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Short communication

Ligation Chain Reaction based gold nanoparticle assembly for ultrasensitive DNA detection

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ABSTRACT

A simple and ultrasensitive DNA biosensor was developed utilizing the color and size changes originating from Ligation Chain Reaction (LCR)-based gold nanoparticle assembly as an indicator. Using synthetic DNA of 10-fold serial dilutions as a target, the limit of detection (LOD) was 1.5 aM with a range of DNA concentrations from 0.01 to 1000 fM by UV–vis absorption and 0.1 aM in the range of 0.01–10,000 fM by dynamic light scattering (DLS). This developed method is low-cost, rapid, and much lower LOD for the detection of specific short DNA sequences, and the results can be directly determined either by the color or by the DLS. The capability of high-throughput detection with the aid of PCR amplification apparatus can also be realized.

1. Introduction

DNA is the carrier of genetic information and plays a critical role in the identification of specific species. The highly sensitive and selective detection of sequence-specific DNA has an increasing number of important applications, such as clinical diagnosis, pathogenic diseases, genetically modified organisms and environmental contamination (Pournaghi-Azar et al., 2006; Mao et al., 2006; Michelini et al., 2008; Marrazza et al., 1999). Therefore, there is an urgent need to exploit convenient and accurate DNA detection methods to meet these requirements.

Numerous techniques have been developed for the detection of DNA, of which, the conventional PCR-based detection strategy is a powerful tool in various applications and is widely used in different DNA detection fields around the world (Heid et al., 1996; Pfaffl, 2001; Giulietti et al., 2001; Freeman et al., 1999; Brittain-Long et al., 2008). However, this approach requires relatively sophisticated and expensive optical instruments, and a professional analytical procedure for processing the data obtained. Organic fluorogenic dye-based RT-PCR has an influence on the accuracy of the results due to the non-selective combination of dyes and double-strand DNA, and it is susceptible to photobleaching resulting in decreased fluorescence signals. In addition, longer target DNA sequences are needed in complex genetic samples to ensure complete accuracy.

With the development of nanotechnology, nanomaterials and their widespread use have attracted more and more attention and research. Each nanomaterial possesses its own specific properties, which can be applied in biochemical substance analysis. Therefore, the fabrication of various detection sensors mediated by nanoparticles, such as gold nanoparticles (AuNPs), silver nanoparticles, silicon nanoparticles, metal-oxide nanoparticles, and quantum dots is one of the main applications of nanomaterials (Sperling et al., 2008; McFarland and Van Duyne, 2003; Shao et al., 2005; Stoimenov et al., 2002; Wu et al., 2002).

AuNPs with their unique optical properties have attracted significant interest and have been widely used in molecular recognition and in the detection of harmful substances (Lee et al., 2007; Wilson, 2008; Liu et al., 2008). They have a higher absorption extinction coefficient, surface plasmon resonance (SPR), size- and distance-dependent optical properties (Daniel and Astruc, 2004; Rechberger et al., 2003). Oligonucleotide functionalized AuNPs have been explored as signaling probes for DNA detection through nucleic acid hybridization or primer extension with the aid of PCR (Deng et al., 2012; Dai et al., 2008; Li and Rothberg, 2004; Song et al., 2010; Kuang et al., 2011; Sato et al., 2003; Chena et al., 2008). After recognition and reaction, AuNPs showed an unordered aggregation state, and the generated specific phenomenon or characters were mostly used as detection

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signals for target DNA detection. In particular, the color change resulting from the aggregation of AuNPs is a simple and convenient detection method, which can be directly observed by the naked eye or monitored by UV–vis absorption spectroscopy (Elghanian et al., 1997; Du et al., 2013; Li, 2004). Meanwhile, the size change of AuNPs assembly measured by DLS can also be utilized for the quantitative determination sensitively (Wang et al., 2010).

