Hindawi Publishing Corporation
Journal of Chemistry
Volume 2013, Article ID 631421, 6 pages
http://dx.doi.org/10.1155/2013/631421

Review Article

Fluorescent DNA Stabilized Silver Nanoclusters as Biosensors

Alfonso Latorre, Romina Lorca, and Álvaro Somoza

Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia), CNB-CSIC-IMDEA Nanociencia Associated Unit "Unidad de Nanobiotecnología", 28049 Madrid, Spain

Correspondence should be addressed to Alfonso Latorre; alfonso.latorre@imdea.org and Álvaro Somoza; alvaro.somoza@imdea.org

Received 26 April 2013; Accepted 18 June 2013

Academic Editor: Eugen Stulz

DNA stabilized fluorescent silver nanoclusters are promising materials, of which fluorescent properties can be exploited to develop sensors. Particularly, the presence of a DNA strand in the structure has promoted the development of gene sensors where one part of the sensor is able to recognize the target gene sequence. Moreover, since oligonucleotides can be designed to have binding properties (aptamers) a variety of sensors for proteins and cells have been developed using silver nanoclusters. In this review the applications of this material as sensors of different biomolecules are summarized.

1. Introduction

The binding capabilities of oligonucleotides have allowed the development of sensors for the detection of different molecules, such as DNA or RNA through base complementarity interactions or proteins and small molecules using properly designed aptamers. Different strategies have been developed depending on the detection method, such as electrochemistry [1, 2], luminescence [3, 4], or surface plasmon resonance [5–7]. However, one of the most used methodologies is fluorescence [8–10], due to the sensitivity and simplicity of this technique. Despite these advantages, this strategy has some drawbacks to be solved. For instance, oligonucleotides have to be modified with a dye and sometimes even with a quencher, as in Molecular Beacons, making the process more tedious and costly. In addition, the organic dyes employed are sensitive to photobleaching and some of them have significant background fluorescence, which could affect the sensitivity.

Fluorescent DNA stabilized silver nanoclusters [11, 12] (DNA-AgNC) have emerged as new powerful nanomaterials in biology. Their unique properties, such as small size [13], biocompatibility, great photostability, and tunable fluorescence [14–16], make these materials excellent candidates to rival quantum dots [17] and organic dyes [18, 19] in different applications, such as biolabels and biosensors.

The introduction of metallic silver in DNA has been explored by other approaches, such as metalation of DNA [20]. But the preparation of DNA AgNCs is quite simple affording discrete number of atoms of silver on the DNA with fluorescent properties. Typically, a solution of an oligonucleotide is incubated with six equivalents of silver nitrate ($AgNO_3$) for 15 min. Then, six equivalents of sodium borohydride ($NaBH_4$) are added, vortexed, and incubated in the dark for several hours. The stabilization of these clusters is mainly favored by the high affinity of Ag^+ by N3 of cytosines [21, 22]. Guanines are able to interact with Ag^+ through the N7 in less extent. Although the influence of the oligonucleotide on the fluorescence properties of AgNC is not clearly understood, it can be easily modulated by the oligonucleotide sequence or length, allowing the preparation of a wide palette of emitters from blue to near IR wavelengths [14]. The fluorescent properties of this nanomaterial are also affected by the environment [23]. Thus, changes in the secondary structures of DNA-AgNC promoted by hybridization of a complementary sequence or complexation with a molecule may promote an emission wavelength shift or a drastic enhancement or decrease in the fluorescence intensity. This property has been exploited to develop sensors for ions [24–27], small molecules [28–30], DNA, RNA, proteins, and cells. In this article we have summarized the biological applications of DNA-AgNCs.

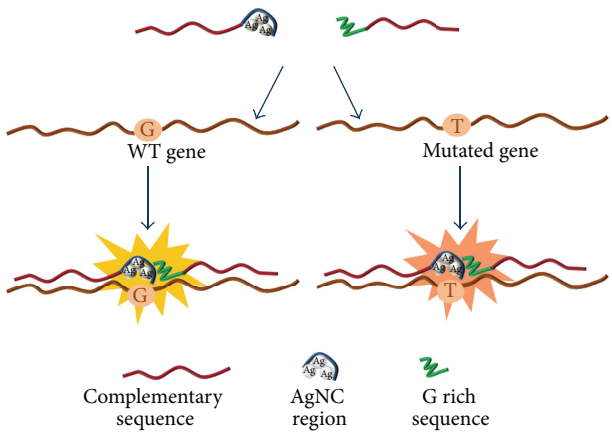

Figure 1: Chameleon Nanocluster Beacon [32, 33]. Nanoclusters interact with different regions of the G fragment depending on the mutated base. The different final structures obtained lead to different colors.

