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A Mini Review on Controlling the Size of Ag Nanoclusters by Changing the Stabilizer to Ag Ratio and by Changing DNA Sequence

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Abstract

Ag nanoclusters have received considerable attention in the past decade due to their distinguished photo-physical properties, which lead to very wide potential applications for biosensing and imaging. To this point, synthesis of well-defined Ag nanoclusters for practical applications is a key issue, in particular, controlling the size (or specific number of silver atoms) of Ag nanoclusters. Herein, we briefly discuss the effect of ratio of reactants, in terms of specific functional groups, on the size of Ag nanoclusters. Also, taking DNA as an example of biopolymer, we review how the DNA sequence can affect the specific number of Ag atoms in Ag nanoclusters. These conducted principles should provide significant guidance for preparation of Ag nanoclusters of precise size.

Key words: Silver nanoclusters; Size control; Stabilizer; Ratio of stablizer to Ag; Functional groups; DNA template

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INTRODUCTION

Ag nanoclusters (AgNC) consist of two to roughly a hundred atoms with the size equal or less than 2 nm. They exhibit molecule-like properties because their small size is comparable to the Fermi wavelength of electrons (~0.5 nm for Ag). This indicates that AgNC has discrete energy levels of electrons, allowing discrete electronic transitions and strong fluorescence (Xu, & Suslick, 2010). The AgNC fluorescence with lifetime and high intensity enables the detection of many ions and compounds for diagnostics (Jameson & Ross, 2010), bioimaging and many other applications (Shang, Dong, & Nienhaus, 2011). AgNC can be used to detect metal ions, like Hg^{2+} (Adhikari, & Banerjee, 2010; MacLean, Morishita, & Liu, 2013) and Cu^{2+} (Lan, Huang, & Chang, 2010), ochratoxin A (Chen et al., 2014), thiol compounds (Huang et al., 2011), melamine (Han et al., 2012), nitrate (Dhanya, Saumya, & Rao, 2013, protein (Li et al., 2012; Qian et al., 2014), bacteria (Wu et al., 2012; Chung et al., 2013) and even miRNA (Shah et al., 2014 ; Shah et al., 2014). Other biological applications include tracing drug delivery (Su et al., 2013), antibacterial activity (Wang et al., 2012), bioimaging (both living cells and tissues) (Antoku et al., 2010; Byers & Hitchman, 2011; Shiang et al., 2012; Dong et al., 2013), probe for drug–DNA interaction (Yuan, Guo, & Wang, 2011), etc..

Although fluorescent AgNC had become a promising strategy in many fields in the past decade, no practical product has been developed yet. The main bottleneck lies in the design and preparation of functional AgNC, namely, controlling over the specific number of Ag atoms or the size of AgNC (Xu & Suslick, (2010). Ag atoms are relatively more reactive than Au atoms, and they are easily oxidized. As a result, stabilizers must be introduced to prepare stable AgNC. The first type of stabilizers is inorganic scaffold/substrate. AgNC containing well-defined number of atoms can be produced under suitable conditions (Díez & Ras, 2011). The most popular technology is vapor deposition of Ag atoms. The Ag atoms are thermally evaporated and transported by a flow of noble gas and then deposited on a cooled transparent substrate to form Ag islands that have strong emission

ranged from UV to near IR. However, the surface Ag atoms are all oxidized by oxygen. It remains unclear how the fluorescence occurs and what the role is of oxygen or substrate plays for the fluorescence (Peyser et al., 2001). Another problem of this type of stabilizer is that the produced particles are not soluble, and they are greatly limited to be applied in biological systems.

To gain more wide applications in biological system, another type of stabilizer is developed. It includes small molecules terminated with –SH groups, and polymer chains containing –SH, -COOH, –OH, -NH_2 and/or =NH groups (Díez & Ras, 2011)[24]. These functional groups exist widely in the natural system and have perfect biocompatibility. More importantly, they all consist of strong electronegative elements, which can strongly interact with Ag atoms to stabilize AgNC. –SH is the most reactive with Ag atom to form S-Ag bond spontaneously so that many small thiol compounds can be used as stabilizers. The affinity of -COOH, –OH, -NH_2 and =NH groups to Ag atoms is much lower than –SH. They often interact with Ag atoms as residues of long polymer chains, whose secondary structure or the folded form contribute to stabilizing AgNC.

