SERS Detection of Viral DNA using Morpholino Oligos Tethered to Colloidal Gold Nanoparticles

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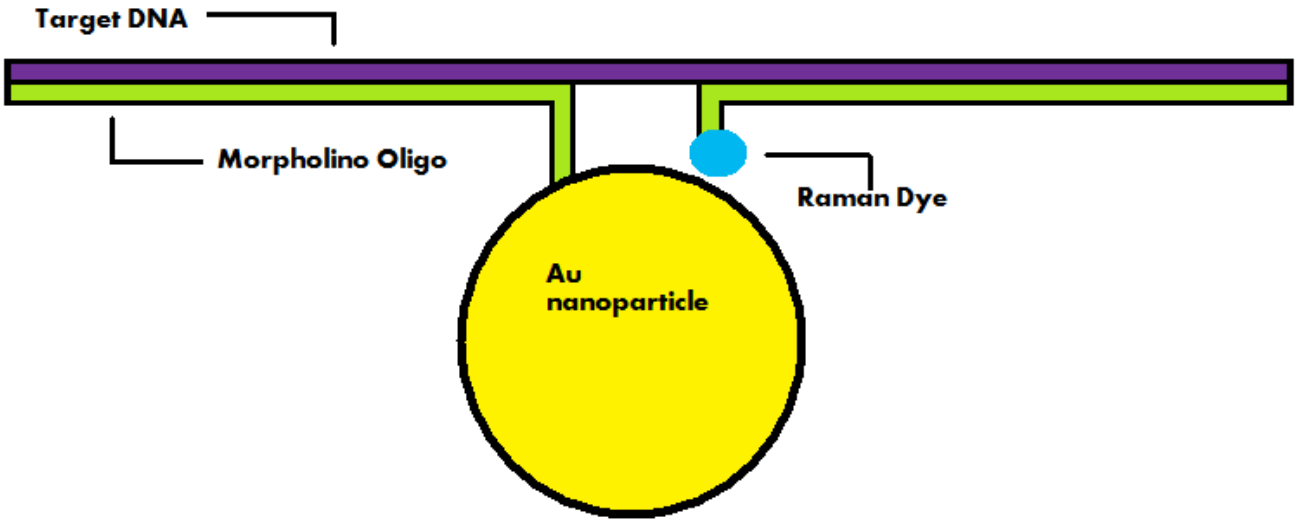
Abstract

In recent years there has been significant interest in DNA sensing technology. Advancements in Surface Enhanced Raman Spectroscopy (SERS) have made devices capable of sensing a particular sequence of DNA in minutes. Applications of these devices include rapid identification of disease, possibly before symptoms arise, and the detection of biological agents[1]. If practical use of such sensors is executed many false diagnoses can be avoided given the device is used for medical purposes and can ensure an efficient response to a biological attack. The variety of sensor I am interested in utilizes a morpholino probe immobilized to a Raman active metal (i.e. gold) and a morpholino-Raman dye hybrid. Each morpholino oligo would be complementary to a distinct sequence on a single strand of DNA from the West Nile Virus. Morpholinos, polymers which are analogous to DNA, have several properties advantageous in DNA sensing. For instance, morpholino-DNA bonds are significantly stronger than DNA-DNA bonds. Additionally, the nucleotide bonding specificity of a morpholino oligo is higher than the analogous strand of DNA[2]. Given the above qualities, I hypothesize that the use of antisense morpholino oligos in DNA biosensors will improve the precision and sensitivity of DNA biosensors.

Description of Proposed Research

Lately there has been interest in the DNA sensing capability of Surface Enhanced Raman Spectroscopy. Enhancements due to plasmon resonance usually range from 10^5 to 10^6 but have been reported to be as high as 10^{14} to 10^{15}.[3] Current methods of DNA detection, such as RT-PCR and gel electrophoresis, are time consuming and burdensome as a result of the many steps involved. DNA detection utilizing SERS exhibits accurate readings, high detection sensitivity and can be performed within a matter of minutes. Applications of SERS based DNA sensing include rapid identification of disease, possibly before symptoms arise, and the detection of biological agents.[1] If practical use of such sensors is executed many false diagnoses can be avoided, given the device is used for medical purposes, and can ensure an efficient response to a biological attack. SERS based detection has been shown to exhibit spectral band gaps significantly more narrow than fluorescence spectroscopy.[1] Recent biosensing studies utilizing SERS can be split into two groups: investigation of an analyte adsorbed to a Raman active surface or in a metal colloid. Surfaces may be roughened[4] or engineered to have a repeating geometric structure.[5] Colloids can be composed of nanoparticles of a single metal or multi-layered structures of various topologies fabricated from several metals.[5] In either of the previous circumstances the colloid may be analyzed in the liquid state or dried on inert glass, the former being the more rapid option. In many instances, a Raman dye is appended to the target DNA sequence as a result of the insufficient specificity and sensitivity of DNA alone for Raman detection.[3] Current research being done on DNA sensing involves using thiol-fuctionalized DNA oligos tethered to colloidal Au nanoparticles coupled with methylene blue hybridized DNA oligos and has been shown to be a valid method of viral DNA detection.[3]



