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Single DNA molecule detection in an optical trap using surface-enhanced Raman scattering

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Raman spectra from single DNA molecules in their natural aqueous environment are presented. A DNA molecule that is anchored between two optically trapped dielectric beads is suspended in a solution with nanosized silver colloid particles. The nonspecific binding of the metal to the DNA enhances the Raman scattering that is excited by a near-infrared beam. A Raman spectrum is first recorded followed by a force-extension curve that verifies the presence of a single DNA molecule. © *2010 American Institute of Physics*. [doi:10.1063/1.3431628]

Raman scattering is a powerful tool for gaining information on the chemical structure of a studied sample. Previously, Raman scattering has even been obtained from single molecules.[1–3] The key element of these measurements is the surface-enhanced Raman scattering (SERS) process where a considerable enhancement of the emitted signal occurs due to coupling of electromagnetic fields with optically excited local surface plasmons in nanosized metal structures.[4]

DNA molecules or their basic constituents in bulk solutions have already been the subject of SERS measurements.[5–10] These works have produced a library of peaks that lead to a well characterized Raman spectrum for DNA. Silver colloids have proven to be effective in detecting single DNA strands as well, however, with the DNA immobilized on the surface, which allows for a higher number of metal nanostructures to be in contact with the molecule and easier positioning of the excitation light field.[11,12]

Here, we believe we demonstrate the use of SERS in the spectroscopic detection of a single DNA molecule that is optically trapped in a natural aqueous environment. Two optically trapped dielectric beads are used as anchors while the DNA is suspended in a solution with nanosized silver colloid particles. The colloidal particles nonspecifically bind to the DNA and enhance the Raman scattering that is excited by a near-infrared beam. Raman spectra show the presence of known vibrational bands of DNA and a force-extension curve verifies the single DNA molecule.

The molecule used was double-stranded λ-DNA amplified at 12 kbp from *E. Coli* using standard polymerase chain reaction (PCR) techniques with sample concentrations of 40 ng/μL. The molecules were tagged with biotin and dioxygenin (DIG) at each end in order to attach to streptavidin and antidioxygenin (anti-DIG) coated polystyrene beads, respectively. DNA molecules were first incubated with the streptavidin coated beads (1.87 μm diameter) for 45 min in phosphate buffer solution (PBS) at pH 7.4. The sample was then washed before being injected along with the anti-DIG coated beads (3.15 μm diameter) into a home built fluid chamber with separate channels for the injection of the beads leading in to a center channel through which passed the optical beams. The final DNA-bead constructs were assembled *in situ*. Silver colloids were produced by the citrate reduction method[13] at a concentration of 5×10^{11} particles/μL. The diameter of the particles was 70 nm $\pm$ 20 nm with the average size and distribution determined from absorption spectroscopy (data not shown). A minute quantity of NaCl was also added which provides the salt-induced activation of the colloids and neutralization of the surface charge of the metal particles thus promoting nonspecific binding to the negatively charged DNA molecules. Silver colloids diluted in PBS were injected in to the chamber at a concentration that achieved an Ag particle:DNA molecule ratio of approximately 5:1. Measurements were conducted after a few minutes wait time in order to allow the diffusing and binding of the metal particles to the DNA.

The experimental setup is a combined optical trapping system with confocal Raman spectroscopy and has been described previously.[14] Briefly, the solution of DNA molecules and functionalized beads were placed inside a flow chamber. The chamber was placed on an inverse microscope equipped with a 100×1.3 numerical aperture oil immersion objective. A 785 nm beam was used for the Raman excitation with a power density of 1.5×10^6 W/cm^2 at the focus. The backscattered light was collected by the same microscope objective and passed through a holographic notch filter before entering the confocal system with a 100 μm pinhole. Raman spectra were recorded with a spectral resolution of 3 cm^{-1}.

1064 nm optical beams were used for the dual-spot trap with an average power of 10 mW at the each bead. For the measurement, we held the beads attached to the DNA and aligned it such that the Raman excitation beam passed between the beads (see Fig. 1). An additional 633 nm optical beam was coaxial to the propagation direction of one of the trapping beams. Its forward scattering intensity was characterized with a pinhole and a quadrant position detector in order to calibrate the optical traps using well established procedures[15] and measure the extension curve of the DNA molecule.