How does this type of stabilizer determine the size of AgNC? The ratio of efficient functional groups of stabilizer to Ag ions should play a dominant role when small molecules are used as stabilizers. Actually, polymer chains are also one type of second stabilizers that containing a large number of various effective functional groups. These functional groups may also play a important role during traping AgNC. However, the sequence of the chain, or in terms of secondary structure, should also play an important role on trapping Ag atoms. For example, DNA is the most commonly used template to prepare AgNC. Its composition and sequence can greatly affect the size of AgNC formation (Petty et al., 2004; Gwinn et al., 2008). The present paper highlights the role of the ratio of the stabilizer to Ag as well as the effect of DNA/RNA bases and sequence, for the size control of synthesized AgNC. Since the size of AgNC can be affected by many ambient factors, like tempreture and pH (Lan, Huang, & Chang, 2010; Shang & Dong, 2008; Liu et al., 2013), the environmental factors are not taken into account here.

1. CONTROL OVER SIZE BY RATIO OF SMALL STABILIZER TO AG IONS

Many small molecules have been used as stabilizers to synthesize AgNC where the interaction between specific functional groups and Ag ions should play a central role in the product. Normally, Ag ions are added into the stabilizer solution to form stabilizer-Ag^{+}_{n} complex or stabilizer protected Ag^{+}_{n} clusters, followed by reduction using chemical or photo/irradiation technologies. The reduction process is carried out in darkness to prevent AgNC excitation by light. Here we compare some representative reports to get insight on the effect of stabilizer to Ag ratio on the size of AgNC, as well as the interaction mechanisms.

1.1 Relative Reactivity of Different Functional Groups

Conducted from previous publications, –SH, -COOH, –OH, -NH_2 and =NH are main groups that interact with Ag atoms to stabilize AgNC. Amino acids contain these functional groups and they have been frequently used to synthesize AgNC. Trp-Ag+n complex has been prepared based on interactions between Ag+ and tryptophan. Ratio of Trp to Ag+ is 1:5. –COOH, -NH_2 and –NH- groups can function as reactive sites and the value of n is 2-5 or 9 (Mitrić et al., 2007; Mitrić et al., 2008). This complex does not undergo reduction process to form AgNC and has no fluorescence. Glutathione can ligate silver clusters to form Ag: SG clusters. A different AgNO3: GSH ratio (1:1, 1:3, 1:4, or 1:10) is used and different ratios can shift the size distribution. However, in PAGE, the relative positions and colors of bands remain the same. This indicates the change of constituent clusters, namely, the overall composition of the product. For instance, compared with 1:4 ratio of $AgNO_3$: GSH, the 1:3 ratio will shift the mass distribution toward larger cluster sizes. The size of Ag: SG is about 2.5 nm while metal core is ~1 nm in diameter (Kumar, Bolan, & Bigioni, 2010). Glutathione contains –COOH, -NH_2, –NH- and –SH groups whereas only –SH bind with Ag+ to form clusters, demonstrating that –SH is much more reactive than other functional groups and has priority to form S-Ag bonds.

Kumacheva et al. (2005) used poly (N-isopropylacrylamide-acrylic acid-2-hydroxyethyl acrylate) microgel as scaffold to form fluorescent AgNC with molar ratio COOH : Ag of 1:1. The formed AgNC contain 2-8 Ag atoms. The microgel consists of both –OH and –COOH, and –COOH is said to be more critical for the formation of nanoclusters. AgNC containing several Ag atoms are also prepared using fourth- and second-generation -OH terminated poly (amidoamine) (G4-OH, G2-OH) (Zheng & Dickson, 2002). Actually, based on the stabilizing effect of –OH and –COOH groups, AgNC can be layer-by-layer assembled as films (Zhang et al., 2013).

