Fluorescent Silver Nanoclusters Self-Assembled on DNA

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Introduction:

Fluorescent chemical groups, known as fluorophores, are essential to fields such as biology and medical imaging. Our group has been investigating a completely new class of fluorophores: silver nanoclusters self-assembled on deoxyribonucleic acid (DNA). These fluorophores are especially interesting because simple changes to their DNA sequence or structure can effectively tune their spectral properties. We are trying to resolve just how sequence and structure influence spectra by investigating and characterizing these fluorescent nanoclusters.

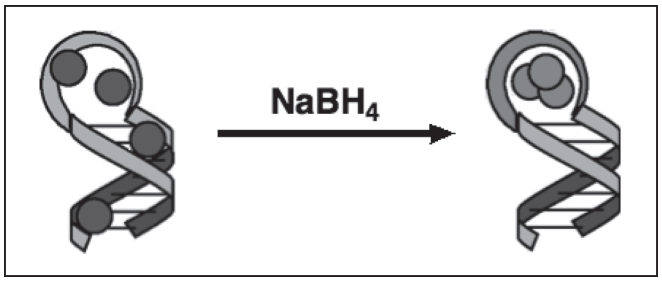

Figure 1: Synthesis of Ag nanocluster.

Synthesis:

Fluorescent silver nanoclusters are hosted in loops comprised of a single strand of DNA bases, also known as hairpins. To synthesize the nanoclusters, we prepare 25 μM of the DNA strand in a solution of 50-500 μM free Ag ions. Adding 25-325 μM $NaBH_4$, a reducing agent, enables the formation of Ag clusters in the hairpins of the DNA (Figure 1).

The synthesis typically produces two or three species of fluorescent nanoclusters, differentiated by their excitation and emission wavelengths. The DNA strand determines which species of fluorescent nanoclusters are produced. Ag and $NaBH_4$ concentrations, as well as the temperature and pH of the solution, determine the proportions of the different nanoclusters.

Experimental Design:

We used fluorescence spectroscopy to characterize the fluorescence of the nanoclusters. Using a fluorimeter, we performed emission scans at incremented excitation wavelengths. From these emission scans we can identify the peak excitation and emission wavelengths of the fluorescent nanoclusters.

We also used mass spectrometry to determine the distribution of cluster sizes in the hairpins. In order to distinguish fluorescent clusters from non-fluorescent clusters at least two sets of data are needed. We identify the fluorescent clusters by correlating a shift in the relative intensity of emitters with a shift in the cluster distribution. To determine the size of the fluorescent clusters we vary the synthesis conditions, often the Ag concentration.

Hairpin loops composed of strictly cytosine bases are the most effective at generating fluorescent nanoclusters. Previous work in this group investigated the 9-cytosine hairpin (9C hairpin). The 9C hairpin hosts a green emitter and a red emitter with emission wavelengths 530 nm and 650 nm, respectively. Fluorescence spectroscopy and mass spectrometry identified the Ag_{11} cluster as the green emitter and the $Ag_{13\text{-}15}$ clusters as red emitters.

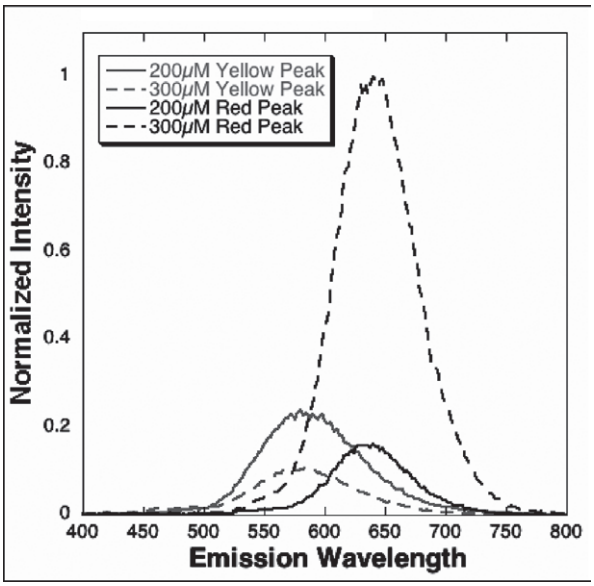

Figure 2: Emission scans of 12C hairpin.

Our research investigated nanoclusters hosted in 12C hairpins. The 12C hairpin hosts a yellow emitter with emission wavelength 580 nm and a red emitter with emission wavelength 650 nm. The red emitters of the 9C and 12C hairpins have the same excitation and emission wavelengths. Our goal was to determine whether the size of the 12C's red emitter was the same size as that of the 9C hairpin. Confirmation would support the hypothesis that the number of Ag atoms comprising the nanocluster is a major determinant of its spectral characteristics.

