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Enhanced fluorescence of silver nanoclusters stabilized with branched oligonucleotides†

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DNA stabilized silver nanoclusters (AgNCs) are promising optical materials, whose fluorescence properties can be tuned by the selection of the DNA sequence employed. In this work we have used modified oligonucleotides in the preparation of AgNCs. The fluorescent intensity obtained was 60 times higher than that achieved with standard oligonucleotides.

The use of fluorescent materials in biology has allowed the discovery of many biological processes in living cells. Structures commonly employed in cells and animals are fluorescent proteins,[1] organic dyes[2] and quantum dots.[2a,3] Fluorescent proteins have been successfully used in many applications such as protein expression or protein–protein interaction studies.[4] However the size of these structures may interfere with some internal cell processes, and aggregation events cause cellular toxicity. The use of organic dyes is limited by their poor photostability and toxicity.[5] In the case of quantum dots, their size and toxicity are important drawbacks that prevent their use in live cells and animals.[3] In this context, DNA-stabilized silver nanoclusters (DNA-AgNCs) are being intensively studied as alternative fluorescent probes.[6] AgNCs have shown good quantum yields, and possess high photostability and small size[7] (<2 nm). The use of DNA as a template enhances the biocompatibility and water solubility of silver clusters. What is more, the specific binding properties of aptamers make these materials promising candidates for specific biolabeling.[8]

The fluorescence properties of AgNCs depend on their structural features and other parameters such as buffer, pH, concentration of reagents, size and oxidation state of the silver clusters.[9] Moreover, the emission of DNA-AgNCs depends strongly on the DNA sequence,[10] allowing the preparation of a wide palette of colours by changing the nucleotide sequence. Interestingly, some of these fluorescent materials have shown good pH, temperature and oxidation resistance, preserving 31% of the initial emission intensity after 10 months.[11]

The environment of AgNCs can also affect their fluorescent properties. In this sense, the structural changes promoted by the interaction with a given analyte have been exploited to develop sensors for different entities such as ions,[12] small molecules,[13] DNA,[14] microRNAs,[15] proteins[16] or tumour cells.[17]

On the other hand, it is well known that DNA is affected in hybrid materials modified with multiples strands, due to an interaction between the neighbouring DNA ion clouds. This behaviour is typically observed in hybrids such as nanoparticles[18] or polymers[19] with a great number of strands, but it is also possible in hybrid materials with only three[20] or two[21] strands around a rigid and small molecule core. However, the effect of neighbouring strands on the fluorescent properties of DNA stabilized silver nanoclusters in this kind of hybrids is still unexplored.

In this work, we report the synthesis of a family of branched DNA structures that can be used in the preparation of DNA-AgNCs. The fluorescent intensities obtained with these derivatives are higher compared with those obtained in the case of AgNCs stabilized with a single stranded DNA. To the best of our knowledge this is the first report of AgNCs prepared with modified oligonucleotides. The derivatives reported herein are composed of two or three ssDNA attached to a small hydrophobic benzene core at different positions. The dimers (**1** and **2**) contain DNA strands at *ortho* and *meta* positions (Fig. 1). On the other hand, the trimers (**3** and **4**) have the strands at alternating positions.

Particularly, the hybrid structure **3** contains a benzene ring where the DNA strands are placed at positions 1, 3 and 5, which should give rise to a planar DNA trimer structure. In the case of hybrid **4**, the benzene ring is further substituted with three ethyl groups at positions 2, 4 and 6. The presence of the ethyl groups in **4** should yield a more rigid structure and probably with a different orientation of the DNA strands (Fig. 1). The benzene scaffold with three ethyl groups has been previously used to force the groups

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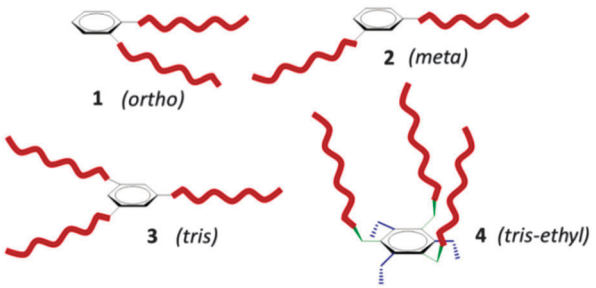

Fig. 1 Schematic representation of the derivatives prepared in this work. The spatial arrangement presented is arbitrary.

placed at alternating positions to be oriented at different sides of the benzene ring.[22] In our case the arrangement of the groups is difficult to predict, but taking into account the sensitivity of DNA-AgNCs to the changes in the surrounding media, we expect variations in the fluorescence properties of trimers **3** and **4** as well in the dimers **1** and **2**.

