http://pmjournal.ir Review Article

Spring 2021,Volume 6, Issue 21 (23-29)

The Application of DNA-Functionalized Gold Nanoparticles for Detection of Metabolites and Nucleic Acids in Personalized Medicine

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DOI:10.22034/pmj.2021.244733

Submitted:2021-05-13
Accepted: 2021-06-04

Keywords:
GNPs
Aptamers
Biosensors
Fluorescence
Metabolites
Personalized medicine

Abstract

In this article, the features of DNA-functionalized Gold Nanoparticles (GNPs), including the size-dependent color, the amount of conjugated DNA, and the fluorescence quenchers will be described. DNA and aptamer conjugated GNPs can be applied for producing the colorimetric and fluorescent biosensors to detect all types of disease markers including DNA, RNA, protein and other small molecular metabolites.

The early phase of this work is performed in clean buffers and serum samples. DNA-conjugated GNPs delivery into the cells is recently used for intracellular diagnosis in personalized medicine. Simultaneously, DNA- functionalized GNPs can be used to deliver the antisense DNA for gene therapy applications.

With targeting both diagnosis and treatment applications, DNA- functionalized GNPs can be used as a suitable approach to reach theranostics purposes (diagnosis and treatment in a simultaneous manner).

INTRODUCTION

Disease diagnosis is an important step in the current health system, allowing treatment interventions to act effectively. Many patients show confirmed molecular markers, and novel markers are constantly targeted in personalized medicine. Molecular markers can be found at several different levels; at the level of DNA, point mutations such as deletion and insertion, and alteration in the DNA copy number that results in variation in the gene expression and features of expressed proteins. Due to the low copy number of DNA in each cell, the direct detection of DNA is difficult, so amplification will be performed by a technique such as polymerase chain reaction (PCR). Genetic information is transcribed into mRNA and protein by central dogma process. Other factors such as microRNAs can regulate effectively the translation and the amount of expressed protein. Enzymatic proteins could be detected by their enzymatic activities, but, non-enzymatic proteins will be detected and measured by immunoassay methods. Due to the difficulties of protein extraction, it is preferred to evaluate the metabolites. Metabolites illustrate pathological conditions. They generally show higher stability and are in more concentration than proteins; the most common example is glucose detection in diabetic patients. Recently, based on the metabolic studies, many of metabolic markers have been detected. For instance, sarcosine has been suggested as a metabolic marker for prostate cancer (1).

Ideally, there is a need for a high- tech approach to detect the disease markers. DNA probes are the best candidate, as they are able to detect DNA or complement RNA. Aptamers are single stranded nucleic acids that selectively attach to the target molecule, too (2). Many of naturally aptamers ((i.e., riboswitches) are found in the non-coding regions of mRNAs of many bacteria which regulate the translation of target mRNAs based on the concentration of the metabolites they(3).

Comparing to antibodies, aptamers are smaller and more stable (specifically DNA aptamers). Although antibody products often affect animals and cause high variations in the products, aptamers can be selected

for attaching to molecules that are not too small such as peptides and proteins. Therefore, the use of DNA as a target recognition element can create a field that detects all the markers of the disease mentioned above. Nevertheless, using proteins for this purpose is difficult. For instance, proteins are poor choices for the detection of nucleic acids. Moreover, if an antibody is used for detecting a small molecule, a competitive assay is required which has a low sensitivity. Over the last 20 years, several signaling methods have been coupled with DNA and aptamers to develop biosensors. GNRs have been a general choice in relation to DNA; as they show vast optical properties (4, 5). In addition, nanoparticles are chemically neutral and non-toxic, and can be detected by microscopic and spectroscopic techniques. In this article, we have focused on the application of nanoparticles as a part of DNA-based biosensors. Recently, it has been discovered that DNA-functionalized GNPs can transfer into the cells and are therefore used in intracellular diagnosis.

PREPARATION AND CHARACTERIZATION OF GNPS

The size of GNPs that are applied to biological and biomedical analysis, is often less than 2 nm up to more than 100 nm (6). Using the surfactants, polymers and other agents simultaneously formed gold nanorods, cubes, and triangles (7).

