Gold Island-Enhanced Multiplex Quantum Dots Fluorescent System for Biomedical Analysis of Circulating Tumor Nucleic Acids

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Abstract

**Background:** Circulating tumor nucleic acids (CTNAs) have been employed as the potential marker for tumor diagnosis and management, which are highly related to the tumorigenesis, progression and metastasis processes. Therefore, it is of significance to develop a highly-sensitive and reliable methods for detection of CTNAs, especially the multiplex point mutation detection of blood-derived CTNAs.

**Results:** Herein, a gold island-enhanced multiplex quantum dots (QDs) fluorescent platform was constructed for highly-sensitive detection of CTNAs in serum. The gold island-enhanced multiplex fluorescent strategy was designed as the highly-efficient signal giving-out mode which could amplify the fluorescence of QDs, realized a homogeneous nano-platform for the enrichment, multiplex detection and point mutations monitoring of CTNAs with the principle of base-stacking. A high sensitivity of 10 amol and desirable specificity were achieved, and the performance index for analysis of clinical CTNAs samples indicated that the gold island-enhanced multiplex QDs fluorescent strategy could realize multiplex point mutations detection of CTNAs in complex blood samples.

**Conclusions:** Hence, this platform achieved high detection rate in clinical samples that suitably met the clinical-requirements for multiplex detection and point mutations monitoring of CTNAs, and thus has the potential to serve as the tumor liquid biopsy strategy based on CTNAs.

Introduction

Circulating tumor nucleic acids (CTNAs),[1-3]the cell-free nucleic acids released from tumor lesion,[4-6] have been approved to be the potential marker for tumor diagnosis and management.[7-10] The diagnosis strategy that relies on CTNAs in blood samples could serve as a liquid biopsy approach that potentially becoming a supplement or replacing of the tumor tissue biopsies.[11-13] The existing researches indicated that tumor patients generally have relatively higher concentration and mutation probability of CTNAs than healthy groups.[14, 15] However, the concentration and mutation probability of CTNAs are drastically varied in plasma or serum samples, and it is very difficult to differentiate tumor patients from healthy person. Furthermore, the low expression level of CTNAs leads to the requirements for large volume of serum in clinical detection process, typically larger than 10 mL to obtain sufficient target CTNAs substrates.[5, 11] Thus, it is of great significance to develop a highly-sensitive, reliable strategy for the detection and mutation analysis of CTNAs.

The existing researches have proved that detection strategies based on the expression level and mutated sequences of CTNAs are the efficient assay for liquid biopsy of tumors.[5, 16-22] These strategies require highly sensitive and specific monitoring approach to detect the low abundance of CTNAs and the mutant fragments in high abundance of wild-type sequences from tumor patients.[23, 24] Among them, the detection methodology based on polymerase chain reaction (PCR) and DNA sequencing provided the possibility for CTNAs.[25, 26] PCR-based CTNAs detection provided sensitive and specific assays for clinical samples, but is not effective for the detection of point mutations in CTNAs. [5, 25, 26] Otherwise,
DNA sequencing provided an excellent assay for detections of the mutant fragments in CTNAs, but the whole implementation was expensive that could not meet the low-cost demands of routine clinical analysis, and time-consuming (2-3 weeks) greatly limited its applications.[16, 27, 28] Therefore, the development of a novel method that is more accurate, and able to detect CTNAs and its mutations directly in serum, especially multiplex point mutation detection in blood, is thus required urgently.

Herein, a homogeneous nanoplatform based on gold island [29-31] -enhanced multiplex quantum dots (QDs)[32-36] fluorescent strategy (Figure 1) was constructed to multiplex detection and point mutations monitoring of CTNAs in serum. This strategy constructed a homogeneous nanoplatform for enrichment and detection of CTNAs in the blood, and the principle of base stacking [37-40] was employed as the force to realize point mutations detection. 8 nt DNA recognition domains were connected to three kind of quantum dots (QDs) with the emission at 525nm (QDs 1), 585nm (QDs 2) and 650nm (QDs 3), to construct multi-functional recognition probes for the detection of multiplex point mutations. The multi-functional recognition probes can be easily excited by a single ultraviolet (UV) light source, [41] and three point mutations can be simultaneously detected with the emission at 525nm, 585nm and 650nm, respectively. With this strategy, a high sensitivity of 10 amol and desirable specificity was achieved. The performance index of gold island-enhanced multiplex QDs fluorescent strategy for analysis of clinical CTNAs samples indicated that it could simultaneously realize multiplex detection and point mutations monitoring of CTNAs in blood samples. Hence, this platform suitably met the strict clinical-requirements for multiplex detection and point mutations monitoring of CTNAs and thus has the potential to serve as an accurate paradigm for the liquid biopsy of tumor.