2. Gene Detection

DNA-AgNCs have been successfully applied in the detection of sequences of DNA and RNA. The most common approach requires the preparation of an oligonucleotide with two different domains. One of them has a sequence complementary to the DNA target, and other domain is able to bind silver ions, which will be reduced to give rise to fluorescent clusters. In this sense, Chang and coworkers were able to detect with high selectivity and sensitivity a gene associated with tyrosinemia type I disease [31]. They found that a C12 sequence directly connected to the probe sequence was able to generate the corresponding fluorescent AgNC, which emission was strongly enhanced after hybridization with the target gene. Interestingly, the system was able to discriminate between the mutated and wild type gene, because the fluorescence intensity for the mutated one was almost 9 times bigger than the wild type. This difference could be due to duplex structures obtained after hybridization, where the most rigid could protect better the AgNC from quenching.

In another strategy employed to detect single stranded DNA, the sensor developed uses two different single strands complementary to the target. One strand has a poly C fragment required for the generation of AgNC, and, the second one has a guanine rich fragment. Upon hybridization with the target, both fragments get close leading to an enhancement and shifting of the fluorescence emission. This system was named NanoCluster Beacon [32] due to the similarities with classical molecular beacons. Based on this work, the same authors reported the preparation of a sensor able to distinguish between the three possible single point mutation of a KRAS gene in clinical samples. In this new probe, named Chameleon NanoCluster Beacon [33], the nanoclusters interact with different regions of the G rich fragment depending on the gene mutation (Figure 1). As a consequence of these interactions, different colors are obtained with enough wavelength separation to be observed by the naked eye under UV irradiation.

DNA duplexes are also able to stabilize AgNC and have been used to develop DNA sensors. In this regard, Wang and coworkers used hybridized duplexes to detect single point mutations in sickle cell disease [34]. In this work, the authors designed a complementary probe sequence to a fragment of the corresponding gene, bearing a C6 loop two nucleotides away from the mutation. After hybridization, AgNCs were generated in the C6 loop, due to the binding affinity of cytosine to silver ions. They observed a strong yellow emission when a match sequence is used. On the other hand, fluorescence was negligible when a mismatch is present. The effect of the distance between the mutation and the loop, as well as the nature of the mismatch, was evaluated, highlighting again the sensitivity of AgNC to the environment. In this sense, they found that the presence of a mutation four nucleotides away from the loop does not affect the emission parameters. Also, it was found that a T or A mismatch produced a higher quenching of the fluorescence than the C or G.

It has also been reported the use of DNA duplexes with abasic sites [35], where the nucleobases are missing leaving the deoxyribose residue in the strand, in the preparation of AgNCs. Shao and coworkers reported the high affinity of silver ions by the unpaired bases opposite to an abasic site, which are able to accommodate few Ag^+ [36]. This finding was applied in the preparation of a sensor for the p53 gene, which is involved in cancer suppression processes [37]. A probe complementary to a fragment of the corresponding gene, with an abasic site opposite to the nucleotide responsible for the mutation, was employed. Hybridization experiments with the wild type fragment, and the three possible mutations and subsequent incubation/reduction procedure with Ag^+ and $NaBH_4$ gave rise to the corresponding silver nanoclusters. The emitters formed with the mutant bases (A, G, and T) opposite to the abasic site showed similar weak intensities. However, when the wild type fragment was used, an increase in fluorescence of 18-fold was observed, indicating the selectivity of the process. The differences observed are due to the affinity of the cytosine to silver, which is present in the wild type example.

3. MicroRNA Detection

MicroRNAs (miRNAs) are single stranded ribonucleotides of around 22 nucleotides long. They can be found in plants, animals, and humans and are involved in gene silence processes avoiding the expression of certain proteins. The detection and quantification of those structures are of great importance since they are involved in the initiation as well as the generation of cancers [38].

