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Functional Gold-Nanoparticles Based Colorimetric Assay for Medical and Industrial Use

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Nanosize materials have been widely available in various research fields as an advanced technology. Gold nanoparticles (GNPs) have unique optical, electrical, and magnetic properties. Especially, the optical spectrum of GNPs shows a localized surface plasmon band in the region of 520–550 nm. The wavelength of spectrum of GNPs change drastically longer when several particles come close to each other. Various types of GNP based assays that was conjugated with functional biomolecules have been developed to take advantage of the optical property. This review introduces the optical assays using GNPs conjugated with biomolecules for medical and industrial use.

Nanoparticles of metals and semiconductors are generally considered to be useful because of their optical, optoelectronic and material properties resulting from their small size [1-4]. These properties may be valuable in applications such as sensors, spectroscopic enhancers and quantum dots [5-6]. Gold nanoparticles (GNPs) have unique properties in electrical, magnetic, and especially optical characteristics [4,7-9]. GNPs in the range of 5–20 nm are usually red in color, as the optical absorption of the nanoparticles due to a localized surface plasmon band is at around 520–550 nm. Because of this property, GNPs have been used in glass staining since the 17th century. When GNPs aggregate within interparticle distance approximately less than the average GNP diameter, the color of the GNP solution changes to purple-blue. This indicates that the absorption peak is shifted towards longer wavelengths. Michael Faraday published a research paper on this widely known phenomenon, constituting the first study on GNPs [10].

Biomolecules, such as DNA, proteins, and antibodies, are used as the molecular recognition element of a biosensor or bioassay because of their specific binding and reaction properties. A convenient optical assay can be comprised using these capabilities combined with the unique properties of GNPs. The utilization of GNPs in colorimetric analytical methods used for various applications is based on their distinctive distance-dependent optical property [7,9,11,12]. Therefore, the GNP-based colorimetric assays have attracted extensive fast-growing interest in recent years because of the potential of visual detection. Various types of GNP bioassays conjugated with biomolecules have been developed and reported by many researchers (Table 1). These biomolecules have exhibited superior molecular recognition ability. By using and conjugating with DNA, antibodies, peptides and proteins, a surprisingly simple assay can be realized. This review presents the highlights of optical assays using GNPs by classifying the conjugated biomolecules.

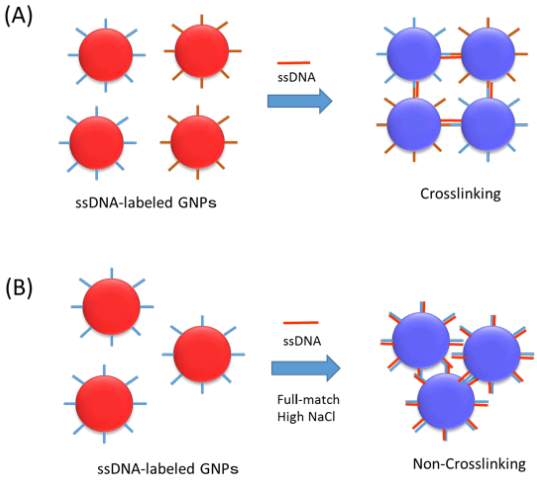

Figure 1. Colorimetric bioassays based on DNA-GNP.
(A) Cross-linking method, (B) Non-cross-linking method.

Table 1 Typical types of conjugated biomolecule on GNPs and their target analytes for GNP-based colorimetric bioassays

Recognition elements on GNPs	Target analysis	References
DNA	DNA	[13-16,18,19,22]
	Enzyme activity	[17]
	Metal ion	[20-21]
	ATP	[23]
Antigen-antibody	Antigen	[24-25]
	Antibody	[26-27]
Peptide	Photon	[31]
	Metal ion	[33-35]
	Receptor	[36]
	Enzyme activity	[39-42]
Protein	Protein	[45-47]
	Substrate	[48-50]

