM image of copper shells grown with nanoparticles of different shapes as nuclei are shown in **Figure S3**. There was no difference in the shape of the grown copper shell particles, which was consistent with previous manuscripts which reported consistent shape regardless of the gold nanoparticles morphology. When the antigen concentration was low, that is, when the number of seed gold nanoparticles was small, the grown copper particles were slightly larger and became about 1 μm.

Prostate Specific Antigen (PSA) detection was chosen as a standard target to verify that our immunoassay system has sufficient sensitivity and detection range. The response curve ranged from 0.01100 ng/mL with purified antigen showing a typical sigmoidal graph with a lower limit of detection value of 50 pg/mL. (**Figure S4**) Moreover, when clinical human serum samples were evaluated using the standard curve, they were in good agreement with the predetermined values.

C - reactive protein (CRP) was discovered as a protein that precipitates with the C-polysaccharide of Streptococcus pneumoniae. It is one of the acute phase proteins which increase in blood when various inflammatory reactions and tissue destruction occur in the body.[22] The molecular configuration is a cyclic pentamer consisting of five 21 KDa monomers. When the blood concentration exceeds 5 to 10 μg/mL, a clinically obvious acute phase reaction occurs. The CRP response curve in our system was saturated over 100 ng/mL, and initial values near the cutoff were recorded to be beyond the dynamic range. Therefore, free CRP antibody was added to the sample in advance to absorb a part of the excess CRP, and the response curve was shifted to the higher concentration range (**Figure S5**). This technique allowed us to quantify the target concentrations near a high cutoff value using our sensitive and delicate assay platform. When the CRP concentration of human serum was determined using this calibration curve, they were in very good agreement with the pre-determined values of the clinical samples. (**Figure 3**)

Procalcitonin is a peptide consisting of 116 amino acids synthesized in the C cells of the thyroid gland as a precursor to calcitonin, and has a

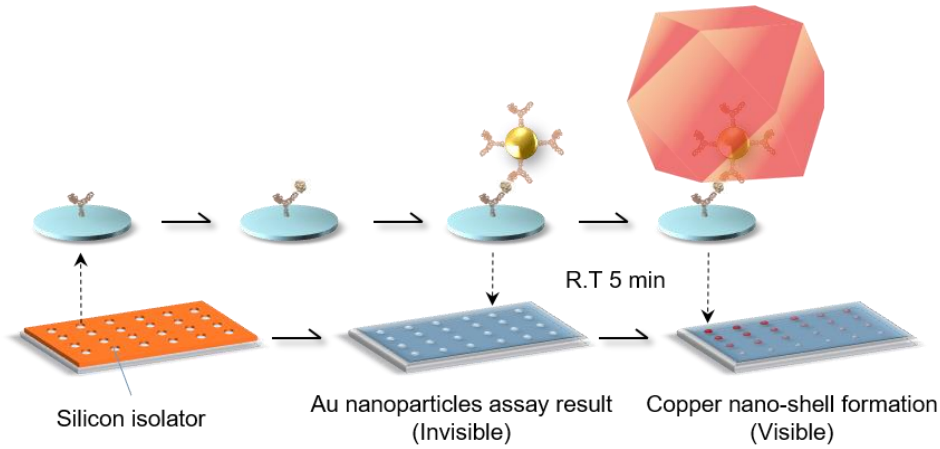
molecular weight of about 13 KDa. During bacterial infections, when inflammatory cytokines such as TNFα increase, PCT is produced in the organs throughout the body and its concentration in the blood level rises.[23] The cut-off value is 0.5 ng / mL in PCT elevated blood levels for severe bacterial infections, whereas there was little change in the blood level during viral or local bacterial infections. Therefore, PCT is greatly suitable for the diagnosis of sepsis compared to IL-6 or IL-8.[24] A total of 6 combinations of 2 monoclonal antibodies (mAb) and 1 polyclonal antibody (pAb) were investigated. The combination using mAb-16B5 on the solid phase side and pAb on the detection side showed best color development in accordance to the standard antigen concentration and correctly reacted with human serum. (**Figure S6**) When the PCT concentration in human serum was quantified using this antibody combination, they showed good agreement with the pre-determined data of the clinical samples. (**Figure 4**)