Results and Conclusions:

With the 12C hairpin, we found that lower concentrations of Ag during synthesis favored the yellow emitter and that higher concentrations of Ag favored the red emitter. Figure 2 depicts normalized emission scans of the red and yellow fluorescence for Ag concentrations of 200 μM and 300 μM. Given the low concentration of emitters in these solutions, we expect a linear relationship between the fluorescence and the number of emitters. Increasing the Ag concentration from 200 μM to 300 μM decreases the number of yellow emitters by a factor of two and increases the number of red emitters by a factor of five.

The cluster size distribution in Figure 3 displays a broad shift in cluster sizes between samples. By contrast with previous work on the 9C hairpin, where specific clusters were identified as emitters, we can only establish ranges of 3-7 atom clusters for the yellow emitter and clusters of 13 atoms or more for the red emitter. We attempted to identify the emitter sizes using selective photobleaching, that is, exposing the sample to intense light that had been filtered to contain only the excitation light of one of the emitters. Due to the limited photostability of these fluorophores, we expected the excited emitter to stop fluorescing and the non-excited emitter to retain its fluorescence. We exposed the nanoclusters for 5 minutes to a mercury lamp filtered to excite only the red emitter. The fluorescence of the yellow emitter was preserved while the fluorescence of the red emitter was diminished by a factor of three. However, the mass spec spectra of two samples showed no difference in distribution of cluster sizes, prohibiting the identification of fluorescent emitters and indicating that the cluster fluorescence is more strongly determined by factors other than cluster size.

Focusing on the yellow emitter of the 12C hairpin, we analyzed a different strand that hosted the same yellow emitter, the 6C dumbbell. The 6C dumbbell consists of a duplex with a 6C hairpin on both ends. Fluorescence of the 6C dumbbell has a temperature sensitivity that we were able to exploit. When a sample, originally synthesized in refrigerated conditions, was incubated for 15 hours at 35°C, the number of yellow emitters decreased by a factor of 1.5 and the number of red emitters increased by a factor of 5. Surprisingly though, the mass spec, shown in Figure 4, again indicates no change in the cluster distribution between samples.

The ranges of cluster sizes established for the emitters are consistent with the hypothesis that the number of Ag atoms determines nanocluster fluorescence. However, our photobleaching and incubation experiments suggest that cluster fluorescence is determined not only by the number of Ag atoms, but also by features such as cluster geometry, cluster charge, or specific Ag-DNA bonds as well. Our work has been instrumental in helping this group determine to redirect further research on these fluorophores towards such features.

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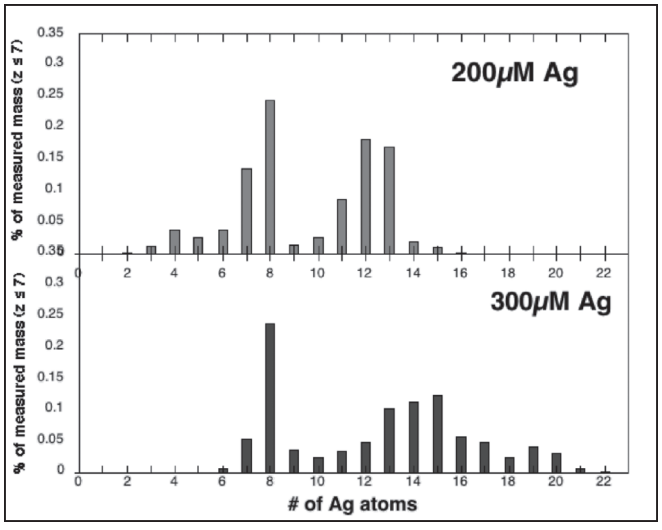

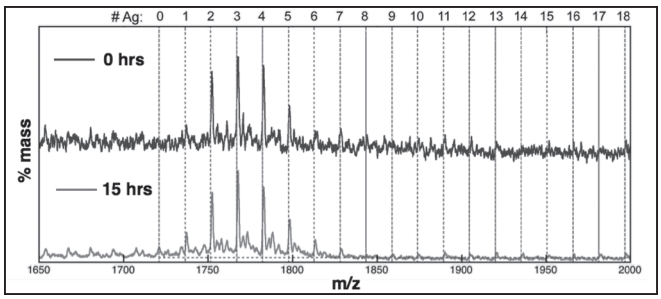

Figure 3, left: Cluster distribution of 12C hairpin.

Figure 4, above: Mass spec of 6C dumbbell.