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dsDNA-templated fluorescent copper nanoparticles: poly(AT-TA)-dependent formation

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In this work, poly(AT-TA) is found as the specific sequence composition which contributes to the formation of dsDNA-templated fluorescent copper nanoparticles. The finding will be helpful in wide fields, such as constructing of DNA-templated nanodevices and designing of biochemical nano-probes.

As well as quantum dots,[1-3] few-atom noble-metal nanoparticles display excellent fluorescent properties, which have been intensely investigated and exploited as alternative fluorophores for biochemical application in the past few years.[4-12] For example, fluorescent metal nanoparticles have been applied for proteins detection, cells detection and *in vivo* imaging.[13-16] During the synthesis of fluorescent metal nanoparticles, ligands are usually used as templates to control growth rates and/or adjust spectra properties.[17,18] By virtue of its abundant and programmable sequence compositions, deoxyribonucleic acid (DNA) has been applied as a prominent template for different fluorescent metal nanoparticles, such as fluorescent gold, silver, and copper nanoparticles.[9,18-27]

As reported, the sequence composition of DNA plays an important role on the fluorescence properties of DNA-templated fluorescent metal nanoparticles. In another word, the formation of a kind of DNA-templated fluorescent metal nanoparticles is generally sequence-dependent. For example, Dickson *et al* reported that differnent oligonucleotides can tune the spectra of silver nanoclusters (AgNCs);[28] Werner *et al* reported that guanine-rich DNA can enhance the intensity of AgNCs;[7] Liu *et al* found that poly(cytosine) DNA at low pH and poly(adenine) DNA at neutral pH can template blue emitting gold nanoclusters (AuNCs);[20] Shao *et al* and our group found that poly(thymine) ssDNA can selectively template red emitting fluorescent copper nanoparticles (CuNPs), compared with poly(adenine), poly(cytosine), poly(guanine), and random ssDNA.[24,25] These findings not only help the understanding of the relationship between sequence composition and DNA-templated fluorescent metal nanoparticles, but also have markedly contributed to a wide range of applications, such as DNA nanotechnology, bioimaging, and biochemical sensing.[29-35]

As best of our knowledge, since dsDNA-templated fluorescent CuNPs has been discovered,[23] there is no investigation on what sequence composition of dsDNA dominates the formation of the fluorescent CuNPs. To identify the specific DNA sequence for the formation of the dsDNA-templated fluorescent CuNPs, we here focus on the relationship between the sequence composition of dsDNA and the formation of dsDNA-templated fluorescent CuNPs. A series of dsDNA of different sequence compositions were systematically investigated for the formation of CuNPs, the fluorescence intensity and fluorescence quantum yield were measured to determine the effect of each dsDNA. After the specific sequence composition was identified, the effects of polymerization degree and strand length on CuNPs' fluorescence were also studied. As a result and shown in the schematic diagram (Fig. 1), poly(AT-TA) is found as the specific dsDNA

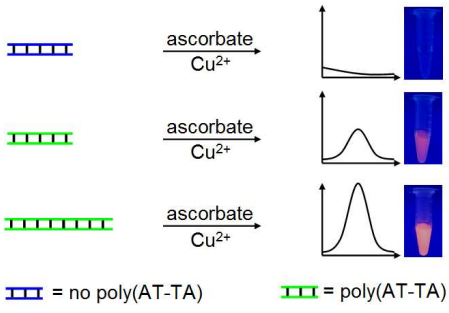

Fig. 1 Schematic illustration of the effects of different dsDNA on the formation of fluorescent CuNPs. Poly(AT-TA) is found as the specific sequence with the capability for forming the fluorescent CuNPs, whose fluorescence intensity is highly dependent on the length of poly(AT-TA).

sequence (green curves) which can act as a highly-efficient template for the formation of the fluorescent CuNPs, the fluorescence intensity is highly-dependent on the polymerization degree and the length of poly(AT-TA), and obvious fluorescence emission can be measured by spectrophotometer or imaged under ultraviolet (UV) light. However, other sequence compositions (blue curves, denoted as "no poly(AT-TA)" in the schematic diagram) have little or no function for supporting the formation of the fluorescent CuNPs.