For the experiment, live Raman spectra were recorded successively at 1 s acquisition time in order to adjust the

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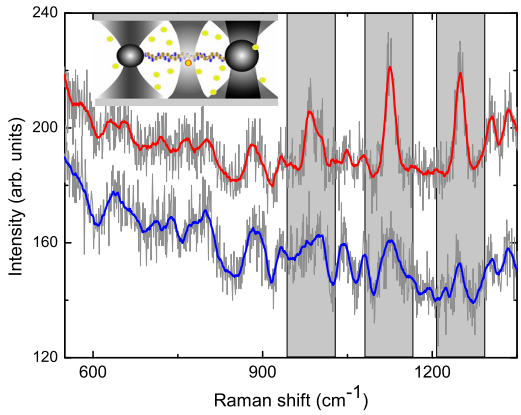

FIG. 1. (Color online) A spectrum (top plot) from a typical SERS measurement of a single DNA molecule plotted against a background spectrum (bottom plot). The raw data (gray line) are plotted with their smoothed counterparts (Savitzky–Galey, 30 points) for both the DNA (top plot) and background (bottom plot) spectra and are shifted for clarity. Inset: an illustration (not drawn to scale) of the DNA-bead construct in the optical trap.

position of the molecule based on the intensity of the Raman peaks. A 10 s acquisition was then performed, and once the DNA Raman peaks were observed, an extension curve was measured by stretching the DNA and recording the position of one of the trapped beads. Figure 1 presents a Raman spectrum that is typical of the successful measurements from the sample set. The spectrum shown in gray is plotted directly from the spectrometer, without any smoothing or background removal. A background curve was also measured, at the same conditions, with the DNA moved away from the trap until the signal disappeared from the short time live scans. The background is not quite as smooth as one would expect from water at near-infrared excitation. During a 10 s acquisition it is possible that loose DNA and other particles along with colloids could diffuse in and out of the excitation volume leading to the nosier background. However, distinct bands emerge when the trapped DNA is brought in to the confocal volume. We consistently observe three bands. The first, at 1265 cm^{-1}, is assigned to the C–C and C–N stretching vibrations (out of phase) of the base residues, in particular to the aromatic skeletal vibrations of cytosine. The peak at 1140 cm^{-1} is attributed to the deoxyribose-phosphate backbone and the C–O stretching in the deoxyribose gives the Raman peak at 1000 cm^{-1}.[16,17] These peaks have previously been observed to be strongly enhanced through interaction with Ag colloids.[12,18] We must point out the absence of the highly SERS active adenine modes which have been observed in single stranded DNA[10] and at single molecule concentrations in solution[9] with silver particles. Initially, one can consider that the double stranded DNA does not allow direct contact of the bases to the metal, however, a strong adenine SERS peak was also observed for double-stranded DNA adsorbed on to gold.[8] Further studies must be performed that consider the DNA-metal interaction in order to shed more light on the specific conformations of DNA structure at the metal surface. The nonspecific binding of the Ag particles means that the distribution of the particles on the DNA is random. This was most likely the major cause for the lack of Raman signal from some of the molecules: at times there were no metal particles in the confocal volume. The fluctuating DNA molecule in the optical trap moves the excited colloids. However, the micron cubed focal volume, which is