For our studies we selected a DNA strand composed of twelve cytosines (C12) because it is known that cytosines stabilize AgNCs better than guanines and much better than adenines and thymines. In addition, this sequence has been previously employed in the preparation of AgNCs, affording fluorescent AgNCs with a maximum absorption at 560 nm, and its corresponding maximum emission at 630 nm.[14b]

The derivatives **1–4** were synthetized using a "click" reaction[23] between a C12 oligonucleotide modified with an alkyne moiety at the 3′ end and the corresponding azide derivative.[24] The required DNA strand (C12 sequence) was synthetized using a MerMade4 DNA synthesizer, a modified CPG prepared in our laboratory, and commercial cytosine phosphoramidites (ESI†).

The Cu-catalyzed Huisgen reaction afforded the corresponding branched derivatives without significant DNA degradation (ESI†).

Then, the compounds **1–4** were employed in the preparation of silver nanoclusters. These derivatives were incubated with silver nitrate (6 equiv. per oligonucleotide strand) for 10 min at room temperature and then reduced with a fresh solution of sodium borohydride.

The fluorescence of the silver nanoclusters generated using compounds **1–4** was measured after 4 hours. The fluorescence obtained with the branched derivatives was higher than the fluorescence obtained with a single strand of DNA (PolyC12) (Fig. 2). Since the effect of the benzene ring on the fluorescence intensity is not relevant (ESI,† Fig. S2) the enhanced fluorescence should be due to the disposition of the strands. Particularly, in the case of the dimers, the *ortho* derivative (**1**) showed higher fluorescence than the *meta* derivative (**2**). This result suggests that the close proximity between strands better promotes the formation of fluorescent AgNCs.

The most striking results were obtained with the trimers where the fluorescence obtained was around 60 times higher than that obtained with the PolyC12 (Fig. 2). Among the trimers, **4** showed the highest fluorescence values compared with all the samples tested, including single strands of DNA with 24 and 36 cytosines (ESI,† Fig. S2). Interestingly, its fluorescence did not decay significantly during the first 24 h as in the single stranded samples (ESI,† Fig. S3). What is more, the fluorescence of the trimers was maintained after 20 days in the dark (ESI,† Fig. S4).

Due to the good results obtained using the trimers we decided to further study these materials. First, a UV analysis of the samples revealed a peak at 440 nm, which is characteristic of silver nanoparticles, and a peak at 560 nm, indicating the presence of AgNCs. In the case of the single stranded PolyC12 the signals were significantly less intense than those of the trimers (Fig. 3). AFM analysis of the samples revealed small particles (*ca.* 2 nm height) in the case of samples **3** and **4**, which could be the AgNCs responsible for the absorbance band at 560 and the intense fluorescence (ESI,† Fig. S13 and S14). In the case of PolyC12 larger structures of 12 nm height were observed, which could be due to the formation of silver nanoparticles during the reduction step (ESI,† Fig. S12).

In addition, we monitored the fluorescence and absorbance during the first few hours to better understand the kinetics of AgNCs formation (ESI,† Fig. S5–S8). We observed that the highest fluorescence is achieved after 10 hours, and then it decreases slowly. In the UV spectrum, the band at 440 nm, that could be related to Ag nanoparticles, decreases overtime, meanwhile the band associated to AgNCs (560 nm) increases.