The dsDNA segments used in this work are named as a general formula: ds(M-N)$_k$, where the logogram ds denotes double-strand, M and N represent sequence composition in each strand, the short bar denotes complementary relationship between M and N, the subscript k is the number of base-pairs (bp) of a certain segment. First, seven kinds of dsDNA, including a random dsDNA (ds(R-R)$_{22}$) and six compositions of four bases (ds(AT-TA)$_{22}$, ds(GC-CG)$_{22}$, ds(AC-TG)$_{22}$, ds(AG-TC)$_{22}$, ds(A-T)$_{22}$, ds(G-C)$_{22}$), were investigated as templates for the formation of fluorescent CuNPs. The length of each dsDNA is 22 bp, and their detailed sequences are listed in the Table S1 (ESI†). From the fluorescence spectra (Fig. 2a), ds(AT-TA)$_{22}$ can induce much stronger fluorescence at around 590 nm with the excitation of 340 nm, while the random dsDNA (ds(R-R)$_{22}$) only result in little fluorescence and no fluorescence is observed in the presence of other sequences. The 3D scans of these fluorescent CuNPs were further investigated and are shown in Fig. S1 (ESI†). The reaction kinetics is demonstrated by real-time scan of fluorescence intensity (Fig. 2b), the ds(AT-TA)$_{22}$-templated CuNPs' fluorescence increases gradually and complete within ten minutes of the begin of the reaction, so fluorescence spectra in all further studies were recorded after reaction beginning for 10 min. Then, to further verify the high effect of ds(AT-TA)$_{22}$ on the formation of CuNPs, fluorescence quantum yields were measured in the presence of ds(AT-TA)$_{22}$ and (ds(R-R)$_{22}$), respectively. As a result, the quantum yield of the ds(AT-TA)$_{22}$-templated fluorescent CuNPs is 7 times greater than that of the ds(R-R)$_{22}$-templated fluorescent CuNPs (Fig. 2c). In addition, the structure and size of the ds(AT-TA)$_{22}$-templated CuNPs were characterized using transmission electron microscopy (TEM) (Fig. 2d). We can see that the size of the ds(AT-TA)$_{22}$-templated fluorescent CuNPs is around 4 nm with obvious crystal lattice structure (inset in Fig. 2d), which indicates that ds(AT-TA)$_{22}$ can serve as a template for Cu^{2+} reduction and fluorescent CuNPs formation. Besides, the sequence selectivity at other reactant concentrations was also demonstrated (Fig. S2, and Fig. S3, ESI†). Thus, from these results, we can preliminarily infer that the sequence composition of A plus T in each strand is crucial for the formation of the fluorescent CuNPs.

To further investigate the effects of different repetitive types of A plus T, another seven kinds of dsDNA were used to template the formation of the fluorescent CuNPs, including ds(AT-TA)$_{22}$, ds(A_2T_2-T_2A_2)$_{22}$, ds(A_3T_3-T_3A_3)$_{22}$, ds(A_4T_4-T_4A_4)$_{22}$, ds(A_5T_5-T_5A_5)$_{22}$, ds(A_6T_6-T_6A_6)$_{22}$, ds($A_{11}T_{11}$-$T_{11}A_{11}$)$_{22}$. The length of each dsDNA is 22 bp, and their detailed sequences are listed in the Table S2 (ESI†). As shown in Fig. 3 and Fig. S4 (ESI†), high fluorescence is observed only in the presence of the sequence ds(AT-TA)$_{22}$, slight fluorescence or no fluorescence can be observed for other repetitive types, which indicates that the type of alternate A plus T in each strand dominates the formation of the fluorescent CuNPs. In another word, poly(AT-TA) is screened as the specific sequence composition which contributes to the formation of the fluorescent CuNPs. The reason for this phenomenon may be due to the different metal affinities between nucleotides and Cu^+,[36] a intermediate in reducing Cu^{2+} to Cu(0).[23] The adenine and thymine have weak binding to Cu^+, facilitating the reduction of Cu^+ to Cu(0), whereas the guanine and cytosine are unfavorable for the reduction reaction owing to the strong complexation.[24] Moreover, the different dsDNA sequences can provide different microenvironments for the formation of