DNA-AgNC has been successfully used in miRNA detection as demonstrated by Yang and Vosch [39]. They were able to detect the miR160, which is a miRNA that targets a plant gene involved in hormone signaling. They designed a probe composed of two parts: a complementary sequence to the miR160 (part A), and directly grafted another fragment able to stabilized red emitting AgNC (part B). The incubation of this probe with $AgNO_3$ and subsequent reduction

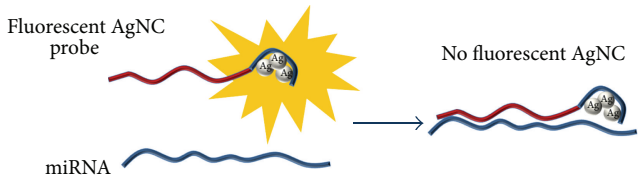

Figure 2: miRNA sensor based in DNA-AgNCs [39, 40]. Fluorescence of DNA-AgNC is quenched upon miRNA target binding.

with $NaBH_4$ afforded the corresponding highly fluorescent material. The incubation of these brighter clusters with the target afforded a drastic decrease of the fluorescence, allowing its detection (Figure 2). This system has been applied to the detection of the miR160 in total RNA extracts of plants. More recently, slight variations on the system described, have led to the same authors detect the miR172 at 82 nM of concentration [40]. Also, the system showed high selectivity, since the experiments with four random miRNA did not produce a comparable fluorescence quench.

Isothermal exponential amplification [41, 42] in conjunction with AgNC has been used to develop an attomolar miRNA sensor [43]. In such system, a single stranded DNA with different fragments acts as template of miRNA recognition and replicator of a reporter DNA molecule. The reporter is obtained upon the miRNA hybridization, and it is used to generate the corresponding AgNC which fluorescence works as positive signal indicator. MiR141, overexpressed in prostate cancer, was the miRNA selected for this study. This target was detected in real cell lysates samples in concentrations of 10 aM, representing one of the most sensitive methods in miRNA sensing. In order to check the specificity of the sensor, two miRNA of the target family and two random miRNA were used as controls. As expected, just the familiar RNA gave a substantial fluorescent signal, but it was lower compared with the fluorescence obtained with the target.

4. Protein Detection

Oligonucleotides can be designed to bind a wide variety of molecules with high selectivity. Those oligonucleotides with such binding capabilities, known as aptamers, have been used coupled with DNA-AgNC to develop protein sensors. Those sensors have some advantages with respect to previously reported [44] since a covalent substitution with a reporter molecule (dye, electrochemical, etc.) is not necessary making the process more efficient and cost effective.

The first example of a protein sensor using aptamer functionalized DNA-AgNC was reported by Martinez and coworkers [45]. In this example, a DNA probe composed of a thrombin aptamer and a C rich fragment was employed to detect such protein with a detection limit of 1 nM. Due to the silver binding capability of cytosines, fluorescent AgNC could be generated selectively on the C rich fragment. Those clusters showed good photostability and stability in bioassay conditions, but in the presence of thrombin the fluorescence was quenched. This system showed a good specificity as deduced by the results obtained with other four proteins used

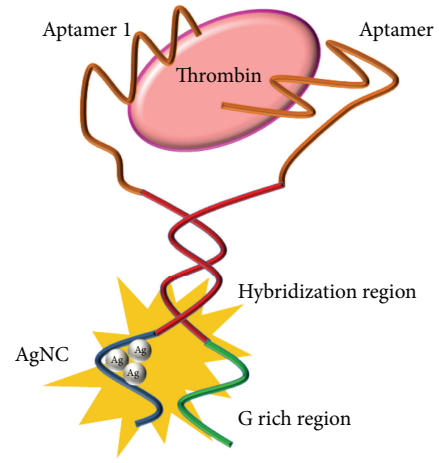

Figure 3: Principle of binding-induced fluorescence turn-on assay for protein detection [46]. After aptamer's binding to human α-thrombin, the hybridization of complementary sequences takes place, bringing together the G rich fragments and AgNCs. This interaction leads to an enhancement of the fluorescence of AgNCs.

as controls, where any quenching effect was detected. The mechanism of quenching was also studied and attributed to a change in the structure of AgNC induced by protein binding to the aptamer.

The same protein was also detected using a turn-on strategy. In this case, the authors manage to detect thrombin at 1 nM by the fluorescence amplification of AgNC when a G rich protein is in close proximity [46]. The design involves two different aptamers modified with two complementary sequences. In addition, one of them has a terminal fragment able to form AgNC, and the other one has a terminal G rich fragment. Once the two aptamers bind the thrombin, the complementary sequences are close enough to hybridize, approaching the G rich fragment to AgNC. As a consequence, the fluorescence is turned from a weak green emission to highly red one (Figure 3). The specificity of the method was tested, comparing the target with other four proteins. In all cases the fluorescence enhancement was lower compared with the thrombin, even when the concentration of controls was 10 times higher.

