Available online at www.sciencedirect.com
ScienceDirect
Vibrational Spectroscopy 42 (2006) 2–8
VIBRATIONAL SPECTROSCOPY
www.elsevier.com/locate/vibspec

Sensitive and selective chem/bio sensing based on surface-enhanced Raman spectroscopy (SERS)

Xiaoyu Zhang [1], Nilam C. Shah [1], Richard P. Van Duyne *

Northwestern University, Department of Chemistry, 2145 Sheridan Rd., Evanston, IL 60208, USA

Available online 5 April 2006

Abstract

This paper summarizes our recent progress toward developing anthrax and glucose sensors based on surface-enhanced Raman spectroscopy (SERS). Ag film over nanosphere (AgFON) substrates was used as the SERS sensor platform in both cases. The AgFON substrates have been optimized for near-infrared (NIR) laser excitations by tuning the extinction maximum of their localized surface plasmon resonance (LSPR). *Bacillus subtilis* spores, harmless simulants for *Bacillus anthracis*, were studied using SERS. Calcium dipicolinate, a biomarker for bacillus spores, was efficiently extracted from spores and rapidly detected by SERS. A limit of detection (LOD) of $\sim 2.6 \times 10^3$ spores, below the anthrax infectious dose of 10^4 spores, has been achieved within 11 min. For glucose detection, a mixed decanethiol (DT)/mercaptohexanol (MH) partition layer is used to bring glucose closer to the AgFON surface. Quantitative detection of glucose in bovine plasma, as well as complete and rapid partitioning and departitioning, was demonstrated. The root mean square error of prediction (RMSEP) is 83.16 mg/dL (4.62 mM) with 85% of the validation points falling within the A and B range of the Clarke error grid.

Keywords: SERS; AgFON substrates; LSPR

Surface-enhanced Raman scattering (SERS) is a powerful technique for the sensitive and selective detection of low concentration analytes [1–5]. SERS produces very large enhancements in the effective Raman cross section of species spatially confined within the electromagnetic fields generated by excitation of the localized surface plasmon resonance (LSPR) of nanostructured noble metal surfaces. The efficiency of the SERS process, and by extension, the sensitivity of the sensor to anthrax spores and glucose, is largely determined by the optical properties of the SERS-active substrate [3,5]. Therefore, this work focuses on: (1) designing and fabricating Ag film over nanosphere (AgFON) substrates with a highly tunable plasmon; and (2) exploiting this tunability to act as a signal transduction mechanism for molecular sensing.

The SERS enhancement factor, EF_{SERS}, is optimized when the energy of the LSPR lies between the energy of the excitation wavelength and the energy of the vibrational band of interest [6,7]. These optimization conditions were exploited to develop SERS-based sensors for two important target molecules: a *Bacillus anthracis* biomarker, calcium dipicolinate (CaDPA) [3] and glucose in bovine plasma [4]. The understanding and application of these optimization conditions are critical for the development of an ultra-sensitive detection scheme based on SERS. For example, we demonstrate a limit of detection (LOD) of $\sim 2.6 \times 10^3$ spores with a data acquisition period of 1 min and a laser power (P_{ex}) of 50 mW. To place these results in context, it should be noted that previous published SERS studies of anthrax detection via the biomarker were 200 times less sensitive and required 3 times more laser power [8,9]. In the following sections, we will detail the quantitative, rapid, real-time detection of an anthrax biomarker, CaDPA, based on SERS.

In order to achieve the lowest limits of SERS detection, both the relationship between the surface nanostructure and laser excitation wavelength, as well as the analyte/surface binding chemistry, must be carefully optimized. While the CaDPA biomarker in *Bacillus subtilis* spores has an affinity towards the rough silver surface required for SERS detection, many important molecules (e.g. glucose) have low or non-existent binding affinity towards the silver surface [10]. The work presented herein demonstrates quantitative glucose detection

* Corresponding author. Tel.: +1 847 491 3516; fax: +1 847 491 7713.
E-mail address: vanduyne@chem.northwestern.edu (R.P. Van Duyne).
[1] These authors contributed equally to this work.