Besides amino acids, pyridine derivates, like diphenylphosphine-2-pyridine (Wang et al., 2004; Jia & Wang, 2009) have also been utilized to prepare AgNC, where =NH group can interact with and stabilize Ag atom. However, the interaction between =NH and Ag atoms is not very strong and it does not play a dominant role in the formation of the product, gold (I) - silver (I) cluster complex.

Overall, relative reactivity for stabilizing Ag nanoclusters is: -SH >> -COOH > -OH > -NH2> =NH groups. –SH has the strongest affinity to Ag atoms and stabilizes AgNC either in small molecule or in micro

polymers. In comparison, the other four functional groups have low affinity and mainly function as residuals of long polyer chains, where the limitted steric space also contributes to stabilize AgNC.

1.2 Effect of Stabilizer: Ag Ratio on the Size of AgNC

To figure out the effect of stabilizer and Ag ratio on the size of AgNC, some research details are compared in table 1, including stabilizer species, stabilizer and Ag ratio, size of AgNC and the reduction methods. Since the experimental parameters vary (like tempreture and pH (Lan, Huang, & Chang, 2010; Shang & Dong, 2008;. Liu et al., 2013), it's hard to conduct some precise rules of how the ratio determines specific number of Ag atoms in AgNC. However, it's certain that the size of AgNC can be controlled less than 2 nm, consisting of several hundreds of Ag atoms or less, adjustable by changing stabilizer and Ag ratio. As shown in figure 1, the super-stable 25kDa $Ag_{152}(SCH_2CH_2Ph)_{60}$ Clusters are prepared in solution of about 5:1 ratio of PETH: Ag (Chakraborty et al., 2012). Ag_5 clusters can be synthesized by conjugating with DHLA under the DHLA: Ag ratio of 100:1 (Adhikari & Banerjee, 2010). Although different stabilizers are used, increase of stabilizer to Ag ratio will lead to the decrease of the size of AgNC. The size decreases from ~ 2.0 nm to ~1.4 nm, when the ratio increases from 1:1 to 10:1 (Liu et al., 2013; Chakraborty et al., 2012; Roy & Banerjee, 2011). If the ratio gradually increase to 100:1, AgNC of less than 1 nm is obtained, containing 4 to 5 Ag atoms (Adhikari & Banerjee, 2010). This is in agreement with that reported by Kumar S. et al (2010). They have found that increasing of Glutathione: AgNO3 ratio (1:1, 3:1, 4:1, or 10:1) will shift the mass distribution toward smaller cluster sizes. These researches indicate that large stabilizer to Ag ratio facilitates formation of small sized AgNC when small molecules act as stabilizers.

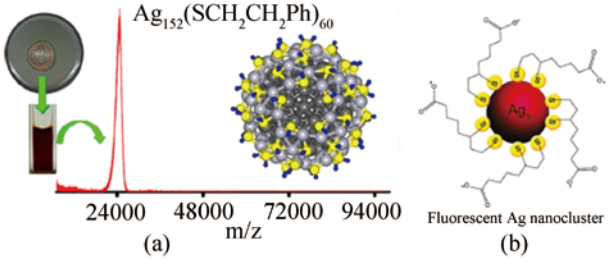

Figure 1
(a) Optimized Icosahedral Structure of $Ag_{152}(SCH_2CH_2Ph)_{60}$ Clusters and the Matrix-Assisted Laser Desorption Mass Spectrum (MALDI MS). This AgNC Is Prepared by Chakraborty et al. (2011), With PETH: Ag Ratio 5:1. (b) Scheme Illustrating the Formation of Ag_5 Nanocluster-DHLA Nanoconjugate, Prepared by Adhikari et al. (2010), With the DHLA: Ag Ratio 100:1
Note. Reproduced from Adhikari & Banerjee, 2010.