As stated before, multiplex detection in one sample is greatly desired during clinical trials. If the two inflammatory markers listed above can be measured simultaneously, it gives weight in making clinical decisions identifying whether sepsis is to be suspected so subsequent decisions such as antibiotic management can be applied before the blood test result or if there is a need for further evaluation of the immunostatus of a patient.[2529] As shown in **Figure 5a**, 1 dot of CRP antibody and 2 dots of PCT antibody were spotted in the same well chamber that immobilized the antibody using a microarrayer. When the reactions to each antigen and each probe antibody were examined, they reacted specifically, and neither cross-reactivity nor non-specific reaction was observed. (**Figure 5b**)

The calibration curve was made with the dot pattern as shown in **Figure 5c** and each serum sample was evaluated. Both measured CRP and PCT values were in good agreement with the pre-determined values of the clinical samples. (**Figure 5d**) B1, B2, B3, specimen were all positive for CRP and negative for PCT. It was suggested that the C1, C3, and C5 were PCT and CRP positive. B4 and C2 were both negative and C4 was PCT positive and CRP negative. This may reflect the pathogenic differences of each specimen, and the background of each patient needs to be investigated in more detail.

4. Conclusion

A rapid and quantitative immunoassay system was constructed using a polyhedral copper nano-shell formation reaction. Even in a reduced reaction volume of 4 μL, PSA could be detected at the lower limit of detection value of 0.05 ng / mL in an overall reaction time of 15 minutes. Optimal assay conditions were examined for CRP and PCT, and the parameters were tailored for the evaluation of clinical specimens. Anti-CRP and anti-PCT antibodies were spotted in same one reaction chamber using a microarrayer, and simultaneous detection of the two biomarkers was attempted. CRP and PCT could be identified by a dot pattern, and both biomarkers could be detected and quantified at the same time in one clinical sample. Our method suggests the possibility of providing a platform that can measure multiple biomarkers arbitrarily selected with different dot pattern in a short time and quantitatively with a very small amount of one sample. Furthermore, if a capture and detection probe capable of hybridizing with a specific target DNA / RNA could be designed and modified into gold nanoparticles, it is considered that it could be applied to a nucleic acid detection system.



Scheme 1. Schematic illustration of the experimental scheme.

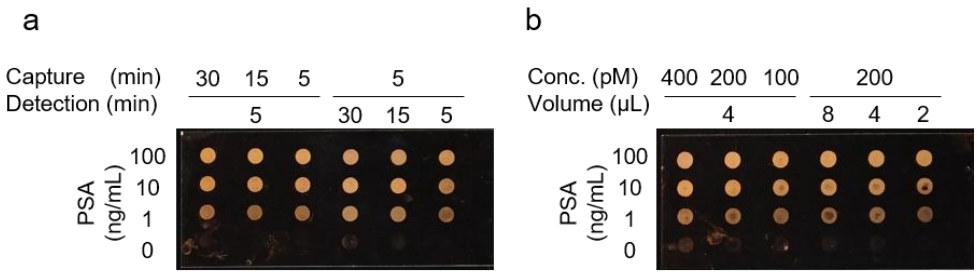


Figure 1. Photograph image for (a) capture and detection time investigation and (b) probe concentration and sample volume.