GNPs are considered as good fluorescence quenchers that show amazing quenching efficiency for a vast range of fluorescence markers (8). High Refractive index along with the color-dependent size and the fluorescence quenching ability, has made the GNPs an appropriate candidate to design the optical biosensors.

CONJUGATION OF THIOL DNA TO GNPS

Thiolated DNAs have been applied to functionalized GNPs frequently. Although GNPs and DNA both are negatively charged, adding some salt can be reduced the electrostatic repulsion. Also, high salt concentration can compromise colloidal stability of the GNPs. This problem can be properly solved by a process named “salt aging” (9, 10) (Figure 1). It has been shown that the density of conjugated DNA is proportional to the NaCl concentration (11, 12). Therefore, even the additional NaCl can increase the amount of conjugated DNA.

Cytosine and Adenine bases can be protonated at low PH, which severely reduces the negative charges on the surface of DNA. Although the high density of DNA causes the high resistance in GNPs against salt, DNA hybridization can prohibit by several surface. For instance, the DNA hybridization can reach its highest efficiency when the loaded DNA is less than 50 DNA Molecules on 13 nM GNPs (13).

The stability of thiol-gold band is basically high, however, the bond between thiol and gold can be detached by high concentration of salt, high temperature and increasing PH level (14, 15).

OPTICAL DETECTION IN BUFFER

Optical sensors are attractive due to their high sensitivity. This type of sensor has made ocular detection possible without requirement of any analytical tools. Similar detection methods can be directly used to observe the concentrations of intracellular analytic, while detection method based on fluorescence is often used, as they are high sensitive and have low background.

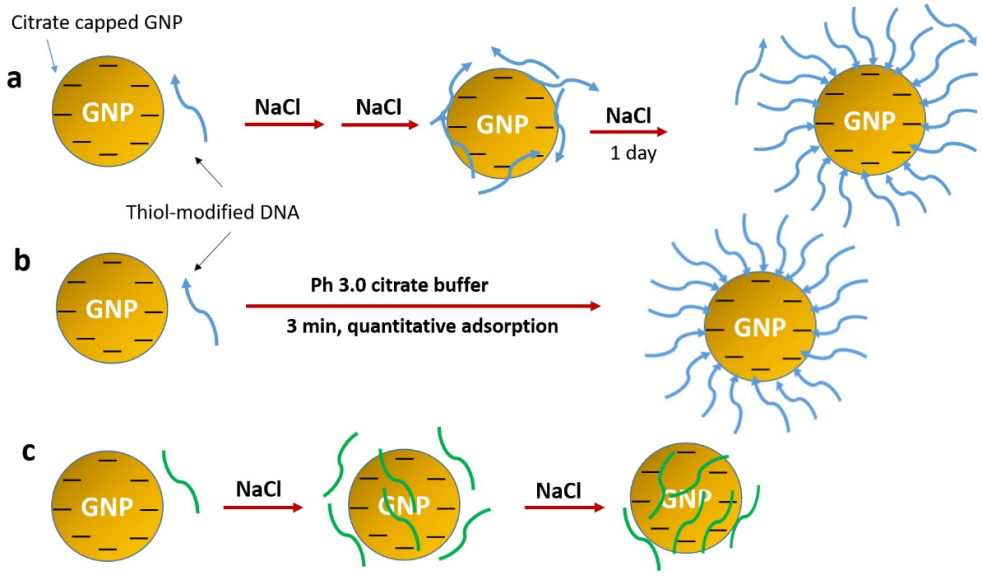

Figure 1. salt-aging method for attaching negatively charged thiolated DNA to negatively charged GNPs (a) and the low pH method (b). (c) Adsorption of non-thiolated DNA by GNPs. (16)