Table 1
Comparison of Different Stabilizer, Stabilizer to Ag^+ Ratio and Their Effect on the Size of Formed AgNC Under Certain Reduction Methods

Stabilizer species	Ratio (Stabilizer: Ag^+)	Reduction method	Size of AgNC or number of Ag	Reference
2-mercaptobenzoic acid (H_2mba)	1:1	Ultrasonic conditions at 50 °C	$[(\mu_6\text{-S})@Ag_{17}(mba)_{16}]$	Sun et al., 2011
2-mercaptonicotinic acid (H_2mna)	2 H_2mna: 1 Ag_2O	\	$[Ag(mna)]_6$ 6-	Tsyba et al., 2003
Tryptophan	1:5	\	Trp–Ag^+_n, n = 2–5,9	Mitrić et al., 2007; Mitrić al., 2008
Glutathione	1:4	$NaBH_4$ solution	~ 1nm	Kumaret al., 2010
Dpenicillium or l-penicillium	1:1	Strong UV irradiation at 365 nm	~2nm	Liu et al., 2013
Phenylethanethiol (PETH)	5.3:1	$NaBH_4$ solution	$Ag_{152}(SCH_2CH_2Ph)_{60}$	Chakraborty et al., 2012
0.1% w/v gel of Fmoc-L-phenylalanine-OH	~ 10:1	Light reduction at pH 7.46 at room tempreture	~1.4 nm	Roy, 2011
Dihydrolipoic acid (DHLA)	100:1	$NaBH_4$ solution	Ag_4 and Ag_5	Adhikari et al., 2010
Poly (N-isopropylacrylamide -acrylic cid-2-hydroxyethyl acrylate)	1:1	UV irradiation	Ag_2-Ag_8	Zhang et al., 2005
Fourth-generation OH-terminated poly (amidoamine) (G4-OH)	1:3	Irradiation of blue light (450-480 nm)	Ag_2-$_A$g8	Zheng et al., 2002
Poly (methacrylic acid) (PMAA)	2:1	Photoreduction	\	Shang et al., 2008

When polymers are used as templates, AgNC is normally composed by several Ag atoms (Shang & Dong, 2008; Zhang, Xu, & Kumacheva, 2005; Zheng, & Dickson, 2002). The polymer to Ag ratio seems to have a weak effect on the size of AgNC. There are mainly two reasons: a) Each polymer chain is made up of large amount of unit molecules, indicating a great number of interacting sites of functional groups, which accumulate to form strong interactions to stabilize AgNC. Even with very low polymer to Ag ratios, the efficient functional group to Ag ratio is very high, facilitating formation of ultra small AgNC. b) The gaps or crevices caused by the folding of the long polymer chain may play a more dominant role on the size of AgNC due to its steric limitations, as illustrated in Figure 2.

Figure 2
Scheme Drawing of How Ag Nanoclusters Are Trapped and Stabilized By Polymer Chain, as Well as the UV Excitation and Emission of Fluorescence
Note. Reproduced from O'Neill, Gwinn, & Fygenson, 2011.

The above studies also suggest that polymer prefers to form ultra small AgNC consisting of several Ag atoms, while small molecule stabilizers may facilitate the formation of large NC. On the other hand, the polymer-templated AgNC may undergo a wider distribution of size of AgNC. This is owing to the uncertain size of steric space caused by random folding structure. As exclusive examples, however, some biopolymers follow certain regular rules to construct the secondary structure, like nucleic acid (Enkin et al., 2014), proteins and DNA/RNA. Taking DNA (or oligonucleotide) as an example, the well-defined sequence and shape (linear, loop or hairpin) can stabilize AgNC consisting of several Ag atoms, with a very narrow size distribution (Díez & Ras, 2011; winn et al., 200; Richardset al., 2008; O'Neill et al., 200; Petty et al., 2013).