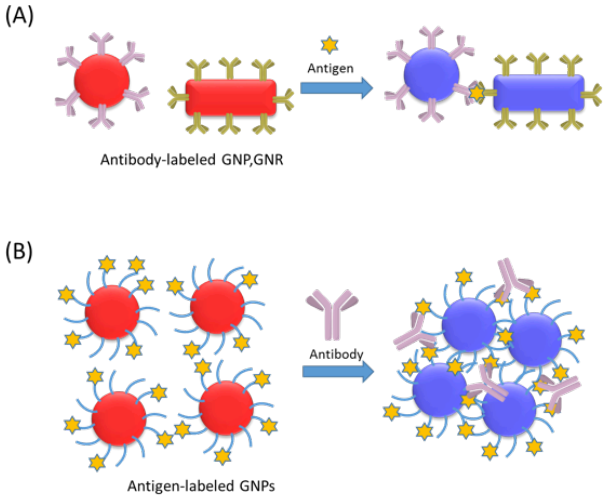

Figure 2. Colorimetric immunoassay based on Antibody-functionalized GNPs. (A) Antigen detection using GNP and GNR that were conjugated with two different primary antibodies. (B) Antibody sensing using antigen-labeled GNPs.

DNA-Functionalized Gold Nanoparticles

Colorimetric bioassays based on DNA-GNP conjugates are divided into two types according to the aggregation function (Figure 1) [13-15]. One type of aggregation method is cross-linking of the GNPs via hybridization (Figure 1A). This cross-linking method is used to not only detect target DNA sequences, but also to identify metal ions or small molecules recognized by DNases [16-21]. The other type of aggregation is the GNP non-cross-linking method which is useful in the detection of genetic polymorphisms (Figure 1B).

Mirkin et al have developed a DNA-based method for assembling colloidal gold nanoparticles rationally into macroscopic aggregates [16]. This method involves two types of GNPs modified each with a non-complementary DNA capped with thiol groups on the surface. When an oligonucleotide duplex with sticky ends complementary to the two grafted sequences is added to the GNP solution, the GNPs self-assembled into aggregates. This assembly process could be reversed by thermal denaturation.

There is a process in which the reverse reaction of the cross-linking assembly method can be applied. Liu et al have designed and developed a colorimetric Pb^{2+} sensor based on the DNAzyme-directed assembly of the GNP system [17]. The DNAzyme consists of an enzyme and a substrate strand, which can be used in the aggregation of DNA-functionalized GNPs. In the presence of the Pb ion, the DNAzyme catalyzes the specific hydrolytic cleavage of the DNA that disrupts the formation of the GNP assembly. After the reaction, the GNP solution becomes red in color.

On the other hand, the aggregation of a GNP-based assay by a non-cross-linking configuration has also been reported [22-23]. Maeda et al have designed and developed GNPs modified with a single-stranded 15mer DNA [22]. Aggregation of GNPs was formed at relatively high NaCl concentration (>/=0.5 M) by the hybridization of surface-anchored DNA with a full-match complementary DNA at room temperature. Single-base mismatches at the distal end stabilized the GNP dispersion. As a result, the colorimetric change did not progress at all. This non-cross-linking aggregation method is applicable for genetic analysis such as SNP typing.

Antibody-Functionalized Gold Nanoparticles

Antibodies are a type of protective protein produced by the immune system in response to invading foreign antigens. Antibodies are also called immunoglobulins, and their function is to identify and eliminate foreign antigens or targets such as viruses and bacteria. To conjugate the antibody with GNPs, a superior immunoassay can be constructed for medical use [24-27].

Liu et al have developed a one-step homogeneous immunoassay using GNPs for the detection of a prostate cancer biomarker [24]. A spherical GNP and a gold nanorod (GNR) were conjugated with two different primary anti-prostate specific antigen (PSA) antibodies in the immunoassay (Figure 2A). PSA is an FDA-approved biomarker for prostate cancer diagnosis. The free PSA (f-PSA) concentration was typically less than 1 ng/mL in 10% of the total PSA. In the presence of the antigen f-PSA in solution, the GNPs and GNRs aggregated with each other into pairs and formed a sandwich-type antibody–antigen–antibody linkage. This washing-free immunoassay could detect f-PSA in the concentration range of 0.1 to 10 ng/mL for dynamic light scattering measurements.