COLORIMETRIC DETECTION USING DIRECTLY AGGREGATED GNPS

Thiolated DNA functionalized GNPs can be used directly for colorimetric detection based on the accumulation or non-accumulation of analytic-induced GNPs. Based on the first report by Mirkin and coworkers, the target DNA causes cross-linking of two types of DNA-functionalized GNPs to make a blue or purple color (17). Detection based on a single nucleotide mismatch will be achieved with measuring the melting point of aggregated GNPs. Due to the role of the multiple DNA linkages in the melting point of DNA, the aggregated GNPs show clear change in melting point than the free DNA (18). As a result, the mismatched DNA can be detected with high accuracy. Using this method for aptamer-based detection is first reported by Leo and Lu (19). The sensor is composed of a linker DNA including an aptamer fragment that links two kinds of DNA-functionalized GNPs (Fig.2). This structure, brings two kinds of GNPs together which results in generating a blue color in solution. When the target is present (for example, Adenosine or cocaine), aptamer formation changes to attach to the target. Therefore, the hybridization is eliminated and the GNP is released. The GNPs appear red as long as they are placed in the adjacency. The results are obtained faster for higher concentrations. Adenosine can be detected in the range of 0.3 to 2 mM, while the detection of cocaine occurs between 50 to 500 µM. Multiple detection was obtained using DNA linker containing double aptameric sequences (20). Detection is also performed using the reverse tendency of the solution to change color from red to blue.

DETECTION BASED ON FLUORESCENCE

Colorimetric sensors are common detection methods for liquid samples and biological fluids such as urine and serum; however, such color change is difficult for intracellular detecting applications (21). Moreover, GNPs as powerful quenching fluorescents, have a high extinction coefficient, Comparing to molecular quenchers, GNPs can quench fluorophores in a big distances, as their quenching mechanism is different (22, 23).

THIOLATED DNA GNPS

The fluorescent quenching ability of GNPs, have made them prevalent for fluorescent-based detection (24). Presence of an analytic target alters the DNA conformation, directs the fluorophore away from the surface of GNPs, and allows the fluorescent to form normally.

Lib Chaubber describes the concept of using the GNPs as a fluorescent quencher (8). Attaching a fluorophore and a GNP with two ends of a hairpin DNA, leads to recovering the quenched fluorescent by DNA hybridization, which causes the breakage in the hairpin and detaching the fluorophore from the surface of the GNPs. Ney and et al have reported similar results. The effective quenching occurs when the fluorophore is near the GNP. In the presence of target molecule (for example Thrombin), fluorophore containing the DNA sequence will be released and can normally act as a fluorescent and produce a signal. Attached fluorophore to the DNA as DNA-GNP hybrids can be released through nucleases cleavages. In this method, a short thiol-DNA which

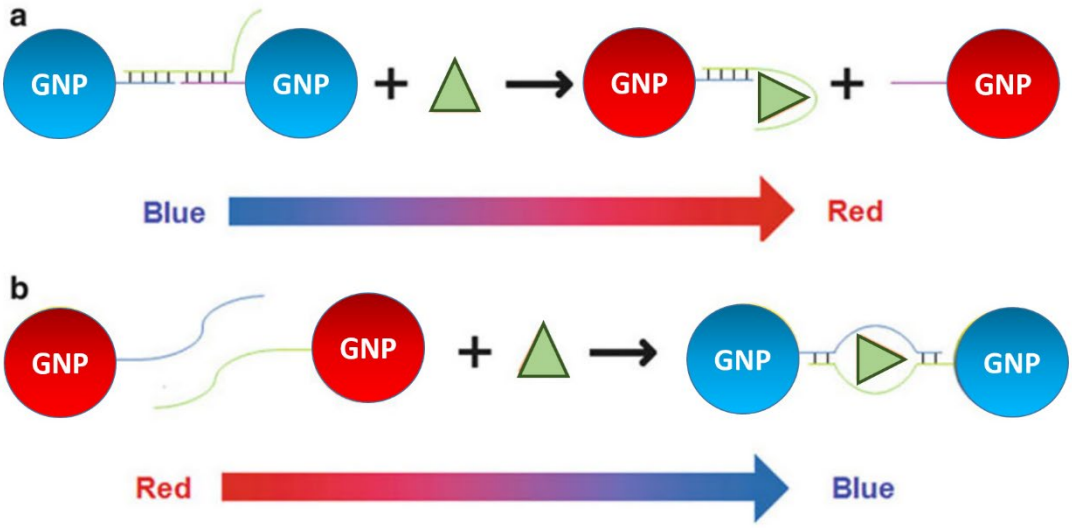

Figure 2. directed disassembly (a) or assembly (b) of aptamer-functionalized gold nanopaticles by target analytes using colorimetric detection method. The triangle shape illusrates the aptamer target