**Materials And Methods**

**Reagents.**

All the chemical reagents, such as HAuCl4, ammonium hydroxide, NaBH4 were obtained from Alfa Aesar Co., Ltd. Diethylpyrocarbonate (DEPC)-treated water and RNase inhibitor were obtained from Takara Biotechnology (Dalian) Co., Ltd. Streptavidin-modified magnetic beads were synthesized by New England BioLabs. Reagents related to electrophoresis and DNA probes were purchased from Shanghai Sangon Biotechnology Co. Ltd. SYBR I and SYBR II were purchased from Invitrogen. Invitrogen synthesized all oligonucleotides.

**Synthesis of Gold-Island.**

The synthesis of gold-island was based on the previously reported methods.30 The glass slides were firstly submerged in HAuCl4 (3 mM) followed by the addition of ammonium hydroxide with shaking for 1 min, the Au ions were absorbed by positive charge on glass slides. The treated glass slide was then washed three times with deionized water to remove the unbound Au ions. And 1 mM NaBH4 was added to reduce the gold clusters to gold seeds. At the end, the slides were incubated in a solution of HAuCl4 and
hydroxylamine at a 1:1 ratio and shaken for 5 min before washing twice with water, followed by incubation for 10 min.

**Construction of QDs Probes.**

The biotin-labeled ssDNA probes and streptavidin-labeled QDs (QDs1 to QDs3, 1 μM) were mixed at a molar ratio of 30:1, and then subjected to thermostatic reactions at 37 °C for 30 min. The free ssDNA probes were filtered with a Nanosep 100K OMEGA tubular ultrafiltration membrane (Pall Corporation, Port Washington, NY). After twice being spun and washed with PBS buffer (1×), the final products were redissolved in PBS buffer (1×) and stored at 4 °C.

**Base-Stacking Hybridization Model.**

The key part of base-stacking hybridization model is the 8 nt DNA recognition domain of signal probe. 50 nM of signal probe was employed as the signal giving-out complex. 50 nM of capture probe was mixed with 10 μL Magnetic beads (1mg/mL) for 30 min. Then, the target was captured by capture probe, and signal probe was added in to construct base-stacking hybridization model at the temperature of 37 °C.

**Cell Culture.**

Tumor cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin, 100 ug/ml streptomycin (GIBCO, Invitro-gen), at 37 °C with an atmosphere of 5% CO₂ and 95% air.

**Results And Discussion**

**Design of Gold Island-Enhanced Multiplex Quantum Dots Fluorescent System.**

A homogeneous nanoplatform based on gold island-enhanced multiplex QDs fluorescent system (Figure 1) was constructed to detect the content and multiplex point mutations of CTNAs. As shown in Figure 1A, the gold ions were reduced to the gold island on the surface of poly-L-lysine-coated glass sheet, and sulfydryl-labeled capture probes were connected to the gold island by Au-S bond for capture of target CTNAs, to form chip-like operation site. Three kind of QDs, with the emission at 525nm, 585nm and 650nm, were labeled with the biotin on three recognition DNAs to form signal probes. The principle of base stacking (Figure S1 to S3) was employed as the force to realize multiplex CTNAs detection and point mutations monitoring. The 3’ terminal of the four recognition DNA were designed as C (wild-type probe), T (135A probe), G (135C probe) and A (135T probe) to recognize the wild-type of 135G and the point mutation of 135A, 135C and 135T in the Kirsten rat sarcoma-2 virus (KRAS) genes of CTNAs. Mutated KRAS gene are proved to be associated with lung cancer, colorectal cancer and ovarian cancer. While the target exists, the capture probe on the gold island can immobilize the target, and then the mixture of signal probes was added in for identifying the target CTNAs with wild-type and point mutation sequence. After the washing steps, the signal probes were excited by UV light (300 nm), and the specific
fluorescent signals were enhanced by the gold island with the principle of surface plasmon resonance. The enhanced signals for target CTNAs were detected by the fluorescence spectrophotometer. The structure of gold island-enhanced multiplex QDs fluorescent system was shown in Figure 1B, the QDs probes were excited by the UV light, and the fluorescent signals were collected by the microscopic system that could recognize the single photon level difference. And the X-Y axis scan was executed to cover all the area of gold island chip.

