Functional Fluorescent Silver Nanoclusters on Single Stranded DNA Scaffolds

Ravyn Solís, Angel Torres, Brock Gascon and Bidisha Sengupta

Department of Chemistry & Biochemistry, Stephen F. Austin State University, P.O. Box 13006, SFA Station, Nacogdoches, TX 75962-3006

Background

The aim of the present study is to determine the effectiveness of silver nanocluster (Ag-NC) against biofilm of *Bacillus Thuringiensis* (BT) and *Pseudomonas Aeruginosa* (PA). A 60 base long DNA aptamer specific for BT tied to the 5' end of a 16 base cytosine rich DNA oligonucleotide is chosen as the template for growing the Ag-NC. Two DNA aptamers specific for PA and part of their sequences were chosen as templates for growing the Ag-NC. Circular dichroism (CD) studies determined the presence of secondary structures. In addition, UV/Vis absorption and fluorescence spectroscopic studies confirmed the formation of the fluorescent Ag-NC on the DNA templates.

Materials and Methods

The objective of this research is to gather data on the oligonucleotide template that produces a larger homogenous population of silver nanoclusters. And to observe the effects that a base substitution can have on the formation of Silver nanocluster populations.

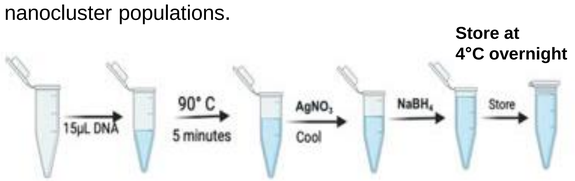

Figure 1 15 µM of single stranded oligonucleotides were heated above 90˚C and then treated with $AgNO_3$ and $NaBH_4$ at room temperature at the ratio (1:6:6), vortexed and incubated at 4˚C for overnight. The solution was made in 10mM pH 6 Citric Buffer Solution.

Conclusion

We have proved that DNA aptamers can be fruitfully designed and exploited to create small silver nanoclusters which can penetrate the exopolysaccharide matrix of biofilm and prevent its formation. The proof of this principle was demonstrated by the decrease in the degree of mass density fluctuation (Ld). The importance of the secondary structures of the aptamer templates in delivering the Ag-NCs to the target cells is also noteworthy.

References

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3.Sengupta, Bidisha, Christa Corley, Keith Cobb, Anthony Saracino, and Steffen Jockusch. 2016. "DNA Scaffolded Silver Clusters: A Critical Study" *Molecules* 21, no. 2: 216. https://doi.org/10.3390/molecules21020216

Acknowledgements

SFASU Department of Chemistry & Biochemistry, Robert Welch Foundation, Grant # AN-0008Justin Lovett

Analyzing the Structure

Single stranded DNA oligonucleotide sequences

5'- CCC ACC CAC CCA CCC A - '3

4GNC 5'- CCC ACC CAC CCA CCC G - '3

i-motif DNA structure

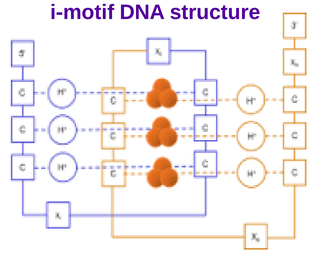
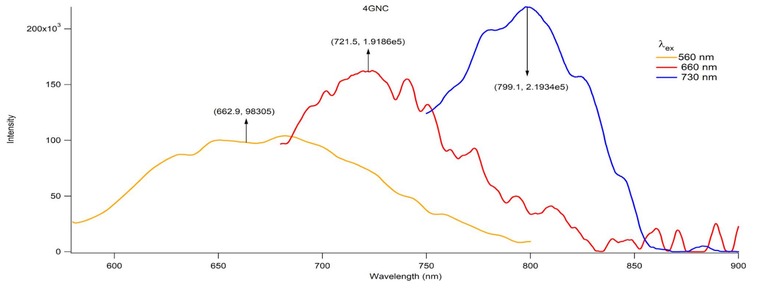


Figure 2 Illustrates the i-motif DNA template that was crucial to the growth of the NIR emitting Ag-NC. The literature data suggests that 9-10 Ag atoms are responsible for the NIR emitting Ag/Oligonucleotide clusters with Guanine being particularly valuable.



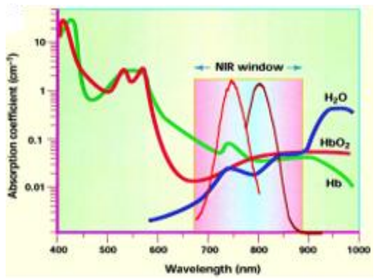


Figure 3 Illustrates the emission spectra of 4GNC at 3 different excitation wavelengths. The 4G template provided a better scaffold for the growth of the NIR species which absorbs at ~730 nm and emits at ~798 nm.

Figure 4 Illustrates the NIR window present in biological conditions. This window is notable because water, oxygenated hemoglobin, and deoxygenated hemoglobin do not have high fluorescence peaks in the present wavelength range.