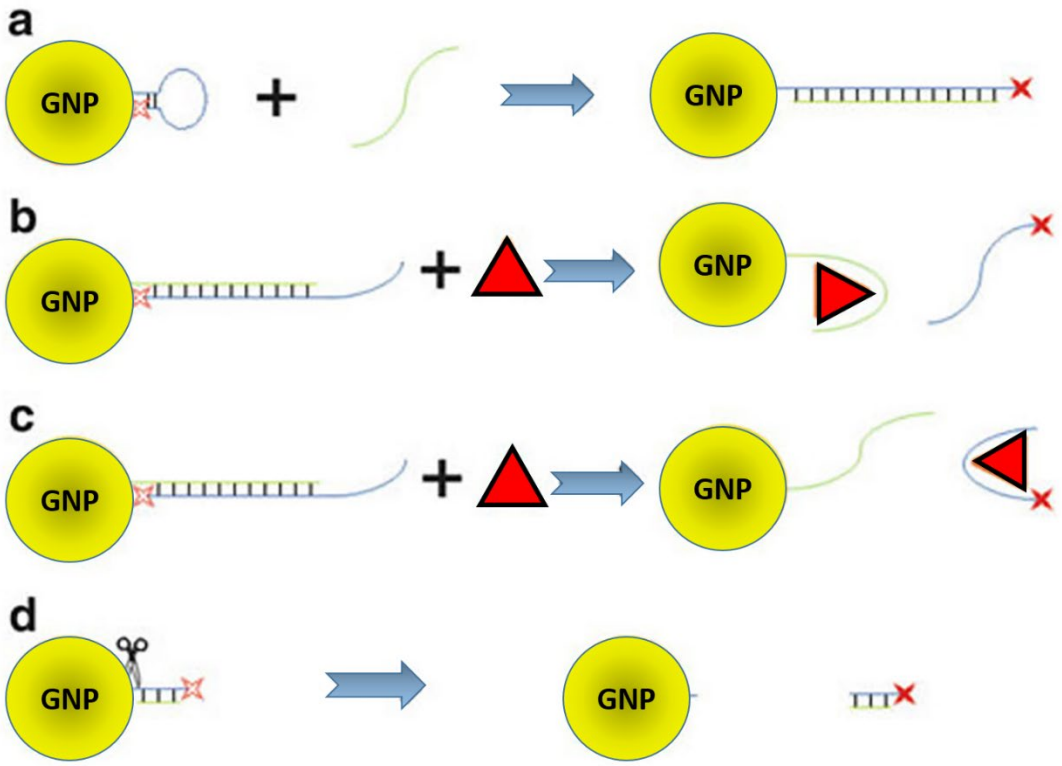

Figure 3. Fluorescent sensors using GNPs as a quencher. (a) A molecular beacon sensor for detection of DNA. Aptamer-based detection where the fluorophore is placed on the nonaptamer strand (b) or the aptamer strand (c). (d) Detection of nuclease activity

is attached to a fluorophore can make a hybrid with its cDNA. This allows the double-stranded DNA to tighten, while keeps the fluorophore within the quenching range (Fig 3D). In the presence of S1 nuclease, DNA is cleaved and fluorophore detaches from the GNP and produces the fluorescence (25).

INTRACELLULAR UPTAKE OF DNA-FUNCTIONALIZED GNPS

The real-time detection of intracellular metabolites using antibodies is extremely difficult. If the target molecule is protein, the cells often need to be fixed to performing the immunoassay.