2. CONTROL OVER SIZE BY DNA BASES AND SEQUENCE

DNA is the most popular biopolymer used to synthesize AgNC (Chen et al., 2014; Qian et al., 2014; Shah et al., 2014; Schultz et al., 2013). A lot of researches have been carried out to investigate how the size and photo-physical properties are controlled by the DNA compositions (in terms of four kinds of bases), sequences and even shapes. These factors, along with various environmental parameters, play a complicated role on determining the size and properties of formed AgNC. Some representative reports are listed and compared in Table 2. Summarized from these researches, we find that ultra-small AgNC with less than 15 Ag atoms can normally be prepared using DNA templates, of which the amount of bases varies between 6 and 46.

Table 2
Comparison of Different DNA Sequences and Their Roles on Determining the Size and Emission Maxima of AgNC

DNA sequence (5'-3')	Size (amount of bases)	Shape	Emission maximal wavelength (nm)	Size of AgNC or number of Ag	Reference
C_{24}	24	Chain	650 & 715	$C_{24}Ag_n$	Antoku et al., 2010.
TGACTAAAAACCCTTAATCCCC	22	\		\	
AGTCACCCCAACCTGCCCTACCACG GACT	29	\		\	
GGCAGGTTGGGGTGACTAAAAACCC TTAATCCC	34	\		\	Sharma et al., 2010
AGTCCGTGGTAGGGCAGGTTGGGGT GACTAAAAACCCTTAATCCCC	46	\		3nm (hydrodynamic radius)	
CCCTTAATCCCC+TATAATAAATTTTA AATATTATTT ATTAAT	12+30				Yeh et al., 2010
AGGTCGCCGCCC	12			<10	Petty et al., 2004
C12	12		665	2-7	Ritchieet al., 2007
$_C$12	12		650 & 700 440 & 560	2, 3	Vosch et al., 2007; Somoza et al., 2013
C_n	6, 10, 12, 24		615		Huang et al., 2011
$C_3AC_3AC_3XC_3Y$, (X, Y) = (T/ G, A) and (X, Y) = (A,G)	16			9, 10	Petty et al., 2011
TATCCGT-C_n-ACGGATA, *n*=6-9, 12	20-23, 26			9-14	O'Neil et al., 2011

To be continued

Continued

DNA sequence (5'-3')	Size (amount of bases)	Shape	Emission maximal wavelength (nm)	Size of AgNC or number of Ag	Reference
C_{20}	20		523		
$AATTC_{12}$	16		562		
$CGAAC_{12}$	16		590	5-11	Petty et al., 2013
$CGCGC_{12}$	16		615		
$ATATC_8$	12		635		
$GGGGC_8$	12		670		
$C_3AC_3AC_3TC_3A$+ CCCGCCGCTGGA	28		400	~11	Petty et al., 2013
TATCCGT-C_n-ACGGATA, *n*=3- 12	17-26			11, 13 (*n*=9)	O'Neillet al., 2009
TATCCGTX_5ACGGATA, X=C, G, T, A	19			2-12	Gwinn et al., 2008
CCCTTTAACCCC	12		485		
CCCTCTTAACCC	12		520		
CCCTTAATCCCC	12		572		Richards et al., 2008
CCTCCTTCCTCC	12		620		
CCCTAACTCCCC	12		705		

Compared with using those stabilizers mentioned in part 2 and other preparation technology (like vapor deposition), utilizing DNA templates appears to be the ideal method to control the size of AgNC. The Ag atom number is easily controlled to be less than 15 and the formed AgNC usually undergo a narrow size distribution (Gwinn et al., 2008; O'Neill et al., 2009). Therefore, the research interest has gradually turned from size control to photo-physical properties of AgNC as well as their applications. Here, we will briefly review the different role of four kinds of bases, how the DNA sequence affects the size and emission band of AgNC, and how DNA sequence is designed to meet the requirement of practical applications.

Notes: Four abbreviated bases: Adenine (A), Guanine (G), Cytosine (C) and Thymine (T).

All "DNA" in present paper represent single strand DNA (ssDNA).