Bacillus Thuringiensis

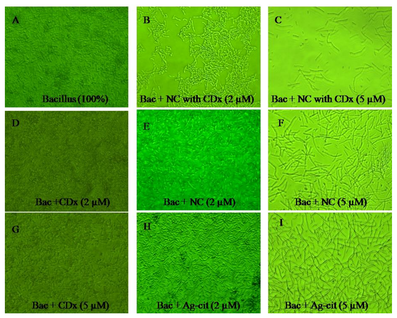


Figure 5 Phase contrast microscopic images of the *Bacillus Thuringiensis* treated with various agents at 30˚C for 30 hours. (A): 100 % bacteria; (B) and (C): Bacteria treated with 2 and 5 M of aptamer-3T-templated silver nanoclusters with methyl-beta-cyclodextrin; (E) and (F): Bacteria treated with 2 and 5 M of aptamer-3T-enclosed silver nanoclusters; (H) and (I): Bacteria treated with 2 and 5 M of Ag+ ions; (D) and (G): Control bacterial samples treated with the same volumes of methyl-beta-cyclodextrin solution in citrate buffer as used for samples in (B) and (C)

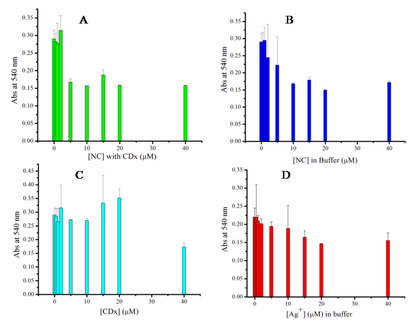


Figure 6 The anti-biofilm activities of (A) aptamer-3T templated silver nanoclusters with methyl-beta-cyclodextrin (NC with CDx), (B) aptamer-3T templated silver nanoclusters (NC in buffer), (C) methyl-beta-cyclodextrin (CDx), and (D) Ag+ ion, were assessed using a crystal violet staining procedure (see text for details). The results are expressed as the mean ± SD of two replicates in three different experiments.

Control of Biofilm

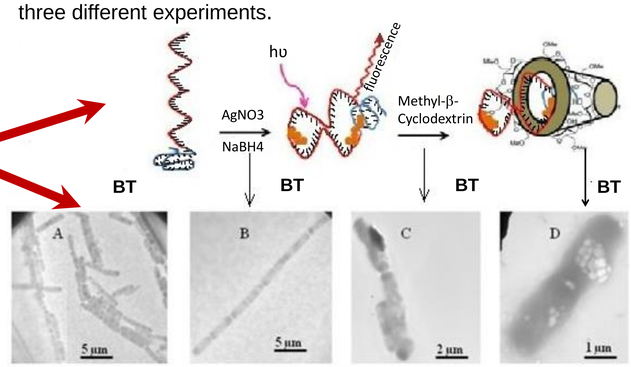


Figure 7 Transmission electron micrographs of *Bacillus Thuringiensis* treated with various agents. (A): 100 % bacteria, (B): Bacteria treated with 5 µM of Ag+ ions, (C): Bacteria treated with 5 µM of aptamer-3T-templated silver nanoclusters, (D): Bacteria treated with 5 µM of aptamer-3T-enclosed silver nanoclusters bound with methyl-beta-cyclodextrin (arrow).

Pseudomonas Aeruginosa

DNA Name	Sequence	Absorption Wavelength (nm) with Absorbance (OD) of the Ag-NC
NC1	5'-TAC TTC CGC ACC CTC CTA CA-3'	442 (0.18), 550 (sh)
NC2	5'-CCC CCG TTG CTT TCG CTT TTC CTT TCG CTT TTG TTC GTT TCG TCC CTG CTT CCT TTC TTG-3'	430 (0.15), 512 (sh)
NC3	5'-CCC TTT CCC TTT CCC ATT CCC GTT CCC TTT CCC TTT CCC ATT CCC GTT A-3'	431 (0.18), 511 (sh)
NC5	5'-ATGAGAGCGTCGGTGTGGTA - CCC TTT CCC TTT CCC ATT CCC GTT CCC TTT CCC TTT CCC ATT CCC GTT A - TACTTCCGCACCCTCCTACA-3'	427 (0.16), 511 (sh)
NC6	5'-ATG AGA GCG TCG GTG TGG TA-3'	454 (0.14), 560 (0.017)

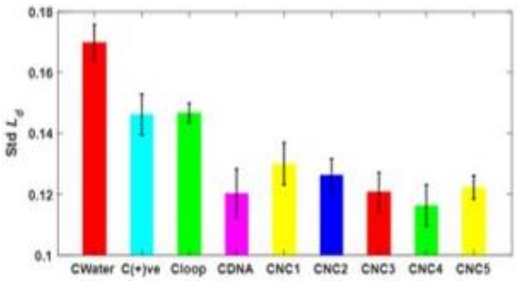


Figure 8 Preliminary result of spectroscopic study of Pseudomonas Aeruginosa bacteria in the presence of Ag-NC. The result shows that the std of the degree of disorder strength (Ld) of *Pseudomonas Aeruginosa* decreases by 13% when treated with DNA and nanocluster (NC 1,5). ($P < 0.001$).

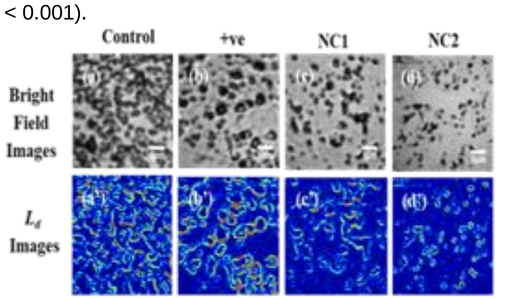


Figure 9 (a)–(g) are the representative bright field images of control, positive control (with $AgNO_3$ + $NaBH_4$) and different (NC1, NC2, NC3, NC5, NC6) aptamer enclosed nanocluster treated samples of *Pseudomonas Aeruginosa* while (a')–(g') are their respective Ld images. In the Ld images, red spots represent a higher degree of refractive index or mass density fluctuations which are easily distinguishable. Long connecting bacterial structures are the sign of biofilm formation.