LCR is a nucleic acid amplification technique, which is mostly used for the diagnostic detection of DNA. This technique employs DNA probes to recognize target DNA and the effect of ampligase to amplify the template exponentially. Li et al. developed a colorimetric method for point mutation detection using high-fidelity DNA ligase. However, this method just used one pair of probes for the recognization of the existing target DNA in the system, and the LOD was limited without the amplification of target DNA (Li et al., 2005). Gao et al. reported another colorimetric sensor for the detection of DNA using AuNP-enabled Real-Time LCR with LOD of 20 aM (Shen et al., 2012). However, the entire experimental procedure was very complicated due to the real-time detection analysis which was time-consuming and more steps were required. On the basis of the above-mentioned studies, we further improved the simplicity and sensitivity of the operation. The improved scheme obtained a much lower and ultrasensitive LOD with less time required and simple operating procedures.

2. Materials and methods

2.1. Materials and reagents

The Ampligase DNA Ligase was purchased from Haoran Biotechnology Co. Ltd. (Shanghai, China). Chloroauric acid ($HAuCl_4$), trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) and bis(p-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt (BPS) were obtained from Sigma-Aldrich (Shanghai, China). Other chemicals were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Water used throughout the procedures was deionized and purified to 18.2 MW (Millipore). All the DNA fragments were synthesized by Shengon Biotechnology Co. Ltd. (Shanghai, China). The selected target DNA was a short oligonucleotide fragment of 24 bp from genetically modified (GM) maize genome (5′-GAGTGGAAGTCTGTCGCGTGGTAC-3′). The complementary probes used for binding with DNA were: 5′-AGACTTCCACTCAAAAAAAAAA-SH-3′ and 5′-SH-AAAAAAAAAAGTACCACGCGAC-3′. Another pair of DNA probes consisted of GAGTGGAAGTCT and GTCGCGTGGTAC.

2.2. Preparation of DNA probe modified AuNPs

AuNPs with a diameter of 15 nm were synthesized by reduction of $HAuCl_4$. The dispersibility of AuNPs in solution was excellent and a clear absorption peak was located at the position of 521 nm (Figs. S1 and S2). The AuNPs were concentrated 10 times and re-suspended in 0.01 M PBS containing 0.01% sodium dodecyl sulfate (SDS). To improve the stability of AuNPs, 8 mg/mL BPS was added and incubated for approximately 6 h with a slow shake. The pair of complementary DNA probes with a final concentration of 4 μM was then added, respectively. In order to attach as many DNA probes as possible onto the surface of AuNPs, salt-aging steps were employed. For instance, 2 M of NaCl solution was gradually added with increments of 50 mM every 2 h, followed by 10 s of ultrasonic dispersion each time. The final concentration of NaCl reached 300 mM. Twenty four hours later, the unbound probes were removed by centrifugation at 12,000 r/min 3 times and dissolved in ultrapure water. The DNA-modified AuNPs were analyzed by agarose gel electrophoresis.

2.3. LCR assay for DNA detection

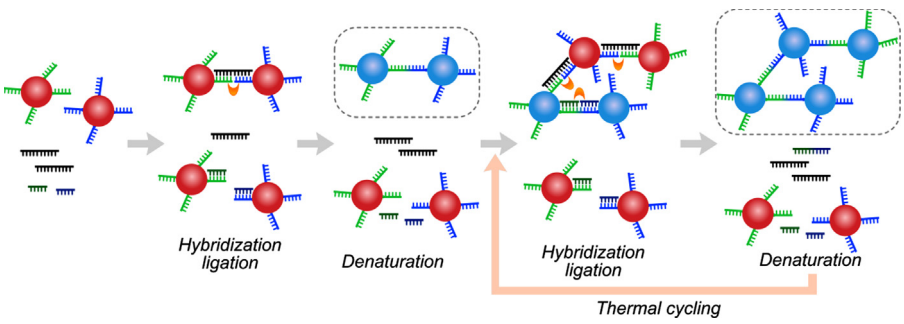
LCR was carried out in a final volume of 35 μL, which consisted of 12.5 μL of complementary probe-modified AuNPs, respectively, 10 U ampligase, 1 × PCR buffer, 500 μM of the other pair of probes and 1 μL of various concentrations of target DNA ranging from 0.01 fM to 10,000 fM. Due to the presence of high concentrations of salt in the matched reaction buffer, DNA polymerase buffer was replaced, in which the enzyme was still active. Based on the catalytic activity of ampligase, LCR cycling parameters were set as 60 °C for 2 min, 90 °C for 1 min, and followed by appropriate cycle numbers. Thirty cycles was selected as the optimum number of cycles, as more than 30 cycles resulted in greater AuNPs aggregations and the assembly was inadequate with no obvious difference at less than 30 cycles. During the whole procedure, all the individual measurements for each concentration were carried out for three experiments.