2.1 Role of Four Kinds of Bases on AgNC

All four kinds of the bases contain effective functional groups that can interact with Ag atoms to stabilizer AgNC. These electron-rich functional groups, $-NH_2$, =NH and C=O, are able to interact with Ag atoms. Based on density functional theory (DFT), Volkov et al. (2013) shows that Ag clusters may be bind to the Watson–Crick guanine–cytosine base pairs and to single DNA bases with about the same affinity. Gwinn et al. (2008) also confirmed that that Ag atoms bind to single stranded DNA via Watson–Crick base pairing. They proved that Ag atoms have comparable affinities to cytosine and guanine using time-of-flight mass spectra (MS). On the other hand, 1H NMR spectra and DFT results indicated that cytosine has a higher affinity to Ag atoms than other three bases (Soto-Verdugo, Metiu, &. Gwinn, 2010). Dickson and coworker (Petty et al., 2004) have also confirmed that cytosine functions as the main sites for Ag atoms attachment.

Guanine, however, has been excluded during designing DNA sequence due to its propensity for self-binding (Richards et al., 2008). Zhou et al. (2007) showed that the G-rich strands prefer to employ "self-association" to forma the G-quadruplex rather than hybridized integration. They tested 15 6-nt oligonucleotides where MS spectra indicated that formation of a parallel tetramer quadruplex requires at least four continuous guanines in the 6-nt sequence.

Four kinds of bases also show different effect on wavelength of absorption and fluorescence of AgNC. Thymine-rich oligonucleotides tend to form of AgNC with only blue/green-emission, whereas cytosine-rich oligonucleotides direct both red- and blue/green-emitting AgNC (Sengupta et al., 2008). The proximity to guanine-rich DNA sequences can enhance the red fluorescence of AgNC for 500-fold (Yeh et al., 2010). This is caused by an increase in the number of Ag nanoclusters "turned on" to be fluorescent due to guanine proximity.

Actually, effects of specific bases on the formation and emission wavelength of AgNC are all studied in the form of DNA chain, instead of individual base molecules. Thus these effects do not represent the behavior of single base molecule but of a certain DNA sequence, which indeed provides some helpful guidance on the design of DNA sequence for AgNC preparation.

2.2 How the DNA Sequence Determine the Size and Properties of AgNC

DNA strands have been proven to be ideal templates to control the size of AgNC since the amount of Ag atoms

can be limited within 15. The photo-physical properties of AgNC, however, are distinct when different DNA strands are used, including fluorescence lifetime, absorption and emission wavelength, and fluorescence quantum yield (Richards et al., 2008; Petty et al., 2013; Sharma et al., 2010). Even though only one or two base(s) of the DNA strand is (are) changed, the fluorescence properties vary a lot (O'Neill, Gwinn, & Fygenson, 2011; Richards et al., 2008). In specific, the roles of DNA sequence are achieved mainly through three aspects, composition, length and shape.

DNA consists of four types of bases that have distinct effect on AgNC formation. Therefore, the proportion of certain base affects AgNC formation. As discussed in part 3.1, cytosine and guanine have comparable affinities to Ag atoms, which are stronger than adenine and thymine. Cytosine acts as the main sites for Ag atoms interaction while G-rich strands prefer self-binding and form quadruplex. Different bases can also be used to tune emission wavelength of AgNC (Petty et al., 2013), since UV excitation of AgNC is completed through the DNA bases (O'Neill, Gwinn, & Fygenson, 2011). Thymine-rich NDA strands prefer formation of AgNC with only blue/green-emission. Cytosine-rich DNA strand direct both red- and blue/green- emitting AgNC. And guanine-rich DNA sequences can enhance the red fluorescence of AgNC. On the other hand, these results suggest it possible to use UV excitation as a universal pathway to provide information about the type, number, and orientation of cluster-bound bases (O'Neill, Gwinn, & Fygenson, 2011).