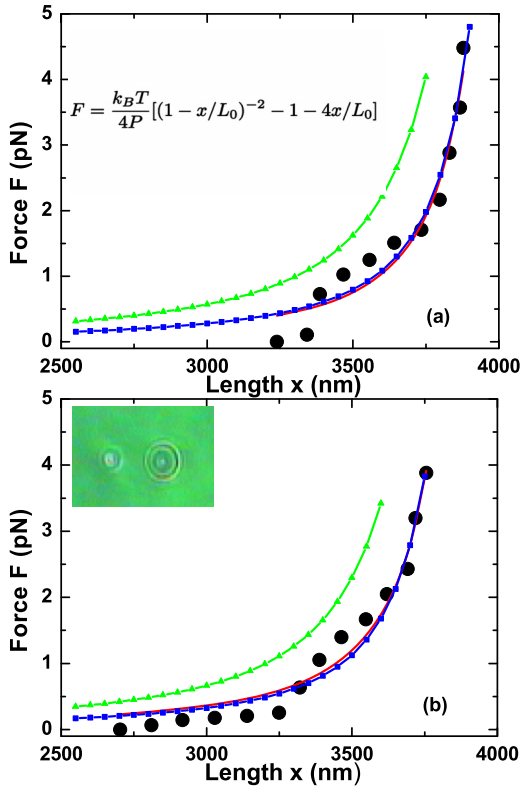

FIG. 2. (Color online) A sample of two extension curves from the data set of DNA molecules that gave a Raman signal at the conditions of Fig. 1. The curves are fit to the WLC model (solid line) [formula in (a) inset] and all curves give the expected contour length (L_0) of 4.1 μm and persistence lengths (P) that vary between 47–57 nm. In the two examples given here, persistence values of (a) 55 nm and (b) 48 nm were found. Error bars are calculated to be far less than the size of the plotting symbols and are, therefore, not shown. Theoretical force-extension curves are included using the expected contour length and persistence lengths of 53 nm (square line) and 26 nm (triangle line) in order to further demonstrate the presence of a single DNA molecule. A camera image of the suspended DNA-bead construct is included in (b) inset.

much bigger than the width of the DNA and metal particle, and our setting of the DNA at about 70% extension before measuring Raman signal combined to minimize this fluctuation, as could be seen in the live 1 s acquisition scans.

To verify that the Raman signals originate from a single DNA molecule, force-extension curves[19] were measured of the DNA molecules that produced a Raman signal. Fitting the curves in Fig. 2 to the well established wormlike chain (WLC) model[20] [formula given in Fig. 2(a) inset], which relates the force applied (F) to the DNA end-to-end length (x), allows two basic parameters to be extracted: the contour length (L_0) and persistence length (P). In all measurements, the measured contour length, at 4.1 μm, was consistent and verified the estimated length found from the electrophoresis gel of the PCR amplification product.

The final confirmation of a single DNA molecule between the beads should be the extension curve fitting to the DNA natural persistence length of 53 nm.[19,21] This value would change proportionally to the number of molecules being extended. In the sample set, the persistence length values ranged between 47–57 nm which is within reasonable error of the well-established value for a single DNA molecule. Most likely due to the metal particles, the fits are not as good as what is typically seen for these types of measurements. Thus, in order to ensure that the curves are beyond a reason-

able error range from a multimolecule ensemble, theoretical force-extensions curves for two persistence lengths, 53 and 26 nm, are included in Fig. 2 with the experimental data and original fit (where all parameters were allowed to vary). The lower persistence length estimates the effective rigidity of two DNA molecules which forces a large deviation of the WLC model from the experimental data. However, the 53 nm value for a single DNA molecule is extremely consistent, not even changing more than 5% at different pH conditions.[19] This leads to our conclusion that although our measured values indicate a single DNA molecule, the mechanics of the DNA are being affected by the attached metal particles.

The WLC fits are weakened by the higher force values in the region between 80% (3.3 μm) and 88% (3.6 μm) extension or 0.5–1.5 pN. The region is representative of the DNA transitioning from entropic dominated forces to acting like a structure with an intrinsic elasticity.[22] The attached metal particles do not seem to affect the entropic forces that are necessary to straighten the DNA nor the linear elastic region at higher forces. The average diameter of the silver particles is larger than the persistence length of DNA. Thus, if there are clusters of few Ag particles, the DNA could tend to coil around the metal rather than taking on a rodlike behavior with just one particle. The force needed to uncoil these segments would certainly occur at low values, because of the nonspecific binding but should be greater than what is needed to straighten the DNA to a rod at normal aqueous and thermal conditions. The absence of an effect at longer extension, or larger forces, is also due to the size of the metal particles but this time in an opposite way. The nanometer sized diameters of the metal particle or clusters are much less than the microns length scale of the DNA, leaving stretching forces in the length direction unaffected when the DNA is acting as a rod with a finite stretch modulus. This is also dependent on the low density of metal on the DNA molecule, which is confirmed from the concentrations used. We expect that ensuring the absence of silver particle aggregates would remove most of the irregular mechanical effects observed here. This could be achieved, for example, by specifically binding the silver to the DNA through refined chemistry, which is a topic of our future work. Nevertheless, in the current form, the DNA–Ag particle constructs would allow for studies in the linear elastic regime where the DNA extension is unaffected by the bound metal. At present, it is unclear what effects the metal would place on the DNA twisting when it is under a force load.