2.4. Specificity analysis

A 24 bp nonspecific DNA fragment was detected to evaluate the specificity of the method. The whole detection procedures were identical to those mentioned above. The final products were analyzed by UV–vis spectrum to observe the difference of the UV–vis absorption.

2.5. Recovery in complex matrix

The DNA detection system was carried out in the solution containing 10 pM of λDNA. Under the above condition, the target DNA at five concentrations 0.05 fM, 0.5 fM, 1 fM, 10 fM, 50 fM were added. The recovery was obtained to assess the feasibility of the method for real sample detection.



Scheme 1. The scheme of the method for DNA detection based on AuNPs assembly induced by LCR.

3. Results and discussion

The experimental strategy for the detection of target DNA is illustrated in Scheme 1. Two pairs of oligonucleotide probes were used, one pair was complementary with the target DNA and was conjugated with AuNPs and the other was complementary with the first pair of probes. Initially, the first pair of probes, which was modified with AuNPs, was hybridized with target DNA, and the nick between the two adjacent probes was ligated by ampligase. After denaturation, along with the process of hybridization of AuNPs-bound probes and target DNA, the second probes recognized and connected with the newly synthetic single strand between the two AuNPs. The second pair of probes formed a strand in the presence of ampligase and served as templates for the next step. During repeated cycles of this process, the target DNA template was exponentially amplified and the AuNPs conjugated by DNA were increasingly assembled together.

The successfully modified AuNPs was used in the LCR (Fig. S3). Photographs of LCR products under different concentrations of target DNA are shown in Fig. 1(a). From left to right, the concentrations of target DNA ranged from 0 fM to 10,000 fM of 10-fold dilutions. With an increase in target DNA concentration, the color of the LCR products gradually changed from red to purple. In the presence of target DNA, DNA probe-modified AuNPs assembled together and displayed a greater aggregation state than the control without target DNA. The higher the concentration of target DNA, the greater the degree of AuNPs assembly. Therefore, the color changed to purple due to the closeness of the AuNPs.

Under optimized conditions, the sensitivity of this method was estimated by the UV signal. UV–vis spectra of 0 fM to 10,000fM of target DNA are shown in Fig. 1(b). With an increase in AuNPs assembly, the specific absorption peak at the position of 521 nm exhibited red-shift, and a new peak appeared under higher concentrations of target DNA. In Fig. 1(c), the standard curve of A640/A521 versus the concentration of target DNA was drawn in the range of 0.01 fM to 1000 fM. An excellent linear relationship with a correlation coefficient of 0.9928 was obtained and the LOD was 1.5 aM. To further substantiate the accuracy of the test, a linear dose–response plot at lower concentrations of 0.01 fM, 0.05 fM, 0.1 fM, 0.5 fM and 1 fM was shown with an excellent liner relationship. By calculating, the A640/A521 of the two added concentrations of 0.05 fM and 0.5 fM were consistent with that of the logarithmic dose–response curve.

To estimate the change rule in the size of the formed AuNPs assembly, size distributions were measured by dynamic light scattering (DLS). The sizes of the AuNPs aggregates showed a gradual increment with increasing concentrations of added target DNA (Fig. 2(a)). In accordance with the diameter change, a standard curve of the sizes versus the concentrations of target DNA was also obtained to analyze the sensitivity of this quantitative method (Fig. 2(b)). A relatively good linear relationship with a correlation coefficient of 0.9801 was obtained in the range of 0.01 fM to 10,000 fM, and the LOD was 0.1 aM. This quantitative method obtained a lower LOD compared with the sensitivity of the signal from UV–vis. The linear dose–response plot at lower concentrations was drawn, and the diameters of the two added concentrations of 0.05 fM and 0.5 fM corresponded well with the values of the logarithmic dose–response curve.