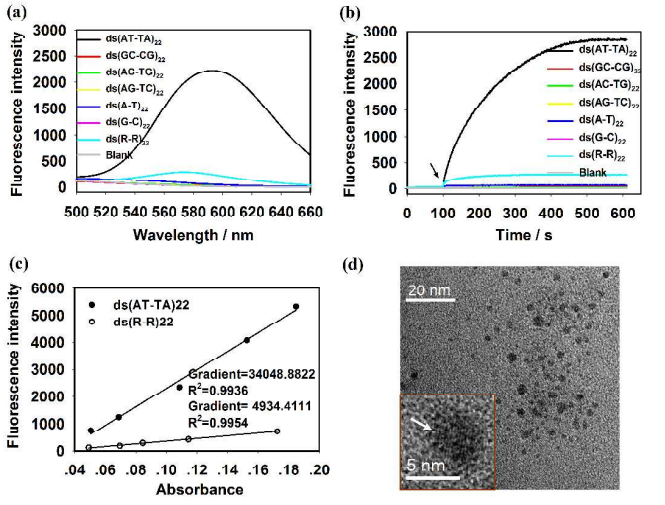

Fig. 2 Sequence specificity for dsDNA-templated fluorescent CuNPs. (a) The fluorescence spectra and (b) real-time fluorescence of fluorescent CuNPs templated by different dsDNA. The arrow marks the addition of Cu^{2+} to the buffered solutions of dsDNA. (c) Linear plots of the relationship between the fluorescence of the fluorescent CuNPs and its absorbance templated by ds(AT-TA)$_{22}$ (●) and ds(R-R)$_{22}$ (○). The gradient is proportional to the fluorescence quantum yield. (d) TEM image of the ds(AT-TA)$_{22}$-templated fluorescent CuNPs. Inset: High-resolution TEM image of the crystal lattice structure of the fluorescent CuNPs formed.

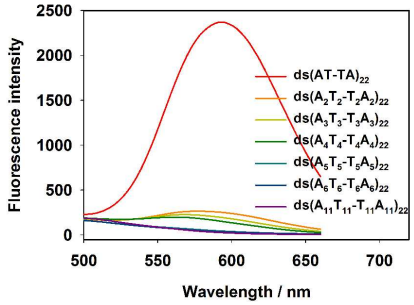

Fig. 3 Effect of the repetitive type of A plus T on the formation of dsDNA-templated fluorescent CuNPs.

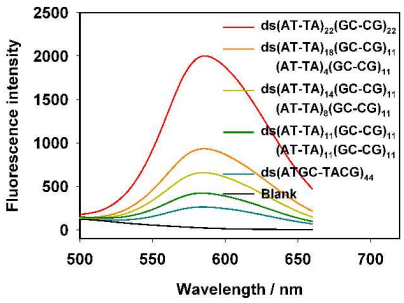

Fig. 4 Fluorescence spectra of resulted solutions in the presence of 44-bp dsDNA with different types of (AT-TA)-rich domains.

fluorescent metal nanoparticles,[37,38] and poly(AT-TA) may be advantageous for the formation of fluorescent CuNPs.

After poly(AT-TA) was found as the specific sequence composition for templating the fluorescent CuNPs, we further tested the effect of polymerization degree of AT-TA on the formation of the fluorescent CuNPs. Five kinds of dsDNA containing different (AT-TA)-rich domains, including ds(AT-TA)$_{22}$(GC-CG)$_{22}$, ds(AT-TA)$_{18}$(GC-CG)$_{11}$(AT-TA)$_4$(GC-CG)$_{11}$, ds(AT-TA)$_{14}$(GC-CG)$_{11}$(AT-TA)$_8$(GC-CG)$_{11}$, ds((AT-TA)$_{11}$(GC-CG)$_{11}$(AT-TA)$_{11}$(GC-CG)$_{11}$, and ds(ATGC-TACG)$_{44}$, were investigated. The length of each dsDNA is 44-bp, the percentage of A plus T in each dsDNA is 50%, (AT-TA)-rich domains in each dsDNA are separated by (GC-CG)-rich domains, and their detailed sequences are listed in the Table S3 (ESI†). As shown in Fig. 4 and Fig. S5 (ESI†), when the polymerization degree of AT-TA increases, obvious increasement in fluorescence intensity is observed. Thus, in a certain dsDNA, the polymerization degree of AT-TA plays an important role in the fluorescence of the CuNPs. Direct regulation of the fluorescence emission intensity of the fluorescent CuNPs can be realized by altering the length of poly(AT-TA). Five kinds of poly(AT-TA) DNA of different lengths, including ds(AT-TA)$_{30}$, ds(AT-TA)$_{26}$, ds(AT-TA)$_{22}$, ds(AT-TA)$_{18}$, and ds(AT-TA)$_{14}$, were investigated for the formation of the fluorescent CuNPs. Their detailed sequences are listed in the Table S4 (ESI†). As shown in Fig. 5 and Fig. S6 (ESI†), a positive relationship between the fluorescence intensity and the length of poly(AT-TA) is found. Moreover, when the total length of the heterozygous dsDNA is fixed, by changing the length of poly(AT-TA) in a dsDNA, the same positive relationship is obtained (Table S5 and Fig. S7, ESI †).