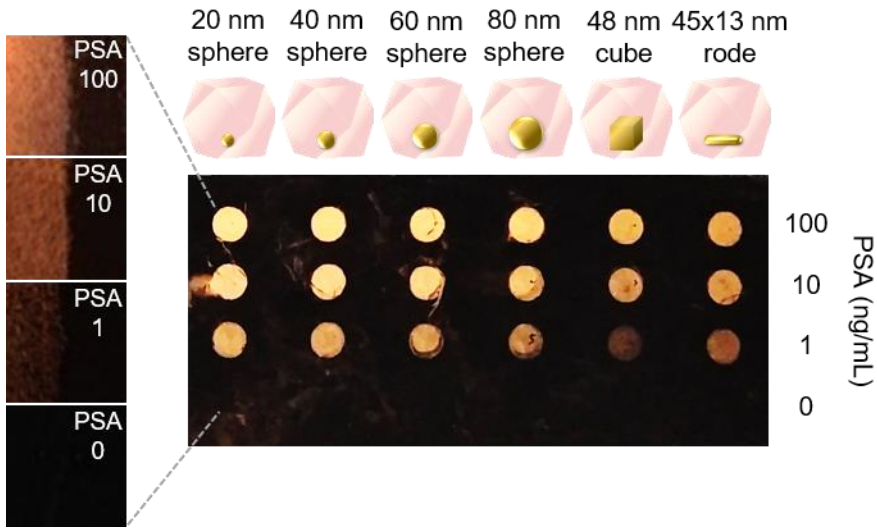
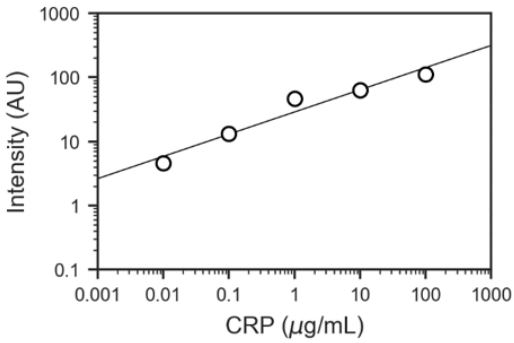


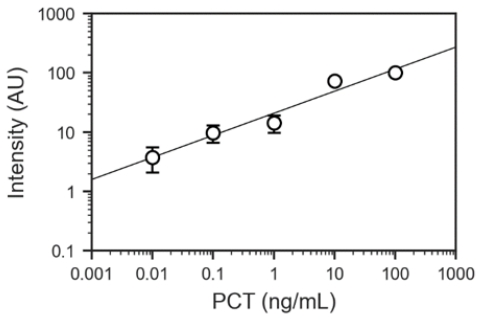
Figure 2. Morphology effect of probe gold nanoparticles. From left to right, 20, 40, 60, and 80 nm gold nanospheres, gold nanocubes, and gold nanorods. The leftmost column shows dark field microscope image in case of 20 nm gold sphere.



	B1	B2	B3	B4
Predetermined*	11	11	11	3.5
This method	**7.2**	**15.2**	**12.6**	**4.9**

*) Immunoturbidimetric assay
(Cobas c 702, Roche)

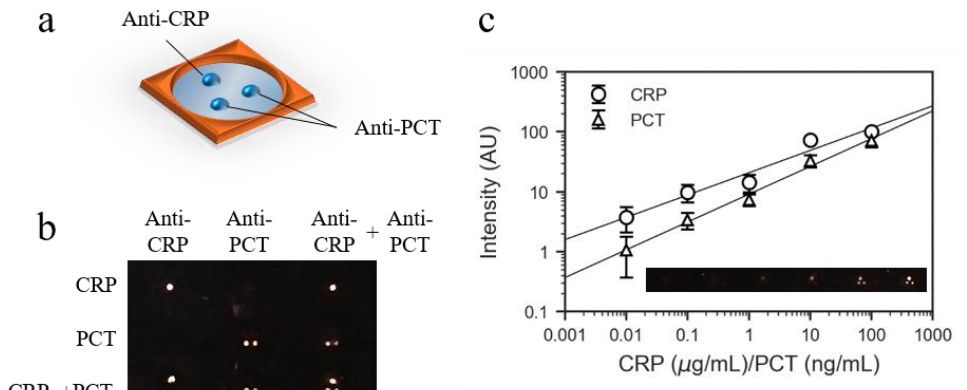
Figure 3. Clinical human serum samples evaluated for CRP.