0924-2031/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.vibspec.2006.02.001

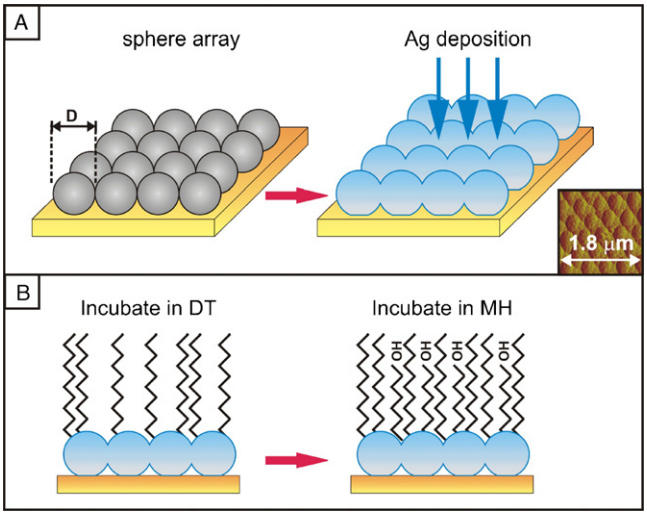

Fig. 1. (A) Schematical illustration of the fabrication of AgFON substrates. The inset shows an AFM image of a AgFON substrate. (B) The AgFON surface was incubated in a solution of 1 mM DT in ethanol for 45 min and then transferred to 1 mM MH in ethanol for at least 12 h. This DT/MH SAM creates a pocket for glucose to fit into, bringing glucose closer to the SERS-active surface.

by tailoring the SERS-active substrate with a self-assembled monolayer (SAM). The SAM layer partitions glucose and brings it closer to the AgFON surface to allow for detection with SERS [2,5,10]. In this study, a mixed SAM that consists of decanethiol (DT) and mercaptohexanol (MH) has been explored (Fig. 1). The dual hydrophobic and hydrophilic properties of the DT/MH SAM make it ideal for partitioning glucose [4]. Although the exact mechanism of this SAM layer has not been well characterized, a space filling computer model shows that combining DT and MH creates a void. It is hypothesized that glucose partitions into this pocket and is brought closer to the surface than was possible with SAMs used previously.

1. Experimental

1.1. Materials

All the chemicals used were of reagent grade or better. Ag (99.99%) was purchased from D.F. Goldsmith (Evanston, IL). Glass substrates were 18 mm diameter, No. 2 cover slips from Fisher Scientific (Pittsburgh, PA). Oxygen-free high-conductivity copper was obtained from McMaster-Carr (Chicago, IL) and cut into 18 mm diameter disks. Pretreatment of substrates required H_2SO_4, H_2O_2, and NH_4OH, all of which were purchased from Fisher Scientific (Fairlawn, NJ). Surfactant-free white carboxyl-functionalized polystyrene latex nanospheres with diameters of 390, 510, 600, and 720 nm were obtained from Duke Scientific Corporation (Palo Alto, CA) and Interfacial Dynamics Corporation (Portland, OR). Tungsten vapor deposition boats were purchased from R.D. Mathis (Long Beach, CA). Nitric acid 70% (Fisher Scientific), dipicolinic acid (2,6-pyridinedicarboxlic acid, DPA), calcium hydroxide, and benzenethiol (Aldrich Chemical Co., Milwaukee, WI) were used as purchased. Water (18.2 MΩ/cm) was obtained from an ultrafilter system (Milli-Q, Millipore, Marlborough, MA). Bovine plasma was obtained from Hemostat Laboratories (Dixon, CA). Glucose, lactate, and urea were purchased from Sigma (St. Louis, MO). Decanethiol ($CH_3(CH_2)_9SH$), and 6-mercapto-1-hexanol ($HS(CH_2)_6OH$) were purchased from Aldrich (Milwaukee, WI). Disposable filters, pore size 0.45 μm, were acquired from Gelman Sciences (Ann Arbor, MI). Calcium dipicolinate was prepared from DPA and calcium hydroxide according to the method of Beiley et al. [11].

1.2. Spore samples

B. subtilis, a widely used stimulant for *Bacillus anthracis*, was purchased from the American Type Culture Collection (Manassas, VA). Spore cultures were cultivated by spreading the vegetative cells on sterile nutrient agar plates (Fisher Scientific), followed by incubating at 30 °C for 6 days. The cultures were washed from the plates using sterile water and centrifuged at 12,000 × *g* for 10 min for five cycles. The lyophilized spores were kept at 2–4 °C prior to use. Approximately 1 g of sample was determined to contain 5.6×10^{10} spores by optical microscopic measurements (data not shown). The spore suspension was made by dissolving spores in 0.02 M HNO_3 solution and with the aid of a 10 min sonication.