The specificity of the assay was estimated using a nonspecific 24 bp DNA fragment as a template. Different concentrations of

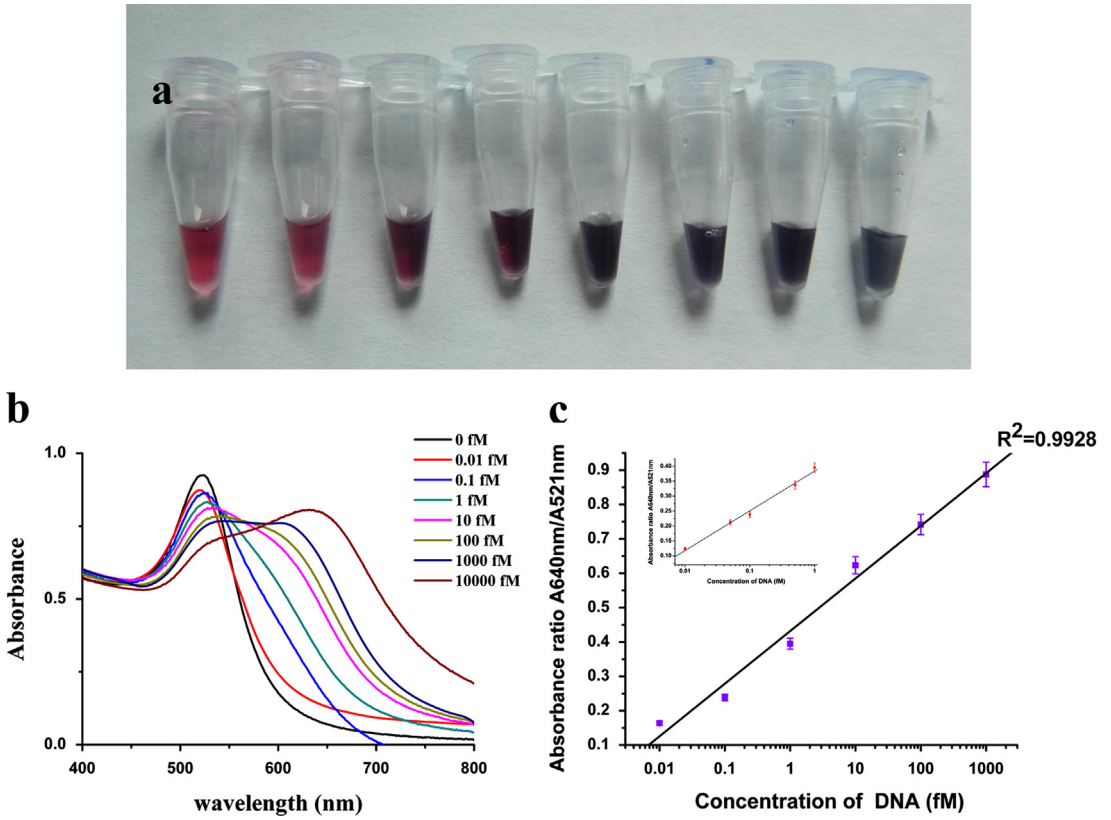


Fig. 1. (a) Photos of LCR based AuNPs assembly in the presence of different concentrations of target DNA. From left to right, the concentrations of target DNA were 0 fM, 0.01 fM, 0.1 fM, 1 fM, 10 fM, 100 fM, 1000 fM, and 10,000 fM. (b) Absorption spectra of AuNPs assembly in the range of 0–10,000 fM. (c) The linear relationship between the concentrations of target DNA and A640/A521 in the range of 0.01–1000 fM. A linear dose–response plot at lower concentrations of 0.01 fM, 0.05 fM, 0.1 fM, 0.5 fM and 1 fM was shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