	C1	C2	C3	C4	C5
Predetermined**	2.13	0.03	21.1	>100	10.5
This method	**0.5**	**N.D.**	**40.9**	**74.9**	**5.1**

**) Electrochemiluminescence immunoassay (Cobas e 601, Roche)

Figure 4. Clinical human serum samples evaluated for PCT.



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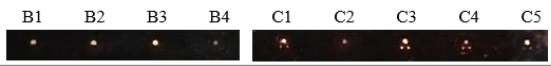

		B1	B2	B3	B4	C1	C2	C3	C4	C5
CRP (μg/mL)	Predetermined*	11	11	11	3.5	-	-	-	-	-
	This method	**4.1**	**8.6**	**21.0**	**1.1**	**44.6**	**1.5**	**37.8**	**5.9**	**24.2**
PCT (ng/mL)	Predetermined**	-	-	-	-	2.1	0.03	21.1	>100	10.5
	This method	**0.1**	**0.1**	**0.3**	**0.05**	**34.9**	**0.3**	**139.8**	**47.8**	**8.9**

*) Immunoturbidimetric assay (Cobas c 702, Roche)
**) Electrochemiluminescence immunoassay (Cobas e 601, Roche)

Figure 5. (a) Schematic diagram of dot patterns of anti-CRP antibody and anti-PCT antibody in the same reaction well chamber. (b) Photograph of the combination reaction pattern of each antigen and each probe antibody. (c) Calibration curve for CRP and PCT with standard antigen. (d) Resulting images and estimated values for each clinical specimen.

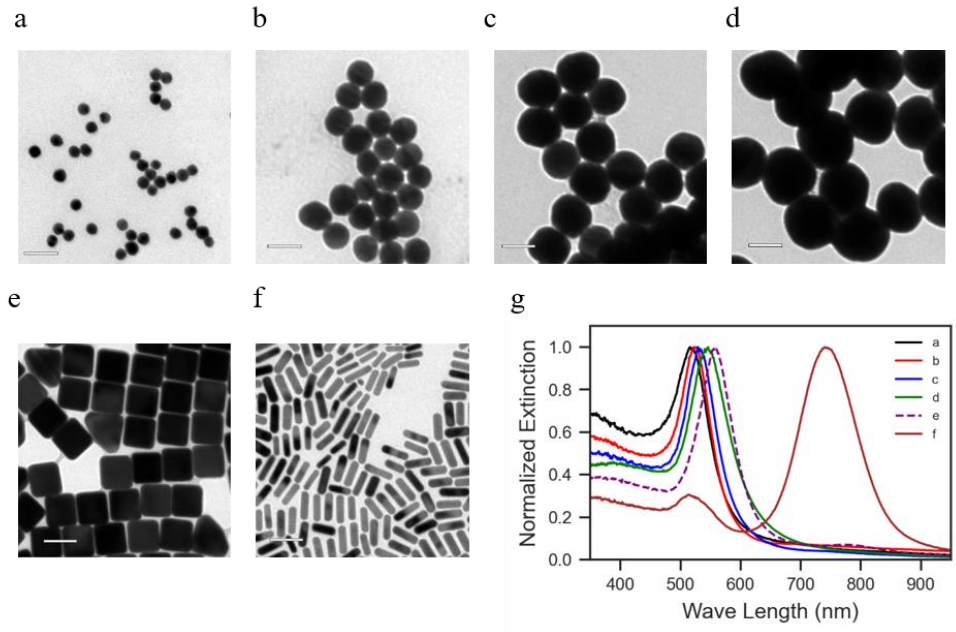

Figure S1. TEM images of gold nanoparticles used in this work. Scale bars are 50 nm. (a - f) UV-Vis spectra for each nanoparticle. (g)

