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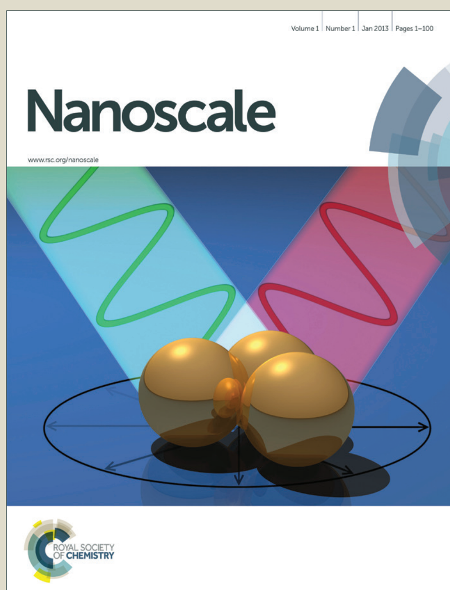

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ARTICLE TYPE

Protein-DNA interactions: A novel approach to improve the fluorescent stability of DNA/Ag nanoclusters

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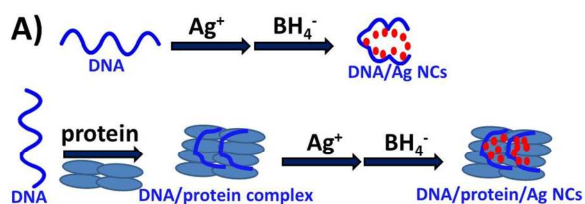
Protein-DNA interactions are known to play an important role in a variety of biological processes. We show here that protein-DNA binding events can also be used to greatly improve the fluorescent stability of DNA-templated Ag nanoclusters (Ag NCs), which would be highly beneficial for Ag NCs in applications of biosensing/imaging.

Although nucleic acids are best known as genetic materials, the use of nucleic acids as templates for the synthesis of inorganic nanoparticles (NPs) or nanoclusters (NCs) has become an active area.[1] In particular, DNA-directed Ag NCs syntheses have been thoroughly investigated, and the highly optically emissive DNA/Ag NCs show a wide range of potential applications especially in biosensing.[2] Compared with organic dyes or quantum dots, DNA-templated Ag NCs may have some advantages, such as ease of synthesis, desirable photophysical properties and smaller size than quantum dots.[3] In addition, DNA/Ag NCs allow flexible modification with aptamers or other recognition elements when they are used in biosensing or bioimaging fields.[2a,2i,2j,4] Moreover, DNA/Ag NCs are particularly responsive to optical modulation of their emission, and from blue to near-infrared (NIR) emissive Ag NCs can be generated with different DNA templates.[5] However, most Ag NCs have the disadvantage of short shelf-lives: the fluorescence of Ag NCs often suffers a dramatic and rapid decrease over time, which is a critical issue that may limit their usefulness as biological probes.[6] Therefore, it would be highly desirable to improve the fluorescent stability of DNA/Ag NCs.[7]



B) N-DNA 5'-CCCACCCACCCTCCCA-3'
G-DNA 5'-GTGGGGGTGTGTGGTG-3'
R-DNA 5'-CCCTTAATCCCC-3'
N2-DNA 5'-CCCACCCACCCGCCCA-3'
N3-DNA 5'- CCCACCCACCCACCCA-3'
O-DNA 5'-CCCCCTCACCCCGCC-3'
R2-DNA 5'-CCCTTAATCCCC-3'

Scheme 1 (A) Schematic showing the enhanced fluorescent stability of DNA-templated Ag NCs caused by protein-DNA interactions. (B) DNA sequences used in the study

Toward this goal, herein, we present a novel approach to rationally improve the stability of DNA/Ag NCs through protein-DNA interactions. According to previous findings,[1a,8] the general model for DNA-templated NPs can be summarized as follows: 1) DNA binds the precursor metal cations and provides the necessary microenvironment for triggering the nucleation event; 2) the localized cations are then reduced to form NPs that follow the contour of the DNA template; 3) DNA serves as a stabilizing cap for NPs and ensures that the NPs remain stably dispersed in aqueous solution. Inspired by these principles, we postulate that the well-defined three-dimensional conformation formed by protein-DNA binding event might offer a more favourable microenvironment to confine and control NC events, and thereby improve the stability of DNA-templated NCs. (Scheme 1A)