Another original turn-on strategy based on fluorescent silver nanoclusters was developed to detect the PDGF-BB protein [47], a protein involved in some human cancers. In this case, two-folded oligonucleotides are required. The first one is an aptamer, which conformation changes upon binding of its target. This structural rearrangement leads to the exposition of a domain of the aptamer, which is able to bind and unfold the other oligonucleotide. Then, the unfolded oligonucleotide is able to stabilize AgNCs, after the addition of $AgNO_3$ and $NaBH_4$ (Figure 4). The limit of detection of this protein using this approach was 0.37 nM. The sensor specificity was demonstrated using different proteins such as PDGF-AB, a dimeric form with 60% of the amino acids identical to the target. Furthermore, the sensor was able to detect the protein in saliva samples at 5 nM concentration

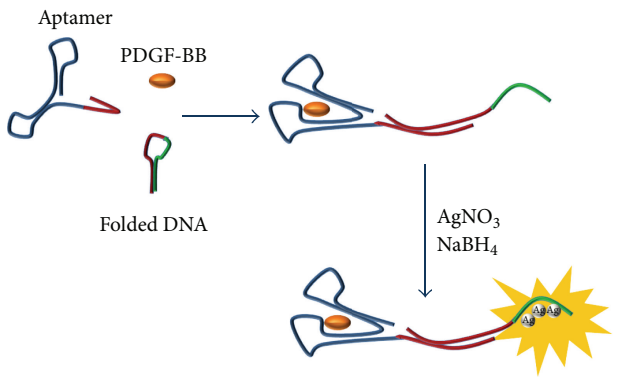

Figure 4: In the absence of PDGF-BB, both aptamer and DNA probes are folded [47]. In this state, fluorescent AgNCs cannot be formed. However, in the presence of PDGF-BB, the nucleation sequence is released and AgNCs can be formed. By monitoring the increase in fluorescence intensity, the protein was detected with high sensitivity.

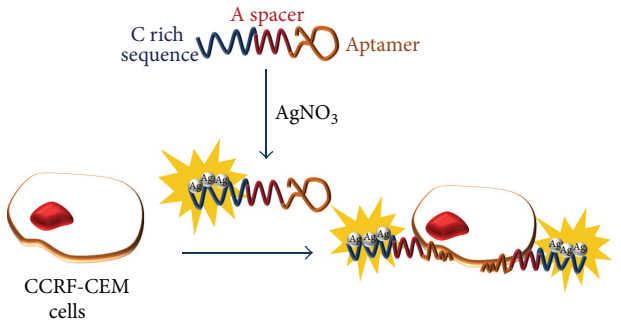

Figure 5: Illustration of the preparation and use of an AgNC-aptamer as specific sensor for the detection of tumor cells [48, 49].

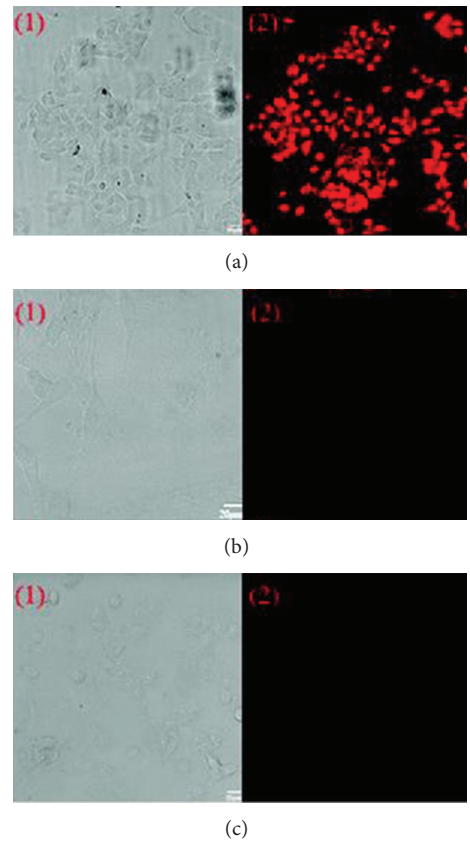

Figure 6: Confocal laser scanning microscopy [50] of (a) MCF-7 human breast cancer cells, (b) NIH-3T3 mouse fibroblast cells, and (c) MCF-10A human normal mammary epithelial cells incubated with aptamer-AgNC: (1) bright-field images; (2) AgNCs fluorescence images (red). Under the same procedure, there were no detectable emissions from the control cells. Reprinted with permission from Li et al. [50]. Copyright (2012) American Chemical Society.

without any interference, showing the capability of the sensor to be used in real samples.