As listed in Table 2, no obvious difference is observed on the size of the formed AgNC when the length of DNA ranges from several to dozens of bases. However, the optimal number of bases is around a dozen to synthesize a few Ag atoms contained AgNC, while 5- 9 bases directly interact with Ag atoms to stabilize AgNC (O'Neillet al., 2009; Petty et al., 2013; Sharma et al., 2010; Petty, et al., 2011). It is very interesting that the emission maxima remain the same even though the length is composed of a single type of bases. Huang et al. (2001) studied a cytosine oligonucleotide (C_n, n=6, 10, 12, 24) bound AgNC, of which the emission bands all centered at 615 nm. At the same time, another three reports (Ritchie et al., 2007; Vosch et al., 2007; Somoza et al., 2013), also use C_{12} as template to prepare AgNC whereas the emission maximum varies a lot. This indicates that the emission maximum is determined by the DNA sequence along with the environmental factors.

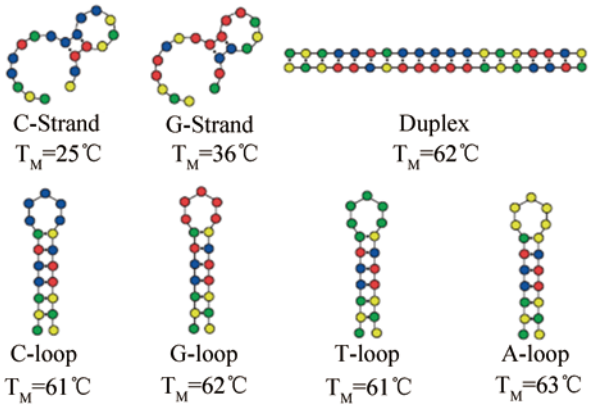

Figure 3
Scheme Draw of the 19-Base DNA Oligomers Used by Gwinn et al. (2008). Blue = C, Green = T, Red = G, and Yellow= A. Black Dots Represent Base Pairing and Solid Lines the Sugar-Phosphate Backbone. Top: C-Strand and G-Strand Form the Duplex When Annealed Together. Bottom: Hairpins C-Loop, G-Loop, T-Loop and A-Loop
Note. Reproduced from Gwinn et al., 2008.

Regarding to the role of the shape of DNA on AgNC preparation, we have to cite the study by Gwinn and coworker (Gwinnet al., 2008). They designed a series of 19-base DNA oligomers to form three different shapes to direct AgNC formation. Shown in Figure 3, a purely ds "Duplex", single-stranded DNA chain and hairpin oligomers (around 2 nm across) are included. The Duplex has the weakest ability to host fluoresencent AgNC because the binding sites of Ag atoms are rendered inaccessible by Watson–Crick base pairing. This is also the reason why we focus on only ssDNA and all DNA represent ssDNA in present paper. The ssDNA directs few-atom AgNC with visible fluorescence whose spectral properties are sensitive to the sequence and secondary structure of DNA. Hairpin-based fluorophores exhibit fluorescence with steady dipole radiation patterns and intermittency. Especially, they also studied how hairpins with Poly-C loops stabilize different types of AgNC (O'Neill et al., 2009). However, the cues that lie in DNA and are responsible for these physical features are still unclear.

2.3 Applications Upon Ag_n-DNA

Although interaction between AgNC and DNA template is complicated, fluorescent Ag_n-DNA have already exhibited wide potential applications on detection and bioimaging. The fluorescence of Ag_n-DNA is sensitive to the presence of metal ions or other compounds. As a result, these ions or compounds can be sensed by the change of fluorescence on Ag_n-DNA. For example, the fluorescence emission is rapidly quenched in the presence of HgII and more quenching occurs with an increase in Hg^{2+} so that Ag_n-DNA can be used for Hg^{2+} sensing (Adhikari & Banerjee, 2010; MacLean, Morishita, & Liu, 2013). The same principles can also be applied on detection of

melamine (Han et al., 2012) and nitrate (Dhanya, Saumya, & Rao, 2013).