Therefore, detection of intracellular metabolites and proteins is challenging for analytical chemistry. Developing GNPs and aptamer sensors might provide an alternative method to reach this purpose. In 2006, the first delivery of DNA-functionalized GNPs into the cell was reported by Merkin and colleagues. They designed a DNA sequence to inhibit the expression of the fluorescent reporter gene (26). Usually, the cell membrane has negative charge and therefore, the common transferring factor is polycationic which allows the electrostatic interaction between the transfer factor and the cell membrane. DNA_ functionalized GNPs show good cellular uptake, even though they are negatively charged. To determine the effective factor, the size and the zeta potential of DNA-functionalized GNPs were measured before and after the absorbance (27). It was shown that there was an increase in size and rise in zeta potential that suggests extracellular proteins adsorption by DNA strands. Further study has revealed that there might be specific proteins that mediate the cellular uptake of DNA-functionalized GNPs (28). The increase in the cellular uptake is made by scavenger receptors which act as a mediator to endocytosis of the DNA-functionalized GNP. Additionally, it has been shown that size, concentration and the aspect ratio of the GNP play roles in the absorbance of the particles. GNPs with 50 nm have the highest absorption, while larger particles need more concentration. Finally, the spherical GNPs are more likely to endocytosis than rod-shaped particles.

INTRACELLULAR DETECTION

Since DNA-functionalized GNPs have been able to produce intense fluorescent and can be absorbed by cells, they have been valuable for use in intracellular detection. GNPs are absorbed by endocytosis. Moreover, the high density of thiol-DNA strands protects their structure from enzymatic degradation. Low toxicity and good biocompatibility causes to decrease the activity of GNPs. DNA-functionalized

GNPs is considered as an alternative option for intracellular detection. For instance, GNPs can be used for intracellular detection of analyst in their natural environment, while the cell remains intact. This method was used for ATP-based intracellular detection (29). While the DNA of nanoparticle is surrounded by ATP molecule, the aptamer releases the reporter. This system can be used for a range of ATP concentrations between 0.1 to 3 mM and only a 2 hours incubation is needed for cells to form a fluorescent signal. This method has been utilized for detecting mRNA as well. Wright and coworkers were able to detect tyrosinase RNA in melanoma cells using a hairpin DNA (30). In this procedure, a hairpin complementary DNAof a portion of the tyrosinase mRNA was thiol-functionalized onto GNPs. The hairpin cDNA includes a fluorophore that has been quenched by energy transfer to the surface of the GNPs. When the DNA-functionalized GNPs are presented into a cell, the mRNA of tyrosinase opens the hairpin for hybridization,keeping the fluorophore out from the GNPs surface and normal signal generats. This method requires 0.5 nM of DNA-functionalized GNPs, and it would take up to 16 h for proper hybridization kinetics to produce a detectable signal.

GNP-BASED DNA DETECTION CLINICAL EVALUATIONS

Recently, the use of bio-barcode systems for the detection of proteins with attomolar sensitivity has been developed. This method involves trapping the analyte using a magnetic particle with detector elements, followed by the bonding of the functionalized GNPs with a second detection agent and “barcode”(marker) DNA strands (31).At first, magnetic separation of sandwich complex accures, after that the DNA barcode are released and the DNA strands are identified and quantified using the gold-nanoprobe sandwich assay method followed by silver intensification (32, 33). This method had been successfully used for measuring the concentration of amyloid-β-derived diffuse ligands, as a potential marker of alzheimer’s disease, and are present in very low concentrations (It is obtained by amplifying the signal by automatography, which leads to an increase of almost 1000 folds in the detection signal) (34).

There has been an improvement in non-crossliking DNA hybridization which was used in SNPs detection in 2006. In this method, the SPR imaging allows the detection of the SNPs to a limit of 32 nmol/L, in 5 minutes without temperature control, which makes it a promising method for SNP detection (35). SPR was also used to improve the sensitivity of p53 cDNA detection at subatomol concentrations using gold nanoprobes, which allows us to detect a 39 µg target at 15 µl of 1.38 fm L^{-1} solution, which promises a bright future for Diagnosis of cancer (36).