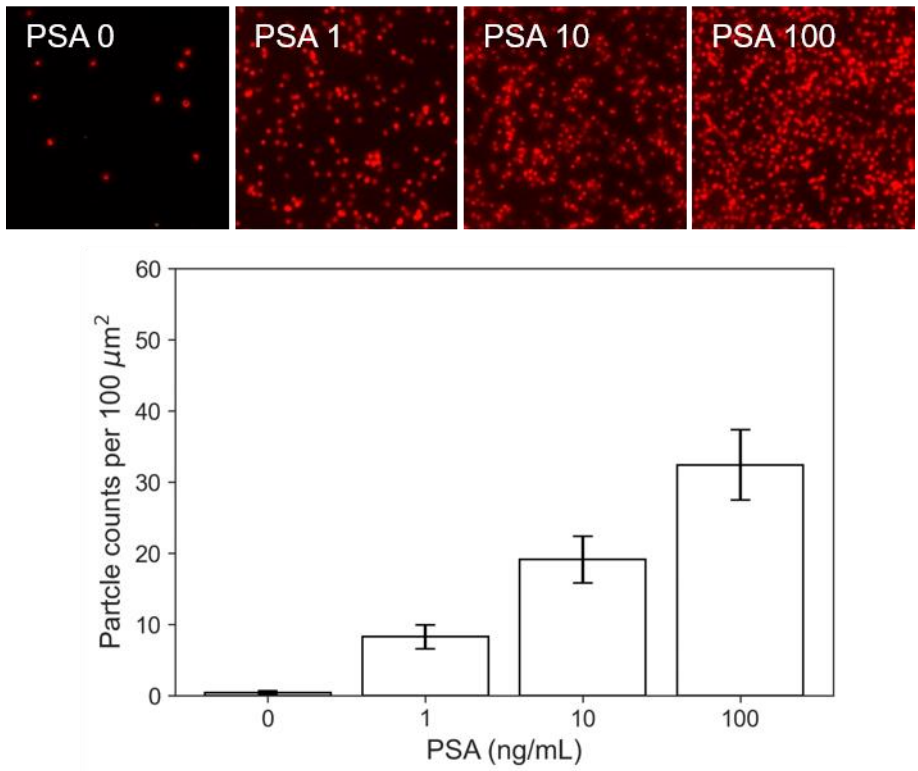

Figure S2. Surface density of the particles by counting spots per 100 μm2 under DFM as a function of PSA concentration. Upper pictures show DFM images at each PSA concentration.

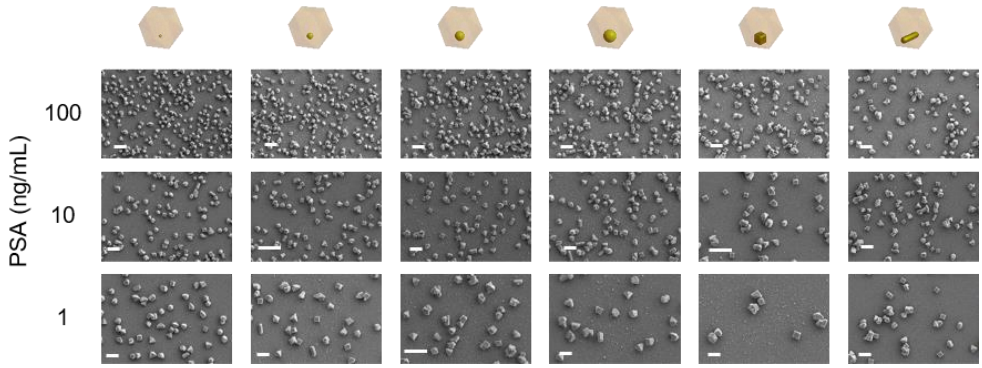

Figure S3. SEM images of grown copper polyhedral shell for each probe gold nanoparticles at different antigen concentration. Small and large scale bars are 1 μm and 2 μm respectively.