5. Cell Labeling

Aptamers functionalized with silver nanocluster have been also commonly employed for surface cell labeling, nuclei staining, and tumor cell sensing, due to their ease of synthesis, specific binding capability, and photochemical properties.

In 2011, Sun and coworkers used an aptamer-DNA-AgNC conjugate to stain specifically the nuclei of CCRF-CEM cells [48]. The probe was synthesized by adding a C8 tail to 5′ end of the aptamer sgc8c, followed by nanocluster generation. This aptamer was able to bind the human protein tyrosine kinase 7 (PTK7) presented in the membrane of CCRF-CEM cells, internalize into the cells, and finally reach the nuclei, as observed by fluorescence microscopy. The specificity of this probe was demonstrated by the incubation of the same aptamer with HeLa cells, lacking the protein PTK7. In this case, no detectable fluorescent signal was observed.

In a similar report, Yin and coworkers developed in 2012 a sensor of CCRF-CEM cells, using the sgc8c aptamer and a poly C tail connected by an A6 spacer (Figure 5) [49]. They found that the fluorescence intensity and photostability of DNA-AgNC generated using this spacer were better compared with others and much better than probes lacking a spacer. Then, the specific sensing capability of CEM cells was demonstrated using flow cytometry and confocal microscopy. Furthermore, the same strategy has allowed the detection of Ramos cells just replacing the proper aptamer sequence.

MCF-7 nuclei were specifically detected using another aptamer-AgNC conjugate [50]. In this case, AS1411 nucleolin antiproliferative aptamer was conjugated to a poly C (12nt) through a 5T spacer. The presence of this spacer was crucial to get high-emission intensities of the AgNC generated. The specificity was demonstrated after the incubation of the aptamer-AgNC conjugate with MCF-7, NIH-3T3 mouse fibroblast, and MCF-10A human normal mammary epithelial cells. Only in the case of MCF-7 cells, fluorescence was detected, due to the strong affinity of AS1411 by the nucleolin presented in the membrane (Figure 6). Furthermore, the antiproliferative function of AS1411 was retained as well, and the efficiency of growth inhibition unexpectedly improved.

Proteins and antibodies have been also employed with success for membrane cell targeting, as Yu and coworkers reported in 2008 [51]. In this work, the authors synthesized an avidin DNA-AgNC conjugate using an orthogonal reaction between a thiolated DNA oligonucleotide and a maleimide group incorporated in the protein. Then, the modified oligonucleotide was incubated with biotinylated live cells, which showed a diffuse fluorescence and some silver aggregates, which were not observed in nonbiotinylated cells. This result evidenced the interaction between the avidin and biotin signaled by the fluorescence of silver nanoclusters. On the other hand, antibodies conjugated to DNA-AgNC, with heparin sulfate polysaccharide (SH receptor) affinity, were used to stain the cell membrane and nuclei of live cells. Thus, when the antibody conjugate was incubated at 4°C during 15 min, fluorescence was detected only in the cell membrane due to the interaction of the antibody with the SH receptor. However, after incubation at 37°C, the fluorescence was centered in the nuclei, suggesting an endocytosis process. Therefore, not only cell surface but internalization processes can be investigated using these materials.

6. Conclusion and Outlook

Fluorescent properties of DNA-AgNC are far to be completely understood. The influence of several parameters such as DNA sequence, environment, and structural changes on the emission of these materials represents a challenge to be solved. In spite of this drawback, the easy preparation and the lack of interferences with the probe targeting properties have promoted the development of DNA-AgNC-based biosensors. The use of DNA-AgNC for biosensing might suppose a considerable advantage, since fluorescent and electrochemical tags as well as the use of nanoparticles can be avoided. In addition, the sensibility and specificity of the biosensors reported open the door to further investigations which might render the discovery of optimized or new sensors with general applications.

Acknowledgments

Spanish Ministry of Science and Innovation (Grant: SAF 2010-15440) and IMDEA Nanociencia are acknowledged for financial support.

References

[1] H. Chang, Y. Wang, and J. Li, "Electrochemical DNA sensors: from nanoconstruction to biosensing," *Current Organic Chemistry*, vol. 15, no. 4, pp. 506–517, 2011.