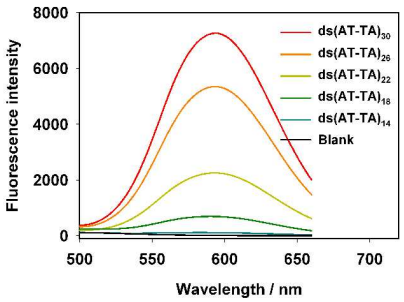

Fig. 5 Fluorescence spectra of resulted solutions in the presence of poly(AT-TA) of different lengths.

In summary, through systematical investigation on the formation of the fluorescent CuNPs templated by dsDNA, poly(AT-TA) is found as the specific dsDNA sequence which can act as a highly-efficient template for the formation of the fluorescent CuNPs, while other sequence compositions have little or no function for supporting the formation of the fluorescent CuNPs. The fluorescence intensity is highly-dependent on the polymerization degree and the length of poly(AT-TA). The reaction conditions are benign and the fluorescence response is very fast. This finding will be helpful for the understanding of the relationship between sequence composition and dsDNA-templated fluorescent CuNPs, and useful for the applications of CuNPs, such as constructing of DNA-templated nanodevices and designing of biochemical nano-probes. Some studies on further applications are currently being undertaken in our laboratory. In addition, this study will impel researchers to uncover other relationships between DNA sequences and formation of DNA-templated fluorescent metal nanoparticles.

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Notes and references

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† Electronic Supplementary Information (ESI) available: chemicals and materials, experimental details, DNA sequence information and additional figures as noted in the text. See DOI: 10.1039/c000000x/.

1 P. Zhang, S. Liu, D. Gao, D. Hu, P. Gong, Z. Sheng, J. Deng, Y. Ma and L. Cai, *J. Am. Chem. Soc.*, 2012, **134**, 8388.
2 J. Liu, X. Yang, K. Wang, R. Yang, H. Ji, L. Yang and C. Wu, *Chem. Commun.*, 2011, **47**, 935.
3 D. Gao, P. Zhang, Z. Sheng, D. Hu, P. Gong, C. Chen, Q. Wan, G. Gao and L. Cai, *Adv. Funct. Mater.* 2014, **24**, 3897.
4 H. A. Becerril and A. T. Woolley, *Chem. Soc. Rev.*, 2009, **38**, 329.
5 H. Wang, T. B. Huff, D. A. Zweifel, W. He, P. S. Low, A. Wei and J. Cheng, *Proc. Natl. Acad. Sci. U.S.A.*, 2005, **102**, 15752.