We believe that the high fluorescence as well as the stability observed in the trimers is due to the spatial arrangement of the AgNCs and the DNA strands. In these cases the AgNCs could be better protected from quenching and also the close proximity of the strands could result in a cooperative effect, leading to the

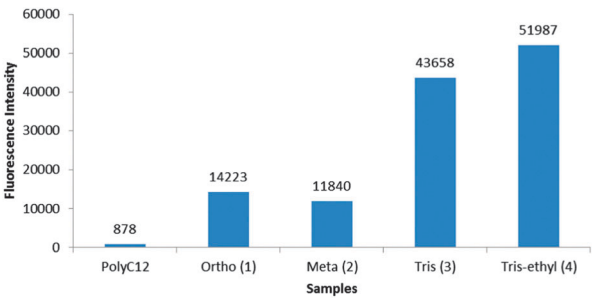

Fig. 2 Fluorescence intensity (ex. 560 nm, em. 630 nm) of AgNCs prepared with PolyC12, *ortho* (**1**), *meta* (**2**) tris (**3**) and tris-ethyl (**4**) measured after 4 h.

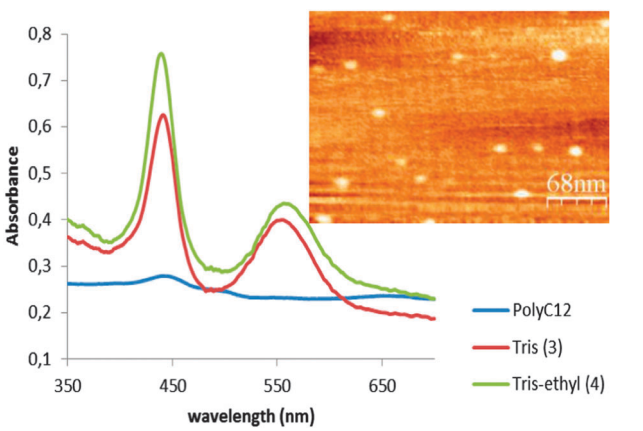

Fig. 3 UV spectra of the three samples: PolyC12, tris (**3**) and tris-ethyl (**4**). The peak at 440 nm indicates the presence of silver nanoparticles, meanwhile the peak at 560 nm arises due to AgNCs. The inset is a zoomed area of an AFM topographic image showing small particles of 2 nm height.

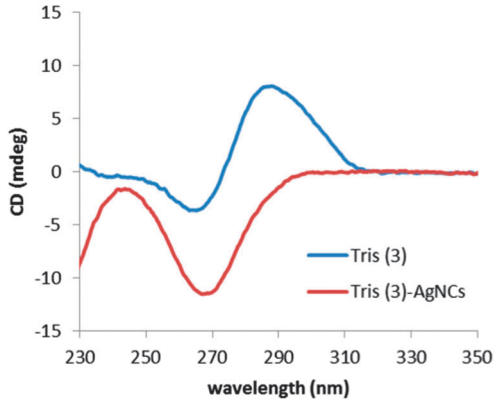

Fig. 4 CD spectra of tris (**3**) before and after AgNC formation. There is a significant change in the structure after the generation of AgNCs.

high fluorescence. In this sense, minimization of the structures using molecular mechanics suggests a globular structure for the trimers. This structure could better protect the AgNCs from quenching, compared with the standard oligonucleotides, which have linear structures (ESI,† Fig. S15).

We also monitored the structural changes in the oligonucleotides promoted by AgNCs formation using circular dichroism (CD). Interestingly, a significant change was observed before and after AgNCs formation where the positive band at 280 nm is completely inverted after AgNCs formation (Fig. 4 and ESI† Fig. S9–S11). What is more, this change in the structure is related to the fluorescence intensity since when the fluorescence of AgNCs decreases the positive band at 280 nm is recovered, whereas the negative band at 270 nm decreases. These data suggest that the structure of the oligonucleotides stabilizing fluorescent AgNCs is different from the structure of the oligonucleotides alone and also that the original DNA structures can be recovered over time leading to an efficient quenching of the fluorescence of AgNCs.

We have prepared fluorescent silver nanoclusters using novel trimers of oligonucleotides connected to a benzene molecule. The fluorescence exhibited by these derivatives is 60 times higher than that obtained with a single strand. We believe that the high fluorescence obtained using the trimers is due to a cooperative effect between vicinal strands, which could better stabilize the AgNCs and prevent their quenching. This work illustrates the use of modified oligonucleotides in the preparation of AgNCs with improved fluorescence properties.

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