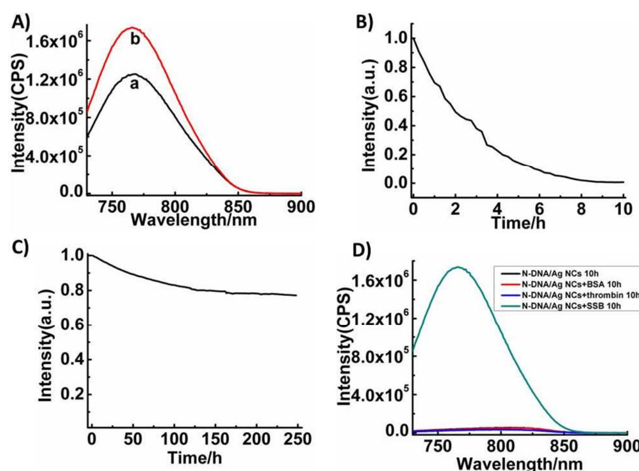


Fig. 1 (A) Fluorescence spectra of N-DNA-stabilized Ag NCs (a) or N-DNA/SSB-stabilized Ag NCs (b) monitored three hours after the initiation of NC nucleation. Decay curves of the fluorescent intensity for N-DNA/Ag NCs (B) and N-DNA/SSB/Ag NCs (C).(D) Fluorescence spectra of N-DNA-stabilized Ag NCs in the presence of various proteins, and the spectra were collected ten hours after the initiation of NC nucleation.

For our proof-of-concept study, we began by exploring a previously reported NIR emissive Ag NCs (quantum yield ~ 30%) synthesized with a single-stranded DNA (ssDNA) template (named N-DNA, sequence see Scheme 1B).[9] We chose to study

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the fluorescent stability of N-DNA/Ag NCs since NIR emitters are particularly promising candidates for biological imaging or sensing applications given the transparency of tissue in certain windows within this spectral regime.[8] Furthermore, the photochemical properties and characterizations of N-DNA/Ag NCs have been investigated by various techniques,[9] which thereby can facilitate our further exploration. Besides, the well-characterized *E. coli* protein, Single-Stranded DNA Binding Protein (SSB), that can bind ssDNA with high affinity,[10] is used as the model protein to interact with N-DNA, and the formed N-DNA/SSB complex is also used as template for the synthesis of Ag NCs. Next, we will compare the fluorescent stability of Ag NCs synthesized with N-DNA and N-DNA/SSB complex templates.

N-DNA-templated Ag NCs are prepared as described previously,[2b] and the formed N-DNA/Ag NCs show a strong emission peak at 768 nm (excited at 713 nm, Fig. 1A, curve a). Meanwhile, synthesis using N-DNA/SSB complex as template (Fig. 1B, curve b) shows similar emission peak (λ_{ex}= 713 nm, λ_{em}=768 nm), indicating that the presence of SSB shows no influence on the silver species. The fluorescent intensity of Ag NCs reached its maximum three hours after the initiation of NC nucleation. In order to quantitatively compare the fluorescent stability of N-DNA/Ag NCs and N-DNA/SSB/Ag NCs, we monitored the decay of fluorescent intensity. According to the decay curves in Fig. 1B and C, we can calculate the values of IT_{50} (defined as the time when the fluorescent intensity reduced to 50%), and use the ratio between $IT_{50(N\text{-}DNA/SSB/Ag\ NCs)}$ and $IT_{50(N\text{-}DNA/Ag\ NCs)}$ to evaluate the stability enhancement efficiency. Results demonstrate that fluorescent stability of N-DNA/Ag NCs can be enhanced >125-fold in the presence of SSB with $IT_{50(N\text{-}DNA/SSB/Ag\ NCs)}$ value of >250 h and $IT_{50(N\text{-}DNA/Ag\ NCs)}$ value of 2 h. These results indicate that N-DNA/SSB complex serves as a competent template for Ag NCs with enhanced stability.