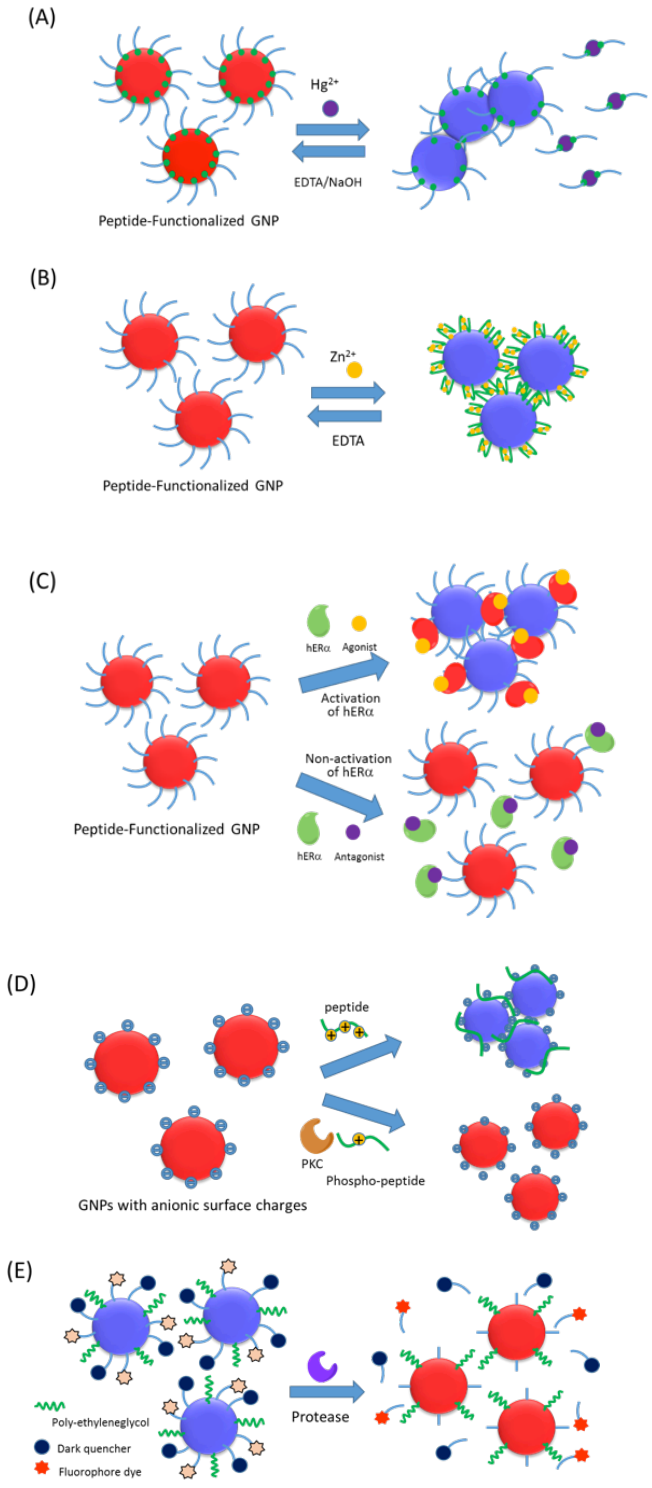

Figure 3. Peptide-Functionalized GNPs for bioassay. (A) Hg^{2+} sensing using peptide-capped GNPs. The aggregation is re-dispersed by addition of EDTA under high pH solution. (B) Zn^{2+} sensing using reversible assembly of GNPs controlled by the homodimerization and folding of an immobilized polypeptide. (C) Peptide functional GNP-based optical assay for ligand screening of human estrogen receptor alpha subtype. (D) GNP-based enzymatic activity assay for protein kinases. (E) Protease assay based on GNPs functionalized with self-assembled heterogeneous monolayers of dye-labeled peptides and poly-ethyleneglycol.

Zhou et al have studied pentapeptide Cys-Ala-Leu-Asn-Asn (CALNN) modified GNPs for aggregation-based colorimetric immunoassays [26](Figure 2B). The colorimetric GNP-based immunoassay was developed for Abscisic Acid glucose ester (ABA-GE) determination. ABA is one of the traditional plant hormones, which plays an important role in regulating plant responses to abiotic stress [28]. This hormone is also an important factor in controlling seed germination, growth, and stomatal aperture. ABA-GE is the most general formation of conjugated ABA and exhibits little or no biological activity in plant cells. The ABA-GE determination is essential to define the relationship between ABA metabolisms and environmental conditions. The ABA-functionalized GNPs were aggregated in the presence of a specific antibody. However, the aggregation was competitively inhibited by the presence of ABA-GE. This GNP-based immunoassay could detect ABA-GE with a linear response range of 5 nM to 10 μM, and the limit of detection was evaluated to be 2.2 nM. This assay is thus a simple and homogeneous method for visual detection of ABA-GE.