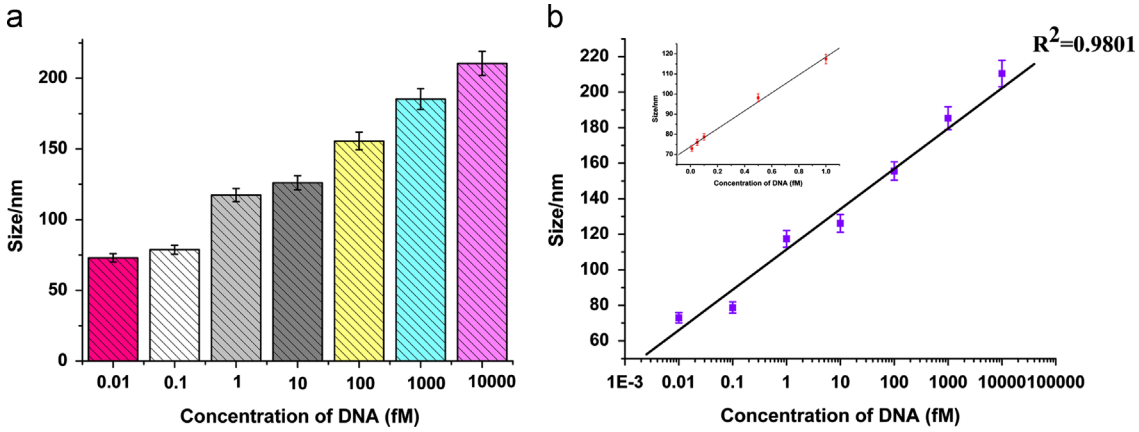


Fig. 2. (a) The sizes of AuNPs assembly under different concentrations of target DNA from 0.01 fM to 10,000 fM. The sizes were the values at the highest intensity. (b) The linear relationship between the concentrations of target DNA and sizes in the range of 0.01–10,000 fM. A linear dose–response plot at lower concentrations of 0.01 fM, 0.05 fM, 0.1 fM, 0.5 fM and 1 fM was shown.

DNA in the range of 0 fM to 1000 fM were added, and all the operation procedures were the same as those for target DNA detection. As shown in Fig. S4, no obvious change in A640/A521 was observed under different concentrations of DNA, which was almost the same as that of the control without DNA template. Therefore, this indicated that this method had excellent specificity for the detection of target DNA.

Matrix interference on the accuracy of detection was estimated by determining the amount of DNA in the solution containing 10 pM of λDNA. Due to the larger and more complex DNA sequence of λDNA, it was used to imitate a complicated environment to assess the accuracy of this method by detecting the recovery ratio. The specificity of the target DNA was compared with λDNA, which showed a perfect result without repeated fragments. Under the five concentrations of the added target DNA, 0.05 fM, 0.5 fM, 1 fM, 10 fM, 50 fM. Satisfactory recovery was obtained in the range of 93.6–97.5% (Table S1), which demonstrated the capacity of this assay in the analysis of real samples where the influence of the matrix could be neglected.

4. Conclusions

Using the UV signal and DLS as an indicator, an ultrasensitive colorimetric sensor was developed by LCR-induced AuNPs assembly. This method was realized by the hybridization of probes and target DNA without the primer extension reaction. Ligation has greater discriminatory power than primer extension reaction. Thus, short DNA fragments can be used as targets for detection, which had no influence on accuracy. The DNA template can also be exponentially amplified by LCR, which amplified the detection signal. A LOD of 1.5 aM was obtained in the range of 0.01 fM to 1000 fM target DNA by UV–vis spectra, and a lower LOD of 0.1 aM was obtained by DLS in the range of 0.01–10,000 fM. Thus, the quantitative determination based on DLS is better than that UV–vis spectroscopy in both sensing range and LOD. Excellent selectivity was demonstrated using a nonspecific DNA sequence and satisfactory recovery was obtained in the solution containing λDNA. This method is simple, low-cost, rapid, and highly sensitive for the detection of specific short DNA sequences, and the results can be directly observed by the color change. Therefore, this method is feasible and has enormous potential for use in DNA detection.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.07.064.