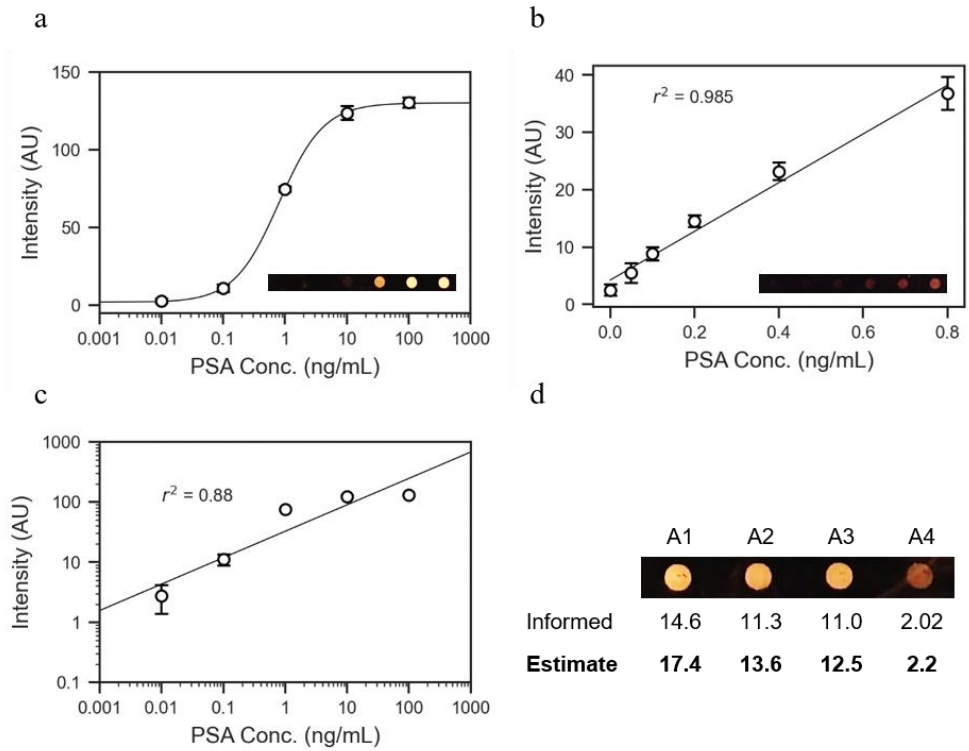

Figure S4. (a) Response curve for the PSA standard antigen ranging from 0.01-100 ng/ mL and (b) in the low concentration region (0 - 0.8 ng/mL). Error bar is standard deviation (n = 3). (c) Log-log axis standard calibration curve. (d) Resulting images and estimated PSA values from human clinical serum samples.

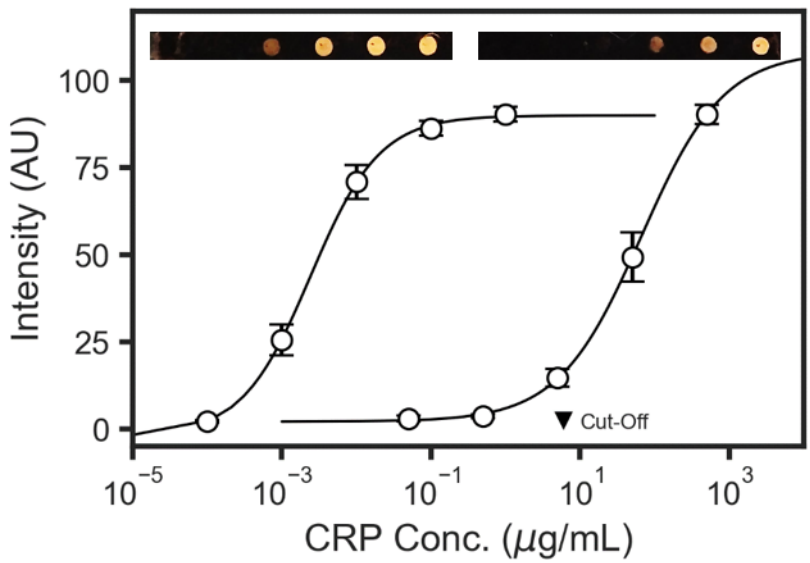

Figure S5. Response curve to CRP without the free antibody (left) and with the antibody (right).