Peptide-Functionalized Gold Nanoparticles

Peptides are short chains of amino acid monomers comprised of 2-50 amino acids. The peptides can be used in various functional capabilities, for example as hormones [29], catalysts [30], photoresponsive [31], and molecular recognition elements [32]. Peptides can be synthesized artificially, which is an advantage for easy mass production. In this section, bioassays using functional peptide modified GNPs are introduced.

Detection of harmful mercury ions in water is essential for water quality management. Si et al have developed carboxylated peptide-functionalized GNPs that are self-assembled one-dimensionally in the presence of Hg^{2+} [33](Figure 3A). The peptide was anchored on the GNP surface through the N-terminus amino groups of the peptide, and the C-terminus carboxylate groups remained free. The peptides on the GNPs were detached from the GNP surface and formed complexes with Hg^{2+} after its addition. On the other hand, the peptides detached from the GNPs assembled into 1D arrays via those bared surfaces because of dipole-dipole interactions. The assembly of GNPs contributed to a change of color of the suspension from red to purple-blue. From the color change, detection of Hg^{2+} was possible up to parts per million levels by the naked eye.

Peptide functionalized GNP-based assays have been developed by using peptide folding. Aili et al reported a reversible assembly of GNPs controlled by the homodimerization and folding of an immobilized polypeptide [34-35](Figure 3B). The peptide folded into a helix–loop–helix four-helix bundle in the presence of Zn^{2+} at neutral pH. The peptide immobilized GNPs were

completely re-dispersed by removal of the Zn^{2+} from the peptide by addition of EDTA.

Takatsuji et al have developed the functional GNP-based optical assay for ligand screening of human estrogen receptor alpha subtype (hERα), a nuclear receptors [36] (Figure 3C). This is a transcription factor that mediates the expression of hormones-responsive genes. The transcriptional responses depend on each nuclear receptor, playing an important role in embryonic development, differentiation, reproduction and metabolic homeostasis [37-38]. Therefore, nuclear receptors have attracted attention as a target of drug development in the research of various human diseases. A synthetic peptide containing LXXLL motif of steroid-receptor coactivator 1 (SRC-1) was used for molecular recognition by ligand-activated nuclear receptors. The colorimetric GNP assay was constructed to modify the SRC-1 peptide on the GNP surface. The absorbance spectrum was altered by decrease of the GNP stability in the solvent at the time when ligand-activated hERα formed complexes with the functional GNPs. This assay shows great promise as a high throughput assay for nuclear receptor-targeted drug screening.

Peptide-functionalized GNPs for a bioassay to determine enzyme activity has also been developed for medical use [39-42]. The protein kinase C (PKC) family is one of the most important families of kinase enzymes that mediate phospholipid-dependent serine/threonine kinases [43]. PKCα belongs to the PKC family, and has been reported to be overexpressed or hyperactivated in several cancer cells or tissues. Katayama et al have developed a GNP-based enzymatic activity assay for protein kinases [40-41] (Figure 3D). Detection of kinase activity was carried out by using aggregation between cationic substrate peptides and citrate-coated GNPs with anionic surface charges. The phosphorylated PKCα-specific peptide substrates suppressed the GNP aggregation, but the color of the GNP solution changed from red to blue in the case of non-phosphorylation. Furthermore, a correlation between the color change of the GNPs and the level of activated PKCα was identified by using cancer cell lines as experimental models. This is the first report of the application of a GNP-based colorimetric assay in the diagnosis of cancer.

Serine proteases are one of the enzymes involved in a vast number of biological processes [44]. The expression level or the activity of this protease is changed in the human body as it becomes susceptible to disease as a result of a weakened immune system. Therefore, evaluating the expression level or the activity of the proteases is essential in understanding human health. Mu et al have designed and developed an in vivo proteolytic activity assay based on GNPs functionalized with self-assembled heterogeneous monolayers of dye-labeled peptides and poly-ethyleneglycol [42](Figure3E). The dye-labeled peptides were designed for sequences to be cleaved by the protease (trypsin and urokinase-type plasminogen activator). The fluorophore dye and GNPs were quenched by a dark quencher, labeled on the GNP surface through a peptide anchor. When the GNP probes were activated by the target protease, the GNPs were transformed from a dark quenched to a near-infrared fluorescent state. This simple GNP-based probe will be a strong tool in optical imaging for tumor and cancer diagnosis.