[2] A. A. Gorodetsky, M. C. Buzzeo, and J. K. Barton, "DNA-mediated electrochemistry," *Bioconjugate Chemistry*, vol. 19, no. 12, pp. 2285–2296, 2008.

[3] L. L. Li, P. Ge, P. R. Selvin, and Y. Lu, "Direct detection of adenosine in undiluted serum using a luminescent aptamer sensor attached to a terbium complex," *Analatical Chemistry*, vol. 84, no. 18, pp. 7852–7856, 2012.

[4] H. Huang, Y. Tan, J. Shi, G. Liang, and J. Zhu, "DNA aptasensor for the detection of ATP based on quantum dots electrochemiluminescence," *Nanoscale*, vol. 2, no. 4, pp. 606–612, 2010.

[5] J. Spadavecchia, A. Barras, J. Lyskawa et al., "Approach for plasmonic based DNA sensing: amplification of the wavelength shift and simultaneous detection of the plasmon modes of gold nanostructures," *Analytical Chemistry*, vol. 85, no. 6, pp. 3288–3296, 2013.

[6] G. Pelossof, R. Tel-Vered, X. Liu, and I. Willner, "Amplified surface plasmon resonance based DNA biosensors, aptasensors, and Hg2+ sensors using hemin/G-quadruplexes and Au nanoparticles," *Chemistry*, vol. 17, no. 32, pp. 8904–8912, 2011.

[7] L. Wang, J. Li, S. Song, D. Li, and C. Fan, "Biomolecular sensing via coupling DNA-based recognition with gold nanoparticles," *Journal of Physics D*, vol. 42, no. 20, Article ID 203001, 2009.

[8] D. Huang, C. Niu, X. Wang, X. Lu, and G. Zeng, "'Turn-on' fluorescent sensor for Hg2+ based on single-stranded DNA functionalized Mn: CdS/ZnS quantum dots and gold nanoparticles by time-gated mode," *Analytical Chemistry*, vol. 85, no. 2, pp. 1164–1170, 2013.

[9] T. Tokunaga, S. Namiki, K. Yamada et al., "Cell surface-anchored fluorescent aptamer sensor enables imaging of chemical transmitter dynamics," *Journal of the American Chemistry Society*, vol. 134, no. 23, pp. 9561–9564, 2012.

[10] X. J. Xing, X. G. Liu, L. Yue-He, Q. Y. Luo, H. W. Tang, and D. W. Pang, "Graphene oxide based fluorescent aptasensor for adenosine deaminase detection using adenosine as the substrate," *Biosensors & Bioelectronics*, vol. 37, no. 1, pp. 61–67, 2012.

[11] A. Latorre and Á. Somoza, "DNA-mediated silver nanoclusters: synthesis, properties and applications," *ChemBioChem*, vol. 13, no. 7, pp. 951–958, 2012.

[12] B. Han and E. Wang, "DNA-templated fluorescent silver nanoclusters," *Analytical and Bioanalytical Chemistry*, vol. 402, no. 1, pp. 129–138, 2012.

[13] J. Zheng, P. R. Nicovich, and R. M. Dickson, "Highly fluorescent noble-metal quantum dots," *Annual Review of Physical Chemistry*, vol. 58, pp. 409–431, 2007.

[14] J. Sharma, R. C. Rocha, M. L. Phipps et al., "A DNA-templated fluorescent silver nanocluster with enhanced stability," *Nanoscale*, vol. 4, no. 14, pp. 4107–4110, 2012.

[15] S. Choi, J. Yu, S. A. Patel, Y. Tzeng, and R. M. Dickson, "Tailoring silver nanodots for intracellular staining," *Photochemical and Photobiological Sciences*, vol. 10, no. 1, pp. 109–115, 2011.

[16] B. Sengupta, C. M. Ritchie, J. G. Buckman, K. R. Johnsen, P. M. Goodwin, and J. T. Petty, "Base-directed formation of fluorescent silver clusters," *Journal of Physical Chemistry C*, vol. 112, no. 48, pp. 18776–18782, 2008.

[17] H. Mattoussi, G. Palui, and H. B. Na, "Luminescent quantum dots as platforms for probing in vitro and in vivo biological processes," *Advanced Drug Delivery Reviews*, vol. 64, no. 2, pp. 138–166, 2012.