6 J. Yu, S. Choi and R. M. Dickson, *Angew. Chem., Int. Ed.*, 2009, **48**, 318.
7 H. C. Yeh, J. Sharma, J. Han, J. Martinez and J. Werner, *Nano Lett.*, 2010, **10**, 3106.
8 J. Xie, Y. Zheng and J. Ying, *J. Am. Chem. Soc.*, 2009, **131**, 888.
9 E. G. Gwinn, P. O'Neill, A. J. Guerrero, D. Bouwmeester and D. K. Fygenson, *Adv. Mater.*, 2008, **20**, 279.
10 H. Xu and K. S. Suslick, *Adv. Mater.*, 2010, **22**, 1078.
11 X. Huang, S. Neretina and M. A. El-Sayed, *Adv. Mater.*, 2009, **21**, 4880.
12 K. Ma, Q. H. Cui, G. Y..Liu, F..Wu, S. J..Xu and Y. Shao, *Nanotechnology*, 2011, **22**, 305502.
13 Z. D. Wang, J. Q. Zhang, J. M. Ekman, P. J. A. Kenis and Y. Lu, *Nano Lett.*, 2010, **10**, 1886.
14 S. Tanaka, J. Miyazaki, D. K. Tiwari, T. Jin and Y. Inouye, *Angew. Chem., Int. Ed.*, 2011, **50**, 431.
15 J. Yin, X. He, K. Wang, F. Xu, J. Shangguan, D. He and H. Shi, *Anal. Chem.*, 2013, **85**, 12011.
16 X. Wu, X. He, K. Wang, C. Xie, B. Zhou and Z. Qing, *Nanoscale*, 2010, **2,** 2244.
17 C. B. Murray, C. R. Kagan and M. G. Bawendi, *Annu. ReV. Mater. Sci*., 2000, **30**, 545.
18 L. Berti and G. A. BurLey, *Nat. Nanotechnol.*, 2008, **3**, 81.
19 A. Houlton, A. R. Pike, M. A. Galindo and B. R. Horrocks, *Chem. Commun.*, 2009, 1797.
20 T. A. C. Kennedy, J. L. MacLean and J. Liu, *Chem. Commun*., 2012, **48**, 6845.
21 G. Y. Liu, Y. Shao, F. Wu, S. J. Xu, J. Peng and L. L. Liu, *Nanotechnology*, 2013, **24**, 015503.
22 J T. Petty, J. Zheng, N. V. Hud and R. M. Dickson, *J. Am. Chem. Soc.*, 2004, **126**, 5207.
23 A. Rotaru, S. Dutta, E. Jentzsch, K. Gothelf and A. Mokhir, *Angew. Chem., Int. Ed.*, 2010, **49**, 5665.
24 G. Liu, Y. Shao, J. Peng, W. Dai, L. Liu, S. Xu and X. Wu, *Nanotechnology*, 2013, **24**, 345502.
25 Z. Qing, X. He, D. He, K. Wang, F. Xu, T. Qing and X. Yang, *Angew. Chem., Int. Ed.*, 2013, **52**, 9719.
26 G. Y. Lan, W. Y. Chen and H. T. Chang, *RSC Adv.*, 2011, **1**, 802.
27 X. P. Wang, B. C. Yin and B. C. Ye, *RSC Adv.*, 2013, **3**, 8633.
28 C. I. Richards, S. Choi, J. C. Hsiang, Y. Antoku, T. Vosch, A. Bongiorno, Y. L. Tzeng and R. M. Dickson, *J. Am. Chem. Soc.*, 2008, **130**, 5038.
29 Z, Qing, X. He, T. Qing, K. Wang, H. Shi, D. He, Z Zou, L. Yan, F. Xu, X. Ye and Z. Mao, *Anal. Chem.*, 2013, **85**, 12138.
30 W. Guo, J. Dong and E. Wang, *J. Am. Chem. Soc.*, 2010, **132**, 932.
31 J. Chen, J. Liu, Z. Fang and L. Zeng, *Chem. Commun.*, 2012, **48**, 1057.
32 L. Zhang, J. Zhao, M. Duan, H. Zhang, J. Jiang and R. Yu, *Anal. Chem.*, 2013, **85**, 3797.
33 J. Xie, Y. Zheng and J. Ying, *Chem. Commun.*, 2010, **46**, 961.
34 T. Li, L. Zhang, J. Ai, S. Dong and E. Wang, *ACS Nano*, 2011, **5**, 6334.
35 F. Xu, H. Shi, X. He, K. Wang, D. He, Q. Guo, Z. Qing, L. Yan, X. Ye, D. Li and J. Tang, *Anal. Chem.*, 2014, **86**, 6976.
36 N. Russo, M. Toscano and A. Grand, *J. Mass Spectrom.*, 2003, **38**, 265.
37 K. Ma, Q. Cui, G. Liu, F. Wu, S. Xu and Y. Shao, *Nanotechnology*, 2011, **22**, 305502.
38 X. Jia, J. Li, L.Han, J. Ren, X. Yang and E. Wang, *ACS nano*, 2012, **6**, 3311.