Protein-Functionalized Gold Nanoparticles

Protein-Functionalized GNPs have also been developed by many researchers [45-50]. Especially, enzymes function as a highly selective catalyst, therefore the enzyme have been used as the molecular recognition element of GNP bioassay because of the specific reaction properties. Radhakumary et al have developed a GNP-based glucose assay using glucose oxidase (GOD) modified GNPs [48](Figure4A). In the presence of D glucose, the GOD-functionalized GNPs are aggregated by reduction in zeta potential of the GNPs that is caused by enzymatic reaction. The color of the colloidal solution changed from red to blue in the presence of ~100 μg/mL glucose. The change can be observed by the naked eye. Therefore, this simple method is useful as the detection of glucose in fluids like urine for a preliminary screening at home itself.

Protein–protein interactions play important roles in structural and functional organization of living cells. Tsai et al have developed a GNP-based competitive colorimetric assay for identification of the binding partners for Concanavalin (ConA) using the ensemble of

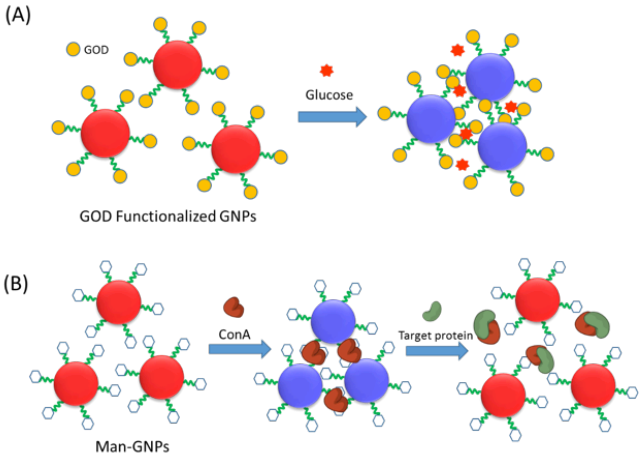

Figure 4 Bioassay using protein-functionalized GNPs. (A) Colorimetric glucose assay using glucose oxidase (GOD) modified GNPs. (B) GNP-based competitive colorimetric assay for identification of the binding protein for Concanavalin.

ConA and mannopyranoside conjugated GNPs (Man-GNPs) [45](Figure4(B)). ConA is a one of lectin, which binds specifically to α-mannose, α-galactose structures found in sugars, glycoproteins and glycolipids. In this study, ConA binds to Man-GNPs, and the complex forms agglomeration via multivalent Man–ConA interactions giving rise to a blue colored solution. However, in the presence of protein that interacts with ConA, the solution color changes from blue to red by inhibition of the binding between ConA and GNPs. The assay can perform detection of protein-protein interaction by the naked eye from the color changes without special protein modifications on the GNPs. This methodology shows great promise as a proteins evaluation for their abilities to interact with the protein of interest in real time.

Conclusion

The introduced GNP-based bioassays are simple methods for detection and have the potential to become excellent throughput assays for industrial use. Analysis equipment is not required for detection as the aggregation can be confirmed by visual observation. However, unexpected contaminants cause the aggregation of nanoparticles, providing false positives. In order to resolve this problem, molecular design of the nanoparticles for improvement of the dispersion characteristics needs to be studied furthermore for practical usage. It is expected that the advancement of technology will affect the future of nanoparticles in many ways.