[18] F. Wang, W. B. Tan, Y. Zhang, X. Fan, and M. Wang, "Luminescent nanomaterials for biological labelling," *Nanotechnology*, vol. 17, no. 1, pp. R1–R13, 2006.

[19] A. Waggoner, "Fluorescent labels for proteomics and genomics," *Current Opinion in Chemical Biology*, vol. 10, no. 1, pp. 62–66, 2006.

[20] C. T. Wirges, J. Timper, M. Fischler et al., "Controlled nucleation of DNA metallization," *Angewandte Chemie*, vol. 48, no. 1, pp. 219–223, 2009.

[21] A. Ono, S. Cao, H. Togashi et al., "Specific interactions between silver(i) ions and cytosine-cytosine pairs in DNA duplexes," *Chemical Communications*, no. 39, pp. 4825–4827, 2008.

[22] D. A. Megger and J. Müller, "Silver(I)-mediated cytosine self-pairing is preferred over hoogsteen-type base pairs with the artificial nucleobase 1,3-dideaza-6-nitropurine," *Nucleosides, Nucleotides and Nucleic Acids*, vol. 29, no. 1, pp. 27–38, 2010.

[23] B. Sengupta, K. Springer, J. G. Buckman et al., "DNA templates for fluorescent silver clusters and i-motif folding," *Journal of Physical Chemistry C*, vol. 113, no. 45, pp. 19518–19524, 2009.

[24] L. Deng, Z. Zhou, J. Li, T. Li, and S. Dong, "Fluorescent silver nanoclusters in hybridized DNA duplexes for the turn-on detection of Hg2+ ions," *Chemical Communications*, vol. 47, no. 39, pp. 11065–11067, 2011.

[25] W. Chen, G. Lan, and H. Chang, "Use of fluorescent DNA-templated gold/silver nanoclusters for the detection of sulfide ions," *Analytical Chemistry*, vol. 83, no. 24, pp. 9450–9455, 2011.

[26] G. Lan, W. Chen, and H. Chang, "Characterization and application to the detection of single-stranded DNA binding protein of fluorescent DNA-templated copper/silver nanoclusters," *Analyst*, vol. 136, no. 18, pp. 3623–3628, 2011.

[27] G. Lan, W. Chen, and H. Chang, "Control of synthesis and optical properties of DNA templated silver nanoclusters by varying DNA length and sequence," *RSC Advances*, vol. 1, no. 5, pp. 802–807, 2011.

[28] X. Guo, L. Deng, and J. Wang, "Oligonucleotide-stabilized silver nanoclusters as fluorescent probes for sensitive detection of hydroquinone," *RSC Advances*, vol. 3, no. 2, pp. 401–407, 2013.

[29] S. Han, S. Zhu, Z. Liu, L. Hu, S. Parveen, and G. Xu, "Oligonucleotide-stabilized fluorescent silver nanoclusters for turn-on detection of melamine," *Biosensors and Bioelectronics*, vol. 36, no. 1, pp. 267–270, 2012.

[30] K. Ma, Q. Cui, Y. Shao, F. Wu, S. Xu, and G. Liu, "Emission modulation of DNA-templated fluorescent silver nanoclusters by divalent magnesium ion," *Journal of Nanoscience and Nanotechnology*, vol. 12, no. 2, pp. 861–869, 2012.

[31] G. Lan, W. Chen, and H. Chang, "One-pot synthesis of fluorescent oligonucleotide Ag nanoclusters for specific and sensitive detection of DNA," *Biosensors and Bioelectronics*, vol. 26, no. 5, pp. 2431–2435, 2011.

[32] H. Yeh, J. Sharma, J. J. Han, J. S. Martinez, and J. H. Werner, "A DNA-silver nanocluster probe that fluoresces upon hybridization," *Nano Letters*, vol. 10, no. 8, pp. 3106–3110, 2010.

[33] H. C. Yeh, J. Sharma, I. M. Shih, D. M. Vu, J. S. Martinez, and J. H. Werner, "A fluorescence light-up Ag nanocluster probe that discriminates single-nucleotide variants by emission color," *Journal of the American Chemical Society*, vol. 134, no. 28, pp. 11550–11558, 2012.

[34] W. Guo, J. Yuan, Q. Dong, and E. Wang, "Highly sequence-dependent formation of fluorescent silver nanoclusters in hybridized DNA duplexes for single nucleotide mutation identification," *Journal of the American Chemical Society*, vol. 132, no. 3, pp. 932–934, 2010.