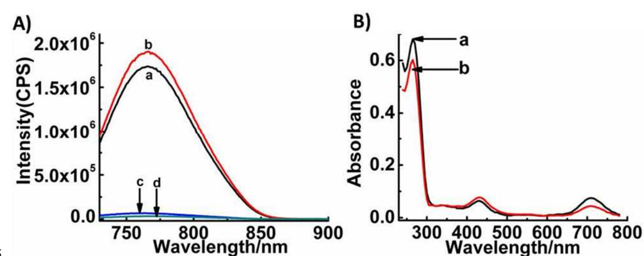

Fig. 2 (A) Fluorescent spectra of various solutions: a) addition of SSB into the freshly prepared N-DNA-templated Ag NCs; b) N-DNA/SSB-stabilized Ag NCs; c) N-DNA-stabilized Ag NCs. The fluorescent spectra (a-c) were monitored ten hours after the initiation of NC nucleation. d) N-DNA-stabilized Ag NCs was prepared, and after ten hours, SSB was introduced. (B) UV-vis spectra of a) N-DNA/SSB/Ag NCs and b) N-DN A/Ag NCs.

To fully understand the mechanism of such phenomena, a series of control experiments were performed: (1) native polyacrylamide gel electrophoresis (PAGE) experiment is utilized to characterize the formation of N-DNA/SSB/Ag NCs. As shown in Fig. S1, in the presence of SSB, the band corresponding to N-DNA/Ag NCs (lane 2) disappears, and the formed large molecular SSB/N-DNA/Ag NCs complex is difficult to migrate in the gel (lane 3). These results provide evidence that SSB really interact with N-DNA/Ag NCs; (2) as controls, bovine serum albumin (BSA) and thrombin, which do not bind N-DNA, were tested for their effect on the fluorescent stability of N-DNA/Ag NCs. We first confirm that BSA or thrombin itself does not quench the fluorescence of N-DNA/Ag NCs, and the fluorescence of N-DNA/Ag NCs in the presence of the two control proteins also reaches its maximum within three hours (Fig. S2). However, the fluorescence is almost completely quenched within ten hours in the presence of BSA or thrombin (Fig. 1D), implying that the fluorescent stability of N-DNA/Ag NCs is not improved. These results suggest that the specific binding between N-DNA and SSB is essential for the enhanced stability of N-DNA/SSB-stabilized Ag NCs; (3) syntheses performed with SSB alone produced non-emissive solutions (Fig. S3, curve a). Given that SSB did not support Ag NCs synthesis, it is clear that for N-DNA/SSB-stabilized Ag NCs system, N-DNA functions as NC-nucleation sequence; (4) a control ssDNA (named G-DNA, sequences see Scheme 1B) that could not facilitate Ag NCs synthesis was investigated, and results demonstrate that syntheses employing G-DNA or G-DNA/SSB complex did not produce Ag NCs or luminescent material (Fig. S3, curves b and c). These results again emphasize the important role of ssDNA template on the formation of Ag NCs; (5) we also investigate the fluorescent stability of Ag NCs when SSB is introduced after the formation of N-DNA-stabilized Ag NCs. It is found that if SSB is introduced into the freshly prepared Ag NCs solution, the stability of Ag NCs still can be enhanced (Fig. 2, curve a). However, if SSB is involved when the fluorescent emission of N-DNA/Ag NCs is very weak (Fig. 2, curve c), the recovery of the decreased fluorescence can not be observed (Fig. 2, curve d). This phenomenon reveals that the process of fluorescence quenching is irreversible, and DNA/protein complex only protect freshly produced Ag NCs from oxidation by air/O_2; (6) we further use UV-vis absorption spectroscopy to characterize Ag NCs synthesized with N-DNA or N-DNA/SSB as template (Fig. 2B). Compared with N-DNA template, the absorption intensity corresponding to Ag nanoparticles (Ag NPs) at 430 nm demonstrates certain decrease (~20%) when N-DNA/SSB complex is used (Fig. 2B, curves b→a). These results suggest that synthesis of Ag NCs with N-DNA/SSB as template can reduce the amount of by-product Ag NPs. According to previous research of ssDNA/SSB complex,[11] ssDNA wraps around the SSB tetramer *via* electrostatic and stacking interactions. In this case, we speculate that the peripheral N-DNA can interact with Ag^+, while the wrapped SSB can provide a better hydrophobic microenvironment for the synthesis of Ag NCs. (7) to demonstrate the generality of DNA/protein interactions-induced fluorescent stability, another ssDNA template (named R-DNA) that has been reported to create red-emitting Ag NCs is also tested.[5] As shown in Fig. 3A, the fluorescence spectra of R-DNA-directed Ag NCs (curve a) and R-DNA/SSB-templated Ag NCs (curve b) showed predominant fluorescence emission peak at 660 nm (excited at 590 nm). The fluorescence reached balance within nine hours. According to the decay curves in Fig. 3 B and C, the presence of SSB also can enhance the stability of R-DNA/Ag NCs with an enhancement ratio greater than 10. To further verify the generality of the proposed strategy, more ssDNA-templated Ag NCs are investigated. Similar phenomena are observed (results are listed in Fig. 3D), namely, SSB can promote the