References

Brittain-Long, R., Nord, S., Olofsson, S., Westin, J., Anderson, L.M., Lindh, M., 2008. Journal of Clinical Virology 41, 53–56.
Chena, L., Leea, S., Leea, M., Lima, C., Chooa, J., Parkb, J.Y., Leeb, S., Jooc, S.W., Leed, K. H., Choi, Y.W., 2008. Biosensors and Bioelectronics 23, 1878–1882.
Daniel, M.C., Astruc, D., 2004. Chemical Reviews 104, 293–346.
Deng, H., Xu, Y., Liu, Y., Che, Z., Guo, H., Shan, S., Sun, Y., Liu, X., Huang, K., Ma, X., Wu, Y., Liang, X.J., 2012. Analytical Chemistry 84, 1253–1258.
Dai, Q., Liu, X., Coutts, J., Austin, L., Huo, Q., 2008. Journal of the American Chemical Society 130, 8138–8139.
Du, J., Yin, S., Jiang, L., Ma, B., Chen, X., 2013. Chemical Communications 49, 4196–4198.
Elghanian, R., Storhoff, J.J., Mucic, R.C., Letsinger, R.L., Mirkin, C.A., 1997. Science 277, 1078–1081.
Freeman, W.M., Walker, S.J., Vrana, K.E., 1999. Biotechniques 26, 112–122.
Giulietti, A., Overbergh, L., Valckx, D., Decallonne, B., Bouillon, R., Mathieu, C., 2001. Methods 25, 386–401.
Heid, C.A., Stevens, J., Livak, K.J., Williams, P.M., 1996. Genome Research 6, 986–994.
Kuang, H., Zhao, S., Chen, W., Ma, W., Yong, Q., Xu, L., Wang, L., Xu, C., 2011. Biosensors and Bioelectronics 26, 2495–2499.
Lee, J.S., Han, M.S., Mirkin, C.A., 2007. Angewandte Chemie 119, 4171–4174.
Liu, X., Dai, Q., Austin, L., Coutts, J., Knowles, G., Zou, J., Chen, H., Huo, Q., 2008. Journal of the American Chemical Society 130, 2780–2782.
Li, H., Rothberg, L.J., 2004. Journal of the American Chemical Society 126, 10958–10961.
Li, H., 2004. Proceedings of the National Academy of Sciences USA 101, 14036–14039.
Li, J., Chu, X., Liu, Y., Jiang, J.H., He, Z., Zhang, Z., Shen, G., Yu, R.Q., 2005. Nucleic Acids Research 33, e168.
Mao, X., Yang, L., Su, X.-L., Li, Y., 2006. Biosensors and Bioelectronics 21, 1178–1185.
Michelini, E., Simoni, P., Cevenini, L., Mezzanotte, L., Roda, A., 2008. Analytical and Bioanalytical Chemistry 392, 355–367.
Marrazza, G., Chianella, I., Mascini, M., 1999. Analytica Chimica Acta 387, 297–307.
McFarland, A.D., Van Duyne, R.P., 2003. Nano Letters 3, 1057–1062.
Pournaghi-Azar, M.H., Hejazi, M.S., Alipour, E., 2006. Analytica Chimica Acta 570, 144–150.
Pfaffl, M.W., 2001. Nucleic Acids Research 29, e45.
Rechberger, W., Hohenau, A., Leitner, A., Krenn, J.R., Lamprecht, B., Aussenegg, F.R., 2003. Optics Communications 220, 137–141.
Sperling, R.A., Rivera Gil, P., Zhang, F., Zanella, M., Parak, W.J., 2008. Chemical Society Reviews 37, 1896–1908.
Shao, M.W., Yao, H., Zhang, M.L., Wong, N.B., Shan, Y.Y., Lee, S.T., 2005. Applied Physics Letters 87, 83103.

Stoimenov, P.K., Klinger, R.L., Marchin, G.L., Klabunde, K.J., 2002. Langmuir 18, 6679–6686.
Song, J., Li, Z., Cheng, Y., Liu, C., 2010. Chemical Communications 46, 5548–5550.
Sato, K., Hosokawa, K., Maeda, M., 2003. Journal of the American Chemical Society 125, 8102–8103.
Shen, W., Deng, H., Gao, Z., 2012. Journal of the American Chemical Society 134, 14678–14681.
Wu, X., Liu, H., Liu, J., Haley, K.N., Treadway, J.A., Larson, J.P., Ge, N., Peale, F., Bruchez, M.P., 2002. Nature Biotechnology 21, 41–46.
Wilson, R., 2008. Chemical Society Reviews 37, 2028–2045.
Wang, L., Zhu, Y., Xu, L., Chen, W., Kuang, H., Liu, L., Agarwal, A., Xu, C., Kotov, N.A., 2010. Angewandte Chemie 49, 5472–5475.