[35] J. Lhomme, J. F. Constant, and M. Demeunynck, "Abasic DNA structure, reactivity, and recognition," *Biopolymers*, vol. 52, no. 2, pp. 65–83, 1999.

[36] K. Ma, Y. Shao, Q. Cui, F. Wu, S. Xu, and G. Liu, "Base-stacking-determined fluorescence emission of DNA abasic site-templated silver nanoclusters," *Langmuir*, vol. 28, no. 43, pp. 15313–15322, 2012.

[37] K. Ma, Q. Cui, G. Liu, F. Wu, S. Xu, and Y. Shao, "DNA abasic site-directed formation of fluorescent silver nanoclusters for selective nucleobase recognition," *Nanotechnology*, vol. 22, no. 30, Article ID 305502, 2011.

[38] C. M. Croce, "Causes and consequences of microRNA dysregulation in cancer," *Nature Reviews Genetics*, vol. 10, no. 10, pp. 704–714, 2009.

[39] S. W. Yang and T. Vosch, "Rapid detection of microRNA by a silver nanocluster DNA probe," *Analytical Chemistry*, vol. 83, no. 18, pp. 6935–6939, 2011.

[40] P. Shah, A. Rørvig-Lund, S. B. Chaabane et al., "Design aspects of bright red emissive silver nanoclusters/DNA probes for MicroRNA detection," *ACSNano*, vol. 6, no. 10, pp. 8803–8814, 2012.

[41] W. Xin-Ping, Y. Bin-Cheng, W. Ping, and Y. Bang-Ce, "Highly sensitive detection of microRNAs based on isothermal exponential amplification-assisted generation of catalytic G-quadruplexDNAzyme," *Biosensors & Bioelectronics*, vol. 42, pp. 131–135, 2013.

[42] Y. Zhang and C. Zhang, "Sensitive detection of microRNA with isothermal amplification and a single-quantum-dot-based nanosensor," *Analytical Chemistry*, vol. 84, no. 1, pp. 224–231, 2012.

[43] Y.-Q. Liu, M. Zhang, B. C. Yin, and B. C. Ye, "Attomolar ultrasensitive MicroRNA detection by DNA-scaffolded silver-nanocluster probe based on isothermal amplification," *Analytical Chemistry*, vol. 84, no. 12, pp. 5165–5169, 2012.

[44] M. Famulok and G. Mayer, "Aptamer modules as sensors and detectors," *Accounts of Chemical Research*, vol. 44, no. 12, pp. 1349–1358, 2011.

[45] J. Sharma, H. Yeh, H. Yoo, J. H. Werner, and J. S. Martinez, "Silver nanocluster aptamers: in situ generation of intrinsically fluorescent recognition ligands for protein detection," *Chemical Communications*, vol. 47, no. 8, pp. 2294–2296, 2011.

[46] J. Li, X. Zhong, H. Zhang, X. C. Le, and J. J. Zhu, "Binding-induced fluorescence turn-on assay using aptamer-functionalized silver nanocluster DNA probes," *Analytical Chemistry*, vol. 84, no. 12, pp. 5170–5174, 2012.

[47] J. J. Liu, X. R. Song, Y. W. Wang, A. X. Zheng, G. N. Chen, and H. H. Yang, "Label-free and fluorescence turn-on aptasensor for protein detection via target-induced silver nanoclusters formation," *Analytica Chimica Acta*, vol. 749, pp. 70–74, 2012.

[48] Z. Sun, Y. Wang, Y. Wei et al., "Ag cluster-aptamer hybrid: specifically marking the nucleus of live cells," *Chemical Communications*, vol. 47, no. 43, pp. 11960–11962, 2011.

[49] J. Yin, X. He, K. Wang et al., "One-step engineering of silver nanoclusters-aptamer assemblies as luminescent labels to target tumor cells," *Nanoscale*, vol. 4, no. 1, pp. 110–112, 2012.

[50] J. Li, X. Zhong, F. Cheng, J. Zhang, L. Jiang, and J. Zhu, "One-pot synthesis of aptamer-functionalized silver nanoclusters for cell-type-specific imaging," *Analytical Chemistry*, vol. 84, no. 9, pp. 4140–4146, 2012.

[51] J. Yu, S. Choi, C. I. Richards, Y. Antoku, and R. M. Dickson, "Live cell surface labeling with fluorescent ag nanocluster conjugates," *Photochemistry and Photobiology*, vol. 84, no. 6, pp. 1435–1439, 2008.