It is proved that there are only a few suitable strategies in clinical diagnosis in which the GNP are directly used in biological samples. Most of the methods based on using the GNP are rapid and easy, especially compared with the most general molecular techniques such as polymerase chain reaction or real-time PCR. Even though these techniques are well-developed, the high costs and the need for highly skilled and trained operations make them very difficult to apply at point-of-care and remote areas, or low resource countries. The non-cross-linking method can compete with other currently available methods at a lower cost but with greater simplicity, due to the use of a single probe. However, it completes in less than 30 minutes. Therefore, methods based on the use of GNP which have reached clinical studies can help to solve this issue. Although there are several methods and techniques using GNP to detect the DNA and RNA, however, most of these methods are controlled in laboratory settings and or implemented using synthetic molecules or molecules that have already been prepared as a target. Nowadays, there are only a few methods for direct detection of DNA/RNA in clinical samples. The advantages of using the complexes of gold-oligonucleotides for delivering into live cells includes efficient intracellular delivery, without any transfection or permeability reagents, and compared to previous approaches, increased nuclease degradation resistance and high target to background detection ratio (37, 38).

The reduction in the fluorescence background is achieved by exploiting the innate quenching features of the gold colloids (7). In this method, the surface of a colloidal GNPs is functionalized with a DNA oligonucleotide sequence as a probe that is targeted to the intracellular RNA sequence. Then, a surface oligonucleotide forms a hybrid with a fluorescent-labeled cDNA, which will be quenched due to its vicinity to the surface of the gold colloid will be quenched. After the intracellular hybridization of the construct with the target RNA, the fluorescent label strand is separated from the oligonucleotide attached to the nanoparticles and will be released into the cytoplasm. This event leads to this event leads to the emission associated with the intracellular presence of target RNA. However, since the fluorescent reporter is not combined with the target RNA and is separated in a competent manner, this strategy cannot be used for the localization of intracellular mRNA.

In this study, a new method of imaging and detecting mRNA in live cells is developed. In addition to the advantages of gold-oligonucleotides constructs such as high intracellular adsorption, maintaining the location of the target mRNA in the cell is important (38).

SUMMARY AND PROSPECTS

Thiol linked ssDNA-functionalized GNP that are used for colorimetric detection of target

DNA markers, can be considered as a low-cost and easy alternative for detections based on the fluorescence or radioactivity (39). If a nanoparticle is functionalized with a multiple of similar ssDNA oligonucleotides, and the complementary DNA is present in the environment, the nanoparticles containing the complementary probes will be brought in close vicinity of each other. Therefore, the crosslink between multiple GNP results in the vast accumulation of nanoparticles and the changes the colorimetry. In this system, the arrangement of GNP is in a tail-to-tail orientation. One of the probes was functionalized by making a crosslink with a 5′-thiol and another one with a 3′-thiol (40, 41).

Recently, the same detection method for DNA sequences has been used as a real-time screening approach to assess the nuclease activity (42). In another simple and inexpensive method, the GNP was used for the expression of eukaryotic gene (RNA) which did not require the repeated transcription or PCR (43). Due to optical characteristics and the ability to use in fluorescent biosensors and colorimetry, the GNPs have many advantages. Since the characteristics of GNP enabled it to be easily absorbed by mammalian cells, the intracellular diagnosis of metabolites and nucleic acids has been made more possible. Future efforts in this field will be possibly focused on generating a specific analytical system. Additionally, better strategies for designing sensors are required so that the produced signals, the fluorescent intensity, and its specificity are enhanced in the biological samples and intracellular. Despite the advantages of using DNA-functionalized GNPs, some still believe that the use of GNPs in clinical settings is limited. The resistance of DNA-functionalized GNPs within cells can be troublesome; especially when using the high dosage of GNPs. Therefore, more fundamental studies need to be conducted to achieve a greater understanding regarding the interaction between GNPs and cells including the toxicity and biocompatibility.

REFRENCE

1. Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature. 2009;457(7231):910-4.

2. Wilson DS, Szostak JW. In vitro selection of functional nucleic acids. Annual review of biochemistry. 1999;68(1):611-47.

3. Winkler WC, Breaker RR. Regulation of bacterial gene expression by riboswitches. Annu Rev Microbiol. 2005;59:487-517.

4. Sharifi M, Hosseinali SH, Alizadeh RH, Hasan A, Attar F, Salihi A, et al. Plasmonic and chiroplasmonic nanobiosensors based on gold nanoparticles. Talanta. 2020;212:120782.

5. Mohebbi S, Moghadam TT, Nikkhah M, Behmanesh M. RGD-HK Peptide-functionalized gold nanorods emerge as targeted biocompatible nanocarriers for biomedical applications. Nanoscale research letters. 2019;14(1):13.

6. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. science. 1990;249(4968):505-10.

7. Xia Y, Xiong Y, Lim B, Skrabalak SE. Shape-controlled synthesis of metal nanocrystals: simple chemistry meets complex physics? Angewandte Chemie International Edition. 2009;48(1):60-103.

8. Dubertret B, Calame M, Libchaber AJ. Single-mismatch detection using gold-quenched fluorescent oligonucleotides. Nature biotechnology. 2001;19(4):365-70.

9. Mirkin CA, Letsinger RL, Mucic RC, Storhoff JJ. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. Nature. 1996;382(6592):607-9.

10. Storhoff JJ, Elghanian R, Mucic RC, Mirkin CA, Letsinger RL. One-pot colorimetric differentiation of polynucleotides with single base imperfections using gold nanoparticle probes. Journal of the American Chemical Society. 1998;120(9):1959-64.

11. Demers LM, Mirkin CA, Mucic RC, Reynolds RA, Letsinger RL, Elghanian R, et al. A fluorescence-based method for determining the surface coverage and hybridization efficiency of thiol-capped oligonucleotides bound to gold thin films and nanoparticles. Analytical chemistry. 2000;72(22):5535-41.

12. Hurst SJ, Lytton-Jean AK, Mirkin CA. Maximizing DNA loading on a range of gold nanoparticle sizes. Analytical chemistry. 2006;78(24):8313-8.

13. Zu Y, Gao Z. Facile and controllable loading of single-stranded DNA on gold nanoparticles. Analytical chemistry. 2009;81(20):8523-8.

14. Bhatt N, Huang P-JJ, Dave N, Liu J. Dissociation and degradation of thiol-modified DNA on gold nanoparticles in aqueous and organic solvents. Langmuir. 2011;27(10):6132-7.

15. Herdt AR, Drawz SM, Kang Y, Taton TA. DNA dissociation and degradation at gold nanoparticle surfaces. Colloids and Surfaces B: Biointerfaces. 2006;51(2):130-9.

16. Zhang X, Servos MR, Liu J. Instantaneous and quantitative functionalization of gold nanoparticles with thiolated DNA using a pH-assisted and surfactant-free route. Journal of the American Chemical Society. 2012;134(17):7266-9.

17. Elghanian R, Storhoff JJ, Mucic RC, Letsinger RL, Mirkin CA. Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. Science. 1997;277(5329):1078-81.

18. Jin R, Wu G, Li Z, Mirkin CA, Schatz GC. What controls the melting properties of DNA-linked gold nanoparticle assemblies? Journal of the American Chemical Society. 2003;125(6):1643-54.

19. Liu J, Lu Y. Preparation of aptamer-linked gold nanoparticle purple aggregates for colorimetric sensing of analytes. Nature protocols. 2006;1(1):246-52.

20. Liu J, Lu Y. Smart nanomaterials responsive to multiple chemical stimuli with controllable cooperativity. Advanced Materials. 2006;18(13):1667-71.

21. António M, Vitorino R, Daniel-da-Silva AL. Gold nanoparticles-based assays for biodetection in urine. Talanta. 2021:122345.

22. Jennings T, Singh M, Strouse G. Fluorescent lifetime quenching near d= 1.5 nm gold nanoparticles: probing NSET validity. Journal of the American Chemical Society. 2006;128(16):5462-7.

23. Yun C, Javier A, Jennings T, Fisher M, Hira S, Peterson S, et al. Nanometal surface energy transfer in optical rulers, breaking the FRET barrier. Journal of the American Chemical Society. 2005;127(9):3115-9.

24. Ray PC, Darbha GK, Ray A, Walker J, Hardy W. Gold nanoparticle based FRET for DNA detection. Plasmonics. 2007;2(4):173-83.