**Principle Validation and Optimization of Gold Island-Enhanced Multiplex Quantum Dots Fluorescent System.**

In this section, we verified the feasibility of this system. The TEM results in Figure 2A and B presents the morphology of the synthesized gold island and the QDs-loaded gold island chip. The sulfydryl-labeled DNA probe (capture probe) was firstly labeled on the gold island, and the QDs probe was added after the target was immobilized by the capture probe. The results indicated that the gold island was punctate distributed (Figure 2A), and larger size particles appeared after the QDs loaded with the target (Figure 2B). These results proved the feasibility of gold island-enhanced multiplex QDs fluorescent system. Meanwhile, the QDs probe used in this work (QDs 1 to 3) were characterized by the dynamic light scattering (Figure 2C and Figure S4) and Zeta potentials (Figure S5). While the loaded QDs excited by the laser source, red dots were emerged. Herein, we observed the single dot in Figure 2D with the extension of time. And the fluorescent signal was detected and treated by the superposition of single photon level charge coupled device (CCD). The results indicated that the fluorescence increased with the data-acquisition time and reached a plateau at the time of 18s. Definitively, we verified the multiplex detection performance with three QDs probes. The results in Figure 2E indicated that the multiplex signals with three kinds of colors were acquired while the target presented, comparing with control group and single target. Hence, the principle of gold island-enhanced multiplex QDs fluorescent system was validated. Then, we evaluated the key factors of experiment, shown in Figure S6, S7 and S8. The results indicated that the optimal experiment conditions of hybridization time, washing time and hybridization temperature were set as 30 min, 4 times and 38°C.

**Sensitivity and Specificity of Gold Island-Enhanced Quantum Dots Fluorescent Strategy.**

With the optimized condition, the sensitivity and specificity were evaluated with the fluorescence spectrograph and gold island-enhanced multiplex QDs fluorescent system. Firstly, we verified the feasibility of multiplex detection for three circulating tumor microRNAs with gold island-enhanced multiplex QDs fluorescent system. The results in Figure 3A approved that the QDs probes could stably response to multiplex circulating tumor microRNAs, and the TEM results in Figure 3B revealed that the three QDs probes were immobilized on the surface with the targets. Then, the ‘golden standard’ fluorescence spectrograph was used for further verification of gold island-enhanced multiplex QDs fluorescent system (Figure 3C). The results in Figure 3D indicated that the QDs1, QDs2 and QDs3 probes could stably response to multiplex circulating tumor microRNAs, and the fluorescence intensity increased with the concentration of circulating tumor microRNAs. Differentiable signals of three emission peaks
corresponding to specific target were obtained. Meanwhile, a good linear response was achieved from 10
to $10^5$ pmol (Figure 3E, F and G), and a high sensitivity of 1 pmol was achieved. Furthermore, the
specificity was also evaluated by comparing with non-target CTNAs M1, M2 and random sequences (RS1
to RS3). The sequences were listed in Table S1. The results in Figure 3H and I indicated that the specific
signals were only detected with targets, and the gold island-enhanced multiplex QDs fluorescent system
achieved excellent specificity. Hereto, the feasibility of multiplex detection for three circulating tumor
microRNAs with gold island-enhanced multiplex QDs fluorescent system was confirmed by fluorescence
spectrograph.