It has been of interest to utilize morpholinos in DNA sensing applications considering the attractive properties possessed by morpholino oligos. Bond strengths of morpholino-DNA configurations are significantly higher than their DNA-DNA counterparts. The nucleotide specificity of morpholino oligos is higher than the corresponding strand of DNA.[2] Additionally,

morpholinos may not need a buffer solution due to its neutral backbone. This is appreciable taking into account that the buffer contributes to “noise” present in SERS spectra. Given the mentioned characteristics, morpholino oligos should enhance the effects of SERS based DNA detection.

The intended experiment will attempt to sense West Nile Virus (WNV) single stranded DNA (ssDNA) with the following mechanisms: antisense morpholino oligos are immobilized onto colloidal Au nanoparticles. The 5' end of target DNA binds to immobilized morpholino strands. A second Morpholino oligo hybridized with a Raman dye binds to the 3' end of the WNV DNA. A signal will only be observed if the Raman dye is sufficiently close to the Au nanoparticle surface. Consequently, only DNA captured by the morpholino probe will emit a Raman signal. As a control, the previous procedure will occur in the presence of Blue Tongue Virus DNA. Quartz crystal microbalance with dissipation (QCM-D) will be utilized to confirm the tertiary structure of the DNA-morpholino hybrid.

Role and Responsibilities

The role and responsibility of the student regarding the proposed research will be to perform experiments in relation to the conjugation of Au nanoparticles to the morpholino capture probe, SERS spectrometry of hybridized WNV ssDNA, evaluation resultant SERS spectra of the specified analyte, QCM-D experimentation and analysis and determination of the procedure’s adequacy based results obtained.

Timeline

The research project will begin in the summer of 2009 and continue into the fall semester of 2009 and spring semester of 2010. During the summer of 2009, Morpholino-DNA hybridizations will be performed along with gel electrophoresis of the hybridized complex to insure that the proper ternary structure is achieved. SERS spectroscopy of the morpholino hybrid and analysis of the resultant spectra will be performed in the fall of 2009. QCM-D experimentation and analysis will be conducted in the spring of 2010.

References

(1) Audrey Sassolas, Béatrice D. Leca-Bouvier, and Loïc J. Blum. “DNA Biosensors and Microassays.” Chemical Review 108 (2008): 109-139.

(2) James E. Summerton. “Morpholino, siRNA, and S-DNA Compared: Impact of Structure and Mechanism of Action on Off-Target Effects and Sequence Specificity.” Current Topics in Medicinal Chemistry 7 ((2007) 651-660.

(3) Mark Harpster, Hao Zhang, Ajaya K. Sankara-Warrier, Bryan H. Ray, Timothy R. Ward, J. Pablo Kollmar, Keith T. Carron, James O. Mecham, Robert C. Corcoran, William C. Wilson, Patrick A. Johnson. “SERS detection of indirect viral DNA capture using colloidal gold and methylene blue as a Raman label.” (unpublished, submitted).

(4) Duncan Graham and Royston Goodacre/ “Chemical and biomedical applications of surface enhanced Raman scattering spectroscopy.” Chemical Society Reviews 37 (2008): 883-884.

(5) Matthew J. Banholzer, Jill E. Millstone, Lidong Qin and Chad A. Mirkin. “Rationally designed nanostructures for surface-enhanced Raman spectroscopy.” Chemical Society Reviews 37 (2008): 885-897.

Budget Summary

Student Stipend:	$5000
Labor by other personnel	$1200
Supplies	$2000
Travel	$0
Total Cost	$8200
Non-Federal cost Sharing	$3200
Amount Requested	$5000

Budget Narrative

The budget begins in the summer of 2009 and continues until the end of the 2010 spring semester. The student stipend should include approximately $3500 for research conducted during the summer and $750 for research conducted during the fall and spring semesters. Labor by other personnel applies to the time invested by the faculty advisor. There will be weekly meetings lasting from to two hours that Dr. Johnson will schedule in order to review the progression of the research and make suggestions regarding the project.

Supplies included in the budget are morpholino oligos with end modifications which will cost $530 per 300nmol. QCM-D crystals, WNV and BTV DNA sequences, gold colloid and other miscellaneous supplies will also need to be obtained for experiments included in the research. The above items will be purchased using funds from Dr. Johnson’s start-up funds account, so are characterized as non-federal cost share.

The entire amount requested will be used for the student stipend for the forthcoming summer and the following fall and spring semesters. The absolute amount requested comes out to be $5000. This is an approximate budget; therefore, it is subject to change throughout the span of the project.