References

1. Anker JN, Hall WP, Lyandres O, Shah NC, Zhao J, Van Duyne RP. Biosensing with plasmonic nanosensors. Nat Mater. 2008; 7: 442-453.
2. Murray RW. Nanoelectrochemistry: Metal nanoparticles, nanoelectrodes, and nanopores. Chem Rev. 2008; 108(7): 2688-2720.
3. Rosi NL, Mirkin CA. Nanostructures in biodiagnostics. Chem Rev. 2005; 105: 1547-1562.
4. Link S, Ei-Sayed MA. Optical properties and ultrafast dynamics of metallic nanocrystals. Annu Rev Phys Chem. 2003; 54: 331-366.
5. Katz E, Willner I. Integrated nanoparticle-biomolecule hybrid systems: Synthesis, properties, and applications. Angew Chem Int Edit. 2004; 43: 6042-6108.
6. Baron R, Willner B, Willner I. Biomolecule-nanoparticle hybrids as functional units for nanobiotechnology. Chem Commun. 2007; 323-332.
7. Ghosh SK, Pal T. Interparticle coupling effect on the surface plasmon resonance of gold nanoparticles: From theory to applications. Chem Rev. 2007; 107: 4797-4862.
8. Link S, El-Sayed MA. Spectral properties and relaxation dynamics of surface plasmon electronic oscillations in gold and silver nanodots and nanorods. J Phys Chem B. 1999; 103: 8410-8426.
9. Daniel MC, Astruc D. Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem Rev. 2004; 104: 293-346.
10. Faraday M. Experimental relations of gold and other metals to light. Philosophical Transactions of the Royal Society of London, 1857;147:145–81.
11. Zhao W, Brook MA, Li YF. Design of Gold Nanoparticle-Based Colorimetric Biosensing Assays. Chembiochem. 2008; 9: 2363-2371.
12. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. Gold Nanoparticles for Biology and Medicine. Angew Chem Int Edit. 2010; 49: 3280-3294.
13. Storhoff JJ, Mirkin CA. Programmed materials synthesis with DNA. Chem Rev. 1999; 99: 1849-1862.
14. Mori T, Maeda M. Stability change of DNA-carrying colloidal particle induced by hybridization with target DNA. Polym J. 2002; 34: 624-628.
15. Sato K, Hosokawa K, Maeda M. Colorimetric biosensors based on DNA-nanoparticle conjugates. Anal Sci. 2007; 23: 17-20.
16. Mirkin CA, Letsinger RL, Mucic RC, Storhoff JJ. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. Nature. 1996; 382: 607-609.
17. Liu JW, Lu Y. Colorimetric biosensors based on DNAzyme-assembled gold nanoparticles. J Fluoresc. 2004; 14: 343-354.
18. Cao YC, Jin RC, Thaxton S, Mirkin CA. A two-color-change, nanoparticle-based method for DNA detection. Talanta. 2005; 67: 449-455.
19. Thaxton CS, Georganopoulou DG, Mirkin CA. Gold nanoparticle probes for the detection of nucleic acid targets. Clin Chim Acta. 2006; 363: 120-126.
20. Lee JS, Han MS, Mirkin CA. Colorimetric detection of mercuric ion (Hg2+) in aqueous media using DNA-functionalized gold nanoparticles. Angew Chem Int Edit. 2007; 46: 4093-4096.
21. Liu J, Lu Y. Colorimetric Cu2+ detection with a ligation DNAzyme and nanopairticles. Chem Commun. 2007; 4872-4874.
22. Sato K, Hosokawa K, Maeda M. Rapid aggregation of gold nanoparticles induced by non-cross-linking DNA hybridization. J Am Chem Soc. 2003; 125: 8102-8103.
23. Miyamoto D, Tang ZL, Takarada T, Maeda M. Turbidimetric detection of ATP using polymeric micelles and DNA aptamers. Chem Commun. 2007; 4743-4745.
24. Liu X, Dai Q, Austin L, Coutts J, Knowles G, Zou JH, Chen H, Huo Q. A one-step homogeneous immunoassay for cancer biomarker detection using gold nanoparticle probes coupled with dynamic light scattering. J Am Chem Soc. 2008; 130: 2780-2782.
25. Wang CG, Chen Y, Wang TT, Ma ZF, Su ZM. Biorecognition-driven self-assembly of gold nanorods: A rapid and sensitive approach toward antibody sensing. Chem Mater. 2007; 19: 5809-5811.
26. Zhou GH, Liu YZ, Luo M, Xu QF, Ji XH, He ZK. Peptide-Capped Gold Nanoparticle for Colorimetric Immunoassay of Conjugated Abscisic Acid. ACS Appl Mater Inter. 2012; 4: 5010-5015.
27. Thanh NTK, Rosenzweig Z. Development of an aggregation based immunoassay for anti protein a using gold nanoparticles. Abstr Pap Am Chem S. 2002; 223: U74-U74.
28. Verslues PE, Zhu JK. New developments in abscisic acid perception and metabolism. Curr Opin Plant Biol. 2007; 10: 447-452.