Subsequently, the sensitivity and specificity of gold island-enhanced multiplex QDs fluorescent strategy
were evaluated. The results in Figure 4 indicated that the QDs1 probe achieved a high sensitivity of 0.1
pmol (Figure 4A), and the QDs2, QDs3 probes were 0.01 pmol (Figure 4B and C). The linear regression
analysis was executed, $R^2$ values of 0.9984, 0.9957 and 0.9811 were achieved for QDs1 to QDs3. These
results revealed a good linear relation. Meanwhile, excellent specificity was achieved by the gold island-
enhanced multiplex QDs fluorescent system with the specific targets (Figure 4A to C). The results in
Figure 4D indicated that the gold island-enhanced multiplex QDs fluorescent strategy could specifically
response to targets (T1 to T3). The above results showed that the gold island-enhanced multiplex QDs
fluorescent strategy realized high sensitivity and excellent specificity, which could respectively response
to single target and multiplex targets of CTNAs.

**Detection of CTNAs from Cultured Tumor Cell Lines and Blood of Tumor Patients.**

To test the performance of detecting complex samples, three tumor cell lines (HepG2, A549, MCF-7) with
high microRNA21 expression levels were detected by the gold island-enhanced multiplex QDs fluorescent
system. The cell samples were processed by a total RNA extraction kit after cell counting ($10^5$ cells). The
results in Figure 5A, B and C revealed that this system could stably response to the target from the tumor
cell lines. Furthermore, the blood samples from tumor patients were detected in Figure 5D, E and F, the
stable signals were also obtained. The outstanding performance for detecting complex samples with
gold island-enhanced multiplex QDs fluorescent system was proved.

Furthermore, the multiplex detection of single-base mutations was constructed for lung cancer with gold
island-enhanced multiplex QDs fluorescent strategy. The single-base mutations of 135A, 135C, 135T of
the KRAS gene were detected with the designed strategy. The detailed strategy was listed in the
Supporting Information (Figure S2 to S3). The results in Figure 6A (135A), B (135C) and C (135T)
indicated that the specific signal was obtained while the target of single-base mutation presented.
Meanwhile, the multiplex signals were obtained with the three mutated targets (Figure 6D). Definitively,
the three mutations were detected with the blood samples of lung cancer patients (Figure 6E to H). The
results showed that the gold island-enhanced multiplex QDs fluorescent strategy could stably response to
mutations of blood samples. Furthermore, we calculated the positive rates of the detections based on
single mutation, two mutations and three mutations, the results in Figure 6I revealed that the positive
rates were increased with the amount of detected mutations, the positive rates of detecting three
mutations was much higher than the two mutations, and the lowest is the single mutation detection. Therefore, the multiplex detection of single-base mutations based on gold island-enhanced multiplex QDs fluorescent strategy could response to multiplex targets with single test process. Hence, this platform achieved high detection rate in clinical samples that suitably met the strict clinical-requirements for multiplex point mutations detection of CTNAs, and thus has the potential to serve as an accurate paradigm for the tumor liquid biopsy based on CTNAs.

Conclusions

A gold island-enhanced multiplex QDs fluorescent platform was constructed for highly-sensitive detection of CTNAs that could also recognize the multiplex point mutations detection of CTNAs in serum. This strategy realized a homogeneous nano-platform for the enrichment, multiplex detection and point mutations monitoring of CTNAs with the principle of base-stacking. Furthermore, a gold island-enhanced multiplex QDs fluorescent strategy was employed as the highly-efficient signal giving-out mode, a high sensitivity of 10 amol and desirable specificity was achieved. The performance index of gold island-enhanced multiplex QDs fluorescent strategy for analysis of clinical CTNAs samples indicated that it could simultaneously detect the multiplex CTNAs and point mutations detection of CTNAs in blood samples. Hence, this platform suitably met the strict clinical-requirements for multiplex point mutations detection of CTNAs and thus has the potential to serve as an accurate paradigm for the liquid biopsy of tumor.

Declarations

Supplementary Information

Supplementary Information The online version contains supplementary material available at

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Authors’ contributions

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Availability of data and materials

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Ethics approval and consent to participate

All animal experiments were approved by the Ethics Committee of Southern Medical University.

Consent for publication

All authors agree for publication.

Competing interests

The authors declare no conflict of interest.

References

1. Aravanis AM, Lee M, Klausner RD: Next-generation sequencing of circulating tumor DNA for early cancer detection. Cell 2017, 168:571-574.

2. Manier S, Park J, Capelletti M, Burstos M, Freeman S, Ha G, Roades J, Liu C, Huynh D, Reed S: Whole-exome sequencing of cell-free DNA and circulating tumor cells in multiple myeloma. Nature communications 2018, 9:1-11.