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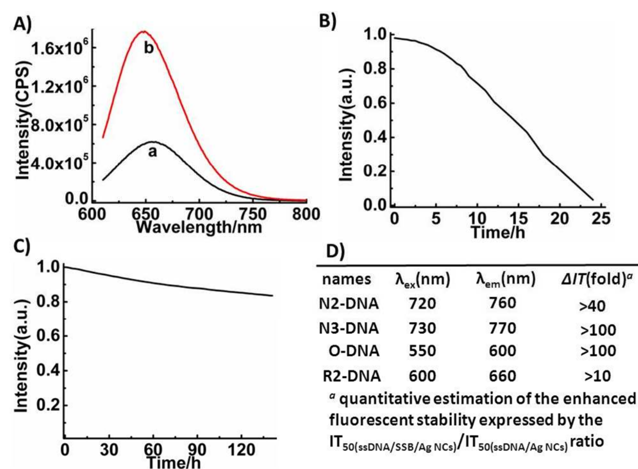
fluorescent stability of ssDNA/Ag NCs upon interaction with ssDNA template. These results clearly demonstrate the generality of such strategy.



names	λ_{ex}(nm)	λ_{em}(nm)	ΔIT(fold)[a]
N2-DNA	720	760	>40
N3-DNA	730	770	>100
O-DNA	550	600	>100
R2-DNA	600	660	>10

[a] quantitative estimation of the enhanced fluorescent stability expressed by the $IT_{50(ssDNA/SSB/Ag\ NCs)}/IT_{50(ssDNA/Ag\ NCs)}$ ratio

Fig. 3 (A) Fluorescent spectra of R-DNA/Ag NCs (a) and R-DNA/SSB/Ag NCs (b). Decay curves of the fluorescent intensity for R-DNA/Ag NCs (B) and R-DNA/SSB/Ag NCs (C). (D) SSB-induced fluorescent stability enhancement efficiencies (ΔIT) of several ssDNA/Ag NCs.

On the basis of the above results, the possible mechanism for the enhanced stability of ssDNA/SSB complex-stabilized Ag NCs can be proposed: 1) ssDNA, as ligand and programmable template, plays an essential role for nucleation; 2) the stabilizing effect of SSB is achieved through its binding with ssDNA template. The results of these studies not only provide insights into the mechanism of DNA-mediated NCs growth but also lay the groundwork for programmable synthesis of NCs with improved properties. Previous studies have argued that nucleic acid structure, composition and sequence can fine tune the optical properties of DNA/Ag NCs.[6c,9,10,12] The proposed strategy in this work can to some extent solve the fluorescent instability of Ag NCs. Taken together, it is hoped that DNA/protein-stabilized Ag NCs would provide a promising and powerful tool for biological imaging and sensing.

Next, we test the stability of ssDNA/SSB/AgNCs in cell culture media (here DMEM). 10×N-DNA/SSB-stabilized Ag NCs are synthesized and diluted with PBS-Buffer (20 mM phosphate, pH 7.4, 1 mM magnesium acetate) or DMEM. The value of F_{DMEM}/F_{Buffer} is used to evaluate the stability in cell culture media, where F_{DMEM} is the fluorescent intensity of Ag NCs diluted with DMEM; F_{Buffer} is the fluorescent intensity of Ag NCs solution diluted with PBS-Buffer. The results are listed at Table S1. Results demonstrate that the fluorescence of Ag NCs can be somewhat quenched (23%) with DMEM-to-DNA/Ag NCs volume ratio of 10:1. It is possible that the high concentration of Cl^- (KCl, NaCl) in DMEM affects the fluorescence of Ag NCs. This can be proved by the following experiments (Fig. S4): when we add NaCl (0.5 M) to the solution of Ag NCs, obvious fluorescent decrease can be observed; however, when adding $NaNO_3$ (0.5 M), no fluorescent decrease appears. These results indicate that the presence of DNA-binding protein can not prevent the influence from Cl^-, which should be countered in future research.