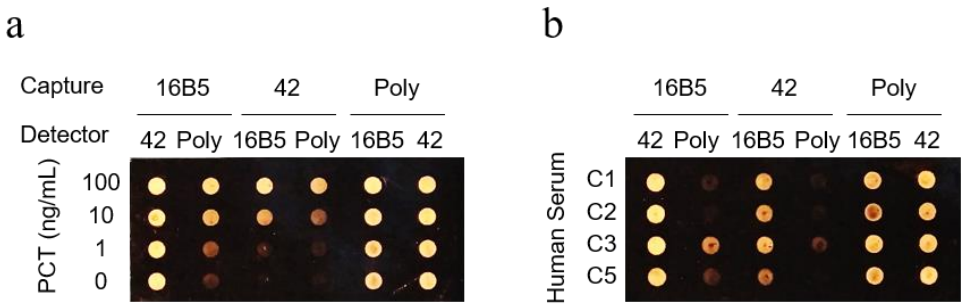

Figure S6. Response with a two monoclonal antibodies and one polyclonal antibody combination against (a) standard PCT antigen and (b) human clinical serum samples.

Table S1. Clinical human serum specimens used in this work.

	A1	A2	A3	A4	
PSA (ng/mL)	14.6	11.3	11.0	2.02	
Cut-off 4 ng/mL	Positive	Positive	Positive	Negative	
	B1	B2	B3	B4	
CRP (μg/mL)	11	11	11	3.5	
Cut-off 8 μg/mL	Positive	Positive	Positive	Negative	
	C1	C2	C3	C4	C5
PCT (ng/mL)	2.13	0.03	21.1	>100	10.5
Cut-off 0.5 ng/mL	Positive	Negative	Positive	Positive	Positive

Serum PSA and PCT levels were measured by electrochemiluminescence immunoassay using Cobas e 601 (Roche). Serum CRP concentrations were measured by an immunoturbidimetric assay using Cobas c 702 (Roche, Basel, Switzerland).

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초 록

Plasmonic Copper Polyhedral Particle에 의한 간단하고 신속한 샌드위치 면역분석 플랫폼

수년에 걸쳐 신속하고 간단한 면역분석 시스템에 대한 수요가 계속 증가해 왔습니다. 이를 인정하기 위해 최근 몇 년 동안 플라스몬 나노입자를 활용하는 여러 비색 면역센서가 제안되었습니다. 그러나 촉매반응에 소요되는 시간과 외부환경의 영향을 받을 가능성 등 해결해야 할 문제가 있다. 시간적 한계를 극복하기 위해 균질 플라즈몬 구리 다면체 나노 쉘을 금 나노 입자 프로브에서 성장 시켰습니다. 이러한 나노 입자는 빛을 휴대폰으로 촬영하고 정량화할 수 있을 정도로 효율적인 방식으로 산란됩니다. 이 방법을 사용하여 우리는 매우 민감하고 빠른 감지 가능한 면역 분석 시스템을 제공합니다.

처음에는 전립선 특이적 항원이 표준 표적으로 선택되었으며, 이의 측정은 총 분석 시간이 15분이고 검출 하한이 50pg/mL(1.5pM, 6 attomole)인 4자리 동적 범위를 가졌습니다. 또한 하나의 샘플에서 점 패턴으로 두 개의 염증성 바이오마커(C - 반응성 단백질 및 Procalcitonin)를 동시에 식별하고 정량화하는 방법을 개발했습니다. 이 방법은 여러 바이오마커를 효율적으로 검출하고 정량화할 수 있는 플랫폼을 제공할 수 있는 가능성을 제시합니다.

주요어 : 면역측정법、금나노입자、구리나노세루、마이크로어레이도트패턴、동시검출、염증성마커

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