3. O’Leary B, Hrebien S, Morden JP, Beaney M, Fribbens C, Huang X, Liu Y, Bartlett CH, Koehler M, Cristofanilli M: Early circulating tumor DNA dynamics and clonal selection with palbociclib and fulvestrant for breast cancer. Nature communications 2018, 9:1-10.

4. Fleischhacker M, Schmidt B: Cell-free DNA resuscitated for tumor testing. Nature medicine 2008, 14:914-915.

5. García-Olmo DC, Domínguez C, García-Arranz M, Anker P, Stroun M, García-Verdugo JM, García-Olmo D: Cell-free nucleic acids circulating in the plasma of colorectal cancer patients induce the oncogenic transformation of susceptible cultured cells. Cancer research 2010, 70:560-567.

6. Schwarzenbach H, Hoon DS, Pantel K: Cell-free nucleic acids as biomarkers in cancer patients. Nature Reviews Cancer 2011, 11:426-437.

7. Bernard V, Kim DU, San Lucas FA, Castillo J, Allenson K, Mulu FC, Stephens BM, Huang J, Semaan A, Guerrero PA: Circulating nucleic acids are associated with outcomes of patients with pancreatic cancer. Gastroenterology 2019, 156:108-118.
8. Gall TM, Belete S, Khanderia E, Frampton AE, Jiao LR: Circulating tumor cells and cell-free DNA in pancreatic ductal adenocarcinoma. The American journal of pathology 2019, 189:71-81.

9. Pallares RM, Thanh NTK, Su X: Sensing of circulating cancer biomarkers with metal nanoparticles. Nanoscale 2019, 11:22152-22171.

10. Soda N, Rehm BH, Sonar P, Nguyen N-T, Shiddiky MJ: Advanced liquid biopsy technologies for circulating biomarker detection. Journal of Materials Chemistry B 2019, 7:6670-6704.

11. Du S, Liu Y, Liu J, Zhao J, Champagne C, Tong L, Zhang R, Zhang F, Qin C-F, Ma P: Aedes mosquitoes acquire and transmit Zika virus by breeding in contaminated aquatic environments. Nature communications 2019, 10:1-11.

12. Das J, Kelley SO: High-Performance Nucleic Acid Sensors for Liquid Biopsy Applications. Angewandte Chemie International Edition 2020, 59:2554-2564.

13. Miranda-Castro R, Palchetti I, de-los-Santos-Álvarez N: The translational potential of electrochemical DNA-based liquid biopsy. Frontiers in Chemistry 2020, 8, 00143.

14. Diehl F, Li M, Dressman D, He Y, Shen D, Szabo S, Diaz LA, Goodman SN, David KA, Juhl H: Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proceedings of the National Academy of Sciences 2005, 102:16368-16373.

15. Liu Y, Liu J, Du S, Shan C, Nie K, Zhang R, Li X-F, Zhang R, Wang T, Qin C-F: Evolutionary enhancement of Zika virus infectivity in Aedes aegypti mosquitoes. Nature 2017, 545:482-486.

16. Thierry AR, Mouliere F, El Messaoudi S, Mollevi C, Lopez-Crapez E, Rolet F, Gillet B, Gongora C, Dechelotte P, Robert B: Clinical validation of the detection of KRAS and BRAF mutations from circulating tumor DNA. Nature medicine 2014, 20:430-435.

17. Bai Y, Lu Y, Wang K, Cheng Z, Qu Y, Qiu S, Zhou L, Wu Z, Liu H, Zhao J: Rapid isolation and multiplexed detection of exosome tumor markers via queued beads combined with quantum dots in a microarray. Nano-Micro Letters 2019, 11:59.

18. Chan K, Lo Y: Circulating tumour-derived nucleic acids in cancer patients: potential applications as tumour markers. British journal of cancer 2007, 96:681-685.

19. Das J, Ivanov I, Montermini L, Rak J, Sargent EH, Kelley SO: An electrochemical clamp assay for direct, rapid analysis of circulating nucleic acids in serum. Nature chemistry 2015, 7:569-575.