Actually, both Ag atoms and DNA strands can function as active sites during the application of AgNC. It's well known that Ag atom can easily bind with –SH group to spontaneously form Ag-S bond. Therefore, Ag_n-DNA can be used for fluorescence turn-on detection of thiol compounds (Huang et al., 2011). On the other hand, a linker terminated with –SH groups can be used to attach AgNC with antibodies and to detect proteins (Liet al., 2012). For further applications, on the basis of detection of certain proteins, Ag_n-DNA can also be used to detect cells and bacteria (Wu et al., 2012; Chung et al., 2013), as well as to image cells or even tissues (Antoku et al., 2010;. Byers & Hitchman, 2011; Shiang et al., 2012).

DNA strand of Ag_n-DNA can also be designed or modified for various applications through hybridization. In this case, DNA is divided to two sections, a nucleation sequence and a hybridization sequence (Yeh et al., 2010; Petty et al., 2013). The nucleation sequence is used to interact with Ag atoms to form AgNC while the hybridization sequence can hybridize with other DNA strand via Watson–Crick base pairing, as illustrated in Figure 4. The photo-physical properties change a lot after hybridization, which can be used to detect certain DNA sequences. Since the hybridization sequence can be freely designed and changed, this type of Ag_n-DNA has bright application potential. However, the application upon hybridization has not been well developed so far.

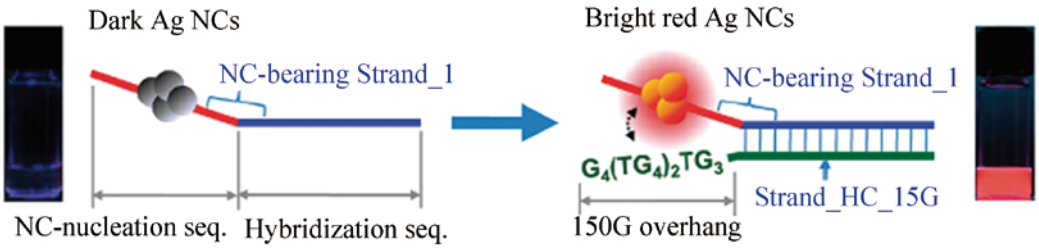

Figure 4
Schematic of the Composition of DNA Bases and Their the Red Fluorescence Enhancement of Ag_n-DNA Caused by Hybridization. Photographs of the Resulting Emission Under UV (366 nm) Irradiation Are Also Shown
Note. Reproduced from Yeh et al., 2010.

CONCLUSION AND PERSPECTIVE

Mainly four types of functional groups interact with Ag atoms to stabilizer AgNC, with the affinity rank of: -SH >> -COOH > -OH > $-NH_2$/=NH groups. The specific number of Ag atoms can be controlled by changing the stabilizer to Ag ratio when small molecules are used as stabilizers. Increase of stabilizer to Ag ratio will lead to the decrease of the size of AgNC and shift the mass distribution toward smaller cluster sizes.

In polymer-templated AgNC synthesis, polymer to Ag ratio plays a less important role because of the contribution of steric limitations caused by secondary structures of polymer chain. Polymer prefers to form AgNC containing several Ag atoms while AgNC may undergo a wide distribution of the number of Ag atoms. As an exclusive example, DNA strands containing several to dozens of bases are ideal templates to prepare few atoms (less than 15) AgNC. DNA sequence has no absolute effect on the size of formed AgNC. However, each DNA strand has a unique fluorescence spectrum, which is determined by DNA sequence along with environmental factors. The role of DNA sequence is achieved through composition, length and shape of DNA strands.

Four types of DNA bases have distinct effect on AgNC formation. T-rich Ag_n-DNA normally has only blue/green-emission while C-rich Ag_n-DNA has both red- and blue/green-emitting AgNC. G-rich DNA sequences enhance the red fluorescence of AgNC while they also tend to employ "self-association" to form the G-quadruplex. These principles should provide significant guidance for the preparation of AgNC whereas the underlying mechanism still remains unclear. DNA templated AgNC has been investigated widely and gradually become promising dector/sensor for many compounds, as well as being used in bioimaging. Further application still needs to be explored and will be hot topic of future research.

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