In addition, we explore the influence of pH and temperature on the fluorescent stability of N-DNA/SSB/Ag NCs. We find that N-DNA/SSB-stabilized Ag NCs are stable between pH 6–8 (Fig. S5A), and the pH value of solution (here we chose pH 5.0) shows no obvious influence on the fluorescent stability (Fig. S5B, IT_{50}>250 h). Compared with the fluorescent intensity at room temperature (~25 °C), only 14% fluorescent decrease occurs at 90 °C (Fig. S5C). However, high temperature (here we chose 80 °C) really influences the fluorescent stability (Fig. S5D, IT_{50} was reduced to ~5h), which might be due to the thermal denaturation of N-DNA and SSB at high temperature.

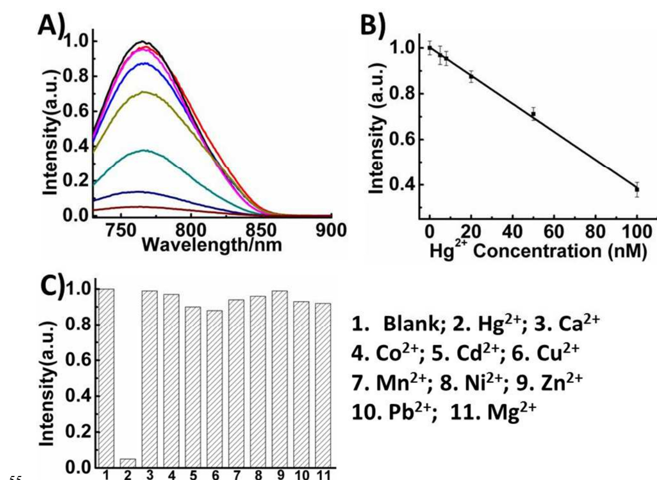


Fig. 4 (A) Fluorescence spectra of N-DNA/SSB-stabilized Ag NCs in the absence and presence of Hg^{2+} with different concentration (0-0.5 μM). (B) Plot of the values of the normalized intensity for DNA/SSB-stabilized Ag NCs with respect to the concentrations of Hg^{2+} (0-0.1μM). (C) Selectivity of N-DNA/SSB-stabilized Ag NCs probes to different metal ions. The concentration of Hg^{2+} is 0.5 μM, and the concentration of other metal ions is 5 μM.

To demonstrate the potential applications in biosensing, we apply N-DNA/SSB-stabilized Ag NCs for the selective and sensitive detection of Hg^{2+}. As shown in Fig. 4, the fluorescence of Ag NCs is quenched with an increasing concentration of Hg^{2+}. There exists a good linear relationship (R^2=0.997) between the fluorescent intensity and the concentration of Hg^{2+} over the range of 0-0.1 μM (Fig. 4B). As low as 8 nM Hg^{2+} can be detected, which meets the requirement of the U.S. Environmental Protection Agency (EPA) standard for the maximum allowable level of inorganic Hg^{2+} in drinking water (10 nM). Besides, N-DNA/SSB-stabilized Ag NCs exhibit high selectivity for Hg^{2+} over other competing metal ions (Fig. 4C). It can be expected that the intrinsic fluorescent stability of N-DNA/SSB-stabilized Ag NCs probes might avoid "false positive" results, and thereby ensure the accuracy of detection.

Conclusions

In conclusion, we have described the first example of DNA/protein complex-stabilized Ag NCs with enhanced fluorescent stability. DNA/protein complex provides an advantageously synthetic microenvironment for the synthesis of Ag NCs: DNA functions as a prerequisite for the production of Ag NCs; the presence of protein can help to stabilize the formed Ag NCs through its interaction with DNA template. In general, DNA-templated Ag NCs have advantage of higher fluorescence

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quantum yields[13] and are particularly responsive to optical modulation of their emission.[9a] In comparison, protein-stabilized Ag NCs often have longer shelf-lives,[14] but display low quantum yields.[13] The present DNA/protein complex-stabilized Ag NCs display advantages of both templates. With rational choice of DNA ligands and corresponding interactive proteins, this concept might be extended to other DNA/protein-mediated NPs synthesis system. Furthermore, the findings presented in this work portend the promise of Ag NCs as fluorescent labels, and have implications in the underlying mechanisms of nucleic acid/nucleotide-directed NPs/NCs synthesis.

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† Electronic Supplementary Information (ESI) available: Experimental details and supporting figures. See DOI: 10.1039/b000000x/

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