20. Schwarzenbach H: Circulating nucleic acids as biomarkers in breast cancer. Breast cancer research 2013, 15:1-9.

21. Li X, Ye M, Zhang W, Tan D, Jaffrezic-Renault N, Yang X, Guo Z: Liquid biopsy of circulating tumor DNA and biosensor applications. Biosensors and Bioelectronics 2019, 126:596-607.

22. Stobiecka M, Ratajczak K, Jakiela S: Toward early cancer detection: Focus on biosensing systems and biosensors for an anti-apoptotic protein survivin and survivin mRNA. Biosensors and Bioelectronics 2019, 137:58-71.

23. Möhrmann L, Huang HJ, Hong DS, Tsimberidou AM, Fu S, Piha-Paul SA, Subbiah V, Karp DD, Naing A, Krug A: Liquid biopsies using plasma exosomal nucleic acids and plasma cell-free DNA compared...
with clinical outcomes of patients with advanced cancers. *Clinical Cancer Research* 2018, **24**:181-188.

24. Hu P, Zhang S, Wu T, Ni D, Fan W, Zhu Y, Qian R, Shi J: **Fe–Au Nanoparticle-Coupling for Ultrasensitive Detections of Circulating Tumor DNA.** *Advanced Materials* 2018, **30**:1801690.

25. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM: **Detection of circulating tumor DNA in early-and late-stage human malignancies.** *Science translational medicine* 2014, **6**:224ra224.

26. Murtaza M, Dawson S-J, Tsui DW, Gale D, Forshew T, Piskorz AM, Parkinson C, Chin S-F, Kingsbury Z, Wong AS: **Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA.** *Nature* 2013, **497**:108-112.

27. Forshew T, Murtaza M, Parkinson C, Gale D, Tsui DW, Kaper F, Dawson S-J, Piskorz AM, Jimenez-Linan M, Bentley D: **Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA.** *Science translational medicine* 2012, **4**:136ra168.

28. Dawson S-J, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin S-F, Dunning MJ, Gale D, Forshew T, Mahler-Araujo B: **Analysis of circulating tumor DNA to monitor metastatic breast cancer.** *New England Journal of Medicine* 2013, **368**:1199-1209.

29. Ruach-Nir I, Bendikov TA, Doron-Mor I, Barkay Z, Vaskevich A, Rubinstein I: **Silica-stabilized gold island films for transmission localized surface plasmon sensing.** *Journal of the American Chemical Society* 2007, **129**:84-92.

30. Bendikov TA, Rabinkov A, Karakouz T, Vaskevich A, Rubinstein I: **Biological sensing and interface design in gold island film based localized plasmon transducers.** *Analytical chemistry* 2008, **80**:7487-7498.

31. Zhang B, Kumar RB, Dai H, Feldman BJ: **A plasmonic chip for biomarker discovery and diagnosis of type 1 diabetes.** *Nature medicine* 2014, **20**:948-953.

32. Shukla N, Nigra MM, Ondeck AD: **One-step synthesis and photoluminescence evaluation of cadmium-containing quantum dots.** *Nano-Micro Letters* 2012, **4**:52-56.

33. Kagan CR, Lifshitz E, Sargent EH, Talapin DV: **Building devices from colloidal quantum dots.** *Science* 2016, **353**: aac5523.

34. Yang Y, Zhao B, Gao Y, Liu H, Tian Y, Qin D, Wu H, Huang W, Hou L: **Novel hybrid ligands for passivating PbS colloidal quantum dots to enhance the performance of solar cells.** *Nano-micro letters* 2015, **7**:325-331.

35. Pietryga JM, Park Y-S, Lim J, Fidler AF, Bae WK, Brovelli S, Klimov VI: **Spectroscopic and device aspects of nanocrystal quantum dots.** *Chemical reviews* 2016, **116**:10513-10622.

36. Xu G, Zeng S, Zhang B, Swihart MT, Yong K-T, Prasad PN: **New generation cadmium-free quantum dots for biophotonics and nanomedicine.** *Chemical reviews* 2016, **116**:12234-12327.

37. Petersheim M, Turner DH: **Base-stacking and base-pairing contributions to helix stability: thermodynamics of double-helix formation with CCGG, CCGGp, CCGGAp, ACCGGp, CCGGU, and ACCGGUp.** *Biochemistry* 1983, **22**:256-263.
38. Friedman RA, Honig B: A free energy analysis of nucleic acid base stacking in aqueous solution. 
   *Biophysical journal* 1995, 69:1528-1535.