25. Ray PC, Fortner A, Darbha GK. Gold nanoparticle based FRET asssay for the detection of DNA cleavage. The Journal of Physical Chemistry B. 2006;110(42):20745-8.
26. Rosi NL, Giljohann DA, Thaxton CS, Lytton-Jean AK, Han MS, Mirkin CA. Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. Science. 2006;312(5776):1027-30.
27. Giljohann DA, Seferos DS, Patel PC, Millstone JE, Rosi NL, Mirkin CA. Oligonucleotide loading determines cellular uptake of DNA-modified gold nanoparticles. Nano letters. 2007;7(12):3818-21.
28. Patel PC, Giljohann DA, Daniel WL, Zheng D, Prigodich AE, Mirkin CA. Scavenger receptors mediate cellular uptake of polyvalent oligonucleotide-functionalized gold nanoparticles. Bioconjugate chemistry. 2010;21(12):2250-6.
29. Zheng D, Seferos DS, Giljohann DA, Patel PC, Mirkin CA. Aptamer nano-flares for molecular detection in living cells. Nano letters. 2009;9(9):3258-61.
30. Harry SR, Hicks DJ, Amiri KI, Wright DW. Hairpin DNA coated gold nanoparticles as intracellular mRNA probes for the detection of tyrosinase gene expression in melanoma cells. Chemical communications. 2010;46(30):5557-9.
31. Taton TA, Lu G, Mirkin CA. Two-color labeling of oligonucleotide arrays via size-selective scattering of nanoparticle probes. Journal of the American Chemical Society. 2001;123(21):5164-5.
32. Hill HD, Mirkin CA. The bio-barcode assay for the detection of protein and nucleic acid targets using DTT-induced ligand exchange. Nature protocols. 2006;1(1):324-36.
33. Nam J-M, Jang K-J, Groves JT. Detection of proteins using a colorimetric bio-barcode assay. Nature Protocols. 2007;2(6):1438-44.
34. Storhoff JJ, Marla SS, Bao P, Hagenow S, Mehta H, Lucas A, et al. Gold nanoparticle-based detection of genomic DNA targets on microarrays using a novel optical detection system. Biosensors and Bioelectronics. 2004;19(8):875-83.
35. Sato Y, Sato K, Hosokawa K, Maeda M. Surface plasmon resonance imaging on a microchip for detection of DNA-modified gold nanoparticles deposited onto the surface in a non-cross-linking configuration. Analytical biochemistry. 2006;355(1):125-31.
36. Yao X, Li X, Toledo F, Zurita-Lopez C, Gutova M, Momand J, et al. Sub-attomole oligonucleotide and p53 cDNA determinations via a high-resolution surface plasmon resonance combined with oligonucleotide-capped gold nanoparticle signal amplification. Analytical biochemistry. 2006;354(2):220-8.
37. Rosi NL, Giljohann DA, Thaxton CS, Lytton-Jean AK, Han MS, Mirkin CA. Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. Science. 2006;312(5776):1027-30.
38. Seferos DS, Giljohann DA, Hill HD, Prigodich AE, Mirkin CA. Nano-Flares: Probes for Transfection and mRNA Detection in Living Cells. Journal of the American Chemical Society. 2007;129(50):15477-9.
39. Storhoff JJ, Lucas AD, Garimella V, Bao YP, Müller UR. Homogeneous detection of unamplified genomic DNA sequences based on colorimetric scatter of gold nanoparticle probes. Nature biotechnology. 2004;22(7):883-7.
40. Beermann B, Carrillo-Nava E, Scheffer A, Buscher W, Jawalekar AM, Seela F, et al. Association temperature governs structure and apparent thermodynamics of DNA–gold nanoparticles. Biophysical chemistry. 2007;126(1-3):124-31.
41. Li Y, Wark AW, Lee HJ, Corn RM. Single-nucleotide polymorphism genotyping by nanoparticle-enhanced surface plasmon resonance imaging measurements of surface ligation reactions. Analytical Chemistry. 2006;78(9):3158-64.
42. Xu X, Han MS, Mirkin CA. A gold-nanoparticle-based real-time colorimetric screening method for endonuclease activity and inhibition. Angewandte Chemie International Edition. 2007;46(19):3468-70.
43. Baptista P, Doria G, Henriques D, Pereira E, Franco R. Colorimetric detection of eukaryotic gene expression with DNA-derivatized gold nanoparticles. Journal of biotechnology. 2005;119(2):111-7.