This technique of the single molecule DNA detection offers distinct advantages over the current methods of studying single DNA spectroscopically that require the DNA to be stuck to a surface in order to realize the reproducibility of the metal coverage[12] or laser excitation in a TERS configuration.[11] This leaves the DNA in a static unnatural state which is sufficient for DNA sequence studies, however, one can envision with the current configuration correlating DNA Raman signal with the force sensing and manipulation aspects of the optical trap. This could add a new dimension to dynamical studies of structural conformation, such as extension or twisting, or even protein diffusion along the DNA.

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[1] S. Nie and S. Emory, Science **275**, 1102 (1997).
[2] K. Kneipp, Y. Wang, H. Kneipp, L. T. Perelman, I. Itzkan, R. R. Dasari, and M. S. Feld, Phys. Rev. Lett. **78**, 1667 (1997).
[3] H. Xu, E. J. Bjerneld, M. Käll, and L. Börjesson, Phys. Rev. Lett. **83**, 4357 (1999).
[4] E. Le Ru and P. Etchegoin, *Principles of Surface-Enhanced Raman Spectroscopy and Related Plasmonic Effects* (Elsevier, Amsterdam, 2009).
[5] B. Prescott, W. Steinmetz, and G. J. Thomas, Biopolymers **23**, 235 (2004).
[6] Y. C. Cao, R. Jin, and C. A. Mirkin, Science **297**, 1536 (2002).
[7] L. Movileanu, J. M. Benevides, and G. J. Thomas, Biopolymers **63**, 181 (2002).
[8] A. Barhoumi, D. Zhang, F. Tam, and N. J. Halas, J. Am. Chem. Soc. **130**, 5523 (2008).
[9] K. Kneipp, H. Kneipp, V. B. Kartha, R. Manoharan, G. Deinum, I. Itzkan, R. R. Dasari, and M. S. Feld, Phys. Rev. E **57**, R6281 (1998).
[10] A. MacAskill, D. Crawford, D. Graham, and K. Faulds, Anal. Chem. **81**, 8134 (2009).
[11] E. Bailo and V. Deckert, Angew. Chem. **47**, 1658 (2008).
[12] H. Wei and H. Xu, Appl. Phys. A: Mater. Sci. Process. **89**, 273 (2007).
[13] P. C. Lee and D. Meisel, J. Phys. Chem. **86**, 3391 (1982).
[14] S. Rao, S. Balint, B. Cossins, V. Guallar, and D. Petrov, Biophys. J. **96**, 209 (2009).
[15] S. F. Tolić-Nørrelykke, E. Schäffer, J. Howard, F. S. Pavone, F. Jülicher, and H. Flyvbjerg, Rev. Sci. Instrum. **77**, 103101 (2006).
[16] G. Thomas, Jr., J. Benevides, S. Overman, T. Ueda, K. Ushizawa, M. Saitoh, and M. Tsuboi, Biophys. J. **68**, 1073 (1995).
[17] R. C. Lord and G. Thomas, Jr., Spectrochim. Acta, Part A **23**, 2551 (1967).
[18] W. Ke, D. Zhou, J. Wu, and K. Ji, Appl. Spectrosc. **59**, 418 (2005).
[19] M. D. Wang, H. Yin, R. Landick, J. Gelles, and S. M. Block, Biophys. J. **72**, 1335 (1997).
[20] C. Bouchiat, M. D. Wang, J. F. Alemand, T. Strick, S. M. Block, and V. Croquette, Biophys. J. **76**, 409 (1999).
[21] A. Carter, Y. Seol, and T. Perkins, Biophys. J. **96**, 2926 (2009).
[22] C. Bustamante, S. Smith, J. Liphardt, and D. Smith, Curr. Opin. Struct. Biol. **10**, 279 (2000).