39. Liu W, Zhou X, Xing D: Rapid and reliable microRNA detection by stacking hybridization on 
   electrochemiluminescent chip system. *Biosensors and Bioelectronics* 2014, 58:388-394.

40. Pörschke D, Eggers F: Thermodynamics and Kinetics of Base-Stacking Interactions. 
   *European journal of biochemistry* 1972, 26:490-498.

41. Liao Y, Zhou X, Xing D: Quantum dots and graphene oxide fluorescent switch based multivariate 
   testing strategy for reliable detection of *Listeria monocytogenes*. *ACS applied materials & interfaces* 
   2014, 6:9988-9996.

Figures
Figure 1

Gold Island-Enhanced Multiplex Quantum Dots Fluorescent System for Combined Diagnosis of Multiplex Circulating Tumor Nucleic Acids. A. Formation of gold island chip and detection process for CTNAs. B. Schematic diagram of gold island-enhanced quantum dots fluorescent system.
Figure 2

Principle Validation of Gold Island-Enhanced Multiplex Quantum Dots Fluorescent System. A. TEM morphology of the synthesized gold island. B. TEM morphology of the QDs-loaded gold island chip. C. Particle size of QDs probes. D. Optimization of the time of data acquisition. E. Multiplex detection performance of gold island-enhanced multiplex quantum dots fluorescent system.
Figure 3

Sensitivity and Specificity of Gold Island-Enhanced Multiplex Quantum Dots Fluorescent System with Fluorescence Spectrograph. A. Detection of multiplex circulating tumor microRNAs. B. TEM morphology of the gold island-enhanced multiplex quantum dots chip with three circulating tumor microRNAs. C. Principle of multiplex circulating tumor microRNAs detection with fluorescence spectrograph. D. Sensitivity of multiplex circulating tumor microRNAs detection with fluorescence spectrograph. E. Linear analysis of QDs1 in Figure 3D. F. Linear analysis of QDs2 in Figure 3D. G. Linear analysis of QDs3 in Figure 3D. H. Specificity of gold island-enhanced multiplex quantum dots fluorescent system with CTNAs.
M1 and M2. I. Specificity of gold island-enhanced multiplex quantum dots fluorescent system with random sequences (RS1 to RS3).

Figure 4

Sensitivity and Specificity of Gold Island-Enhanced Multiplex Quantum Dots Fluorescent Strategy. A. Detection of T1 target with different concentration and specificity of QDs1 probe. B. Detection of T2 target with different concentration and specificity of QDs2 probe. C. Detection of T3 target with different concentration and specificity of QDs3 probe. The concentration of T1 to T3 was varied from 10^-3 pmol to 10^3 pmol. D. Single and multiplex target detection of T1, T2 and T3 targets.
Figure 5

Detection of MicroRNA from Cell Lines (HepG2, A549, MCF-7) and CTNAs in Blood of Tumor Patients. A. Detection of microRNA from HepG2 cell lines. B. Detection of microRNA from A549 cell lines. C. Detection of microRNA from MCF-7 cell lines. D. Detection of CTNAs in blood of liver cancer patients. E. Detection of CTNAs in blood of lung cancer patients. F. Detection of CTNAs in blood of breast cancer patients.
Figure 6

Multiplex Detection of Single-Base Mutations. A. Detection of 135A mutation with gold island-enhanced multiplex quantum dots fluorescent strategy. B. Detection of 135C mutation with gold island-enhanced multiplex quantum dots fluorescent strategy. C. Detection of 135T mutation with gold island-enhanced multiplex quantum dots fluorescent strategy. D. Multiplex detection of 135A, C and T with gold island-enhanced multiplex quantum dots fluorescent strategy. E. Detection of multiplex single-base mutations in sample ID 1 to 10. F. Detection of multiplex single-base mutations in sample ID 11 to 20. G. Detection of multiplex single-base mutations in sample ID 21 to 30. H. Detection of multiplex single-base mutations in
sample ID 31 to 40. I. Positive rate of the single, two and three mutation detection results in Figure 6E to H.

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