29. Rosenzweig A, Seidman CE. Atrial-Natriuretic-Factor and Related Peptide-Hormones. Annu Rev Biochem. 1991; 60: 229-255.
30. Akagawa K, Akabane H, Sakamoto S, Kudo K. Organocatalytic asymmetric transfer hydrogenation in aqueous media using resin-supported peptide having a polyleucine tether. Org Lett. 2008; 10: 2035-2037.
31. Slocik JM, Tam F, Halas NJ, Naik RR. Peptide-assembled optically responsive nanoparticle complexes. Nano Lett. 2007; 7: 1054-1058.
32. Sano KI, Sasaki H, Shiba K. Specificity and biomineralization activities of Ti-binding peptide-1 (TBP-1). Langmuir. 2005; 21: 3090-3095.
33. Si S, Kotal A, Mandal TK. One-dimensional assembly of peptide-functionalized gold nanoparticles: An approach toward mercury ion sensing. J Phys Chem C. 2007; 111: 1248-1255.
34. Aili D, Enander K, Baltzer L, Liedberg B. Assembly of polypeptide-functionalized gold nanoparticles through a heteroassociation- and folding-dependent bridging. Nano Lett. 2008; 8: 2473-2478.
35. Aili D, Enander K, Rydberg J, Nesterenko I, Bjorefors F, Baltzer L, Liedberg B. Folding induced assembly of polypeptide decorated gold nanoparticles. J Am Chem Soc. 2008; 130: 5780-5788.
36. Takatsuji Y, Ikeno S, Haruyama T. Gold Nanoparticles Functionalized with Peptides for Specific Affinity Aggregation Assays of Estrogen Receptors and Their Agonists. Sensors-Basel. 2012; 12: 4952-4961.
37. Aranda A, Pascual A. Nuclear hormone receptors and gene expression. Physiol Rev. 2001; 81: 1269-1304.
38. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ. Nuclear receptors and lipid physiology: Opening the X-files. Science. 2001; 294: 1866-1870.
39. Chen CK, Huang CC, Chang HT. Label-free colorimetric detection of picomolar thrombin in blood plasma using a gold nanoparticle-based assay. Biosens Bioelectron. 2010; 25: 1922-1927.
40. Oishi J, Asami Y, Mori T, Kang JH, Niidome T, Katayama Y. Colorimetric enzymatic activity assay based on noncrosslinking aggregation of gold nanoparticles induced by adsorption of substrate peptides. Biomacromolecules. 2008; 9: 2301-2308.
41. Kang JH, Asami Y, Murata M, Kitazaki H, Sadanaga N, Tokunaga E, Shiotani S, Okada S, Maehara Y, Niidome T, Hashizume M, Mori T, Katayama Y. Gold nanoparticle-based colorimetric assay for cancer diagnosis. Biosens Bioelectron. 2010; 25: 1869-1874.
42. Mu CJ, LaVan DA, Langer RS, Zetter BR. Self-Assembled Gold Nanoparticle Molecular Probes for Detecting Proteolytic Activity In Vivo. ACS Nano. 2010; 4: 1511 -1520.
43. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science. 2002; 298: 1912-1934.
44. Hedstrom L. Serine protease mechanism and specificity. Chem Rev. 2002; 102: 4501-4523.
45. Tsai CS, Yu TB, Chen CT. Gold nanoparticle-based competitive colorimetric assay for detection of protein-protein interactions. Chem Commun. 2005; 4273-4275.
46. Lin TE, Chen WH, Shiang YC, Huang CC, Chang HT. Colorimetric detection of platelet-derived growth factors through competitive interactions between proteins and functional gold nanoparticles. Biosens Bioelectron. 2011; 29: 204-209.
47. Chen YY, Tseng CW, Chang HY, Hung YL, Huang CC. Gold nanoparticle-based colorimetric assays for coagulation-related proteins and their inhibition reactions. Biosens Bioelectron. 2011; 26: 3160-3166.
48. Radhakumary C, Sreenivasan K. Naked Eye Detection of Glucose in Urine Using Glucose Oxidase Immobilized Gold Nanoparticles. Anal Chem. 2011; 83: 2829-2833.
49. Pandey P, Singh SP, Arya SK, Gupta V, Datta M, Singh S, Malhotra BD. Application of thiolated gold nanoparticles for the enhancement of glucose oxidase activity. Langmuir. 2007; 23: 3333-3337.
50. Li DX, He Q, Cui Y, Duan L, Li JB. Immobilization of glucose oxidase onto gold nanoparticles with enhanced thermostability. Biochem Bioph Res Co. 2007; 355: 488-493.