1.3. AgFON substrate fabrication

Glass substrates were pretreated in two steps: (1) piranha etch (caution: piranha solution should be handled with great care), 3:1 H_2SO_4:30% H_2O_2 at 80 °C for 1 h, was used to clean the substrate, and (2) base treatment, 5:1:1 H_2O:NH_4OH:30% H_2O_2 with sonication for 1 h, was used to render the surface hydrophilic. Approximately 2 μL of the nanosphere suspension (4% solids) was drop-coated onto each glass substrate and allowed to dry in ambient conditions. The copper substrates were cleaned by sonicating in 5:1:1 H_2O:30% H_2O_2:NH_4OH. Approximately 10 μL of 390 nm nanosphere solution was drop-coated onto a clean copper substrate and allowed to dry at room temperature. The metal films were deposited in a modified Consolidated Vacuum Corporation vapor deposition system with a base pressure of 10^{-6} Torr. The deposition rates for each film (10 Å/s) were measured using a Leybold Inficon XTM/2 quartz crystal microbalance (QCM) (East Syracuse, NY). AgFON substrates were stored in dark at room temperature prior to use.

For glucose sensing experiments, AgFON substrates were first incubated in 1 mM DT in ethanol for 45 min and then transferred to 1 mM MH in ethanol for at least 12 h (Fig. 1B). Then the SAM-functionalized surfaces were mounted into a small-volume flow cell for SERS measurements.

1.4. LSPR reflectance spectroscopy

Measurements were carried out using Ocean Optics (Dunedin, FL) SD2000 spectrometer coupled to a reflection probe (Ocean Optics) and a halogen lamp (Model F-O-Lite H, World Precision Instruments, Sarasota, FL). The reflection probe consists of a tight bundle of 13 optical fibers (12

illumination fibers around a collection fiber) with a useable wavelength range of 400–900 nm. All reflectance spectra were collected against a mirror-like Ag film over glass substrate as a reference.

1.5. SERS apparatus

A titanium-sapphire laser (CW Ti:Sa, model 3900, Spectra Physics, Mountain View, CA) pumped by a solid-state diode laser (model Millenia Vs, Spectra Physics) was used to generate λ_{ex} of 750 or 785 nm. The SERS measurement system consists of an interference filter (Coherent, Santa Clara, CA), a 1″ holographic edge filter (Physical Optics Corporation, Torrance, CA), a single-grating monochromator with the entrance slit set at 100 μm (model VM-505, Acton Research Corporation, Acton, MA), a liquid-N_2-cooled CCD detector (model Spec-10:400B, Roper Scientific, Trenton, NJ), and a data acquisition system (Photometrics, Tucson, AZ). The spectral positions of the CCD pixels were calibrated using emission lines of known wavelengths from a Neon lamp. For the detection of spores, all the measurements were performed in ambient conditions. For the detection of glucose, a small-volume flow cell was used to control the external environment of the AgFON surfaces.

1.6. Quantitative multivariate analysis

All data processing was performed using MATLAB (MathWorks, Inc., Natick, MA) and PLS_Toolbox (Eigenvector Research, Inc., Manson, WA). Prior to analysis, the spectra were smoothed using the Savitsky–Golay method with a second-order polynomial and window size of 9. Cosmic rays were removed from the spectra using a derivative filter. The slowly varying background, commonly seen in SERS experiments, was removed by subtracting a fourth-order polynomial fit. This method greatly reduced varying background levels with minimum effect on the SERS peaks. The chemometric analysis was performed using the partial least-squares (PLS) method and leave-one-out (LOO) cross-validation algorithm.

1.7. Time constant analysis

The data were processed using PeakFit 4.12 software (Systat Software Inc., Richmond, CA). To remove the varying background in the SER spectra, a fourth-order polynomial was subtracted from the baseline using MATLAB software. The spectra were further preprocessed in PeakFit with linear best-fit baseline correction and Savitsky–Golay smoothing. The amplitude of the Raman bands was obtained by fitting the data to the superposition of the Lorentzian amplitude line shapes.

2. Results and discussion

2.1. AgFON substrates with tunable LSPR optimized for SERS

Previously, an important correlation between the nanoparticle structure, as reported by the spectral position of the LSPR relative to the laser excitation wavelength, and the SERS intensity was demonstrated. The maximum SERS enhancement factor is shown to occur for excitation wavelengths that are blue-shifted with respect to the LSPR peak of adsorbate-covered nanoparticle arrays [7]. In this work, near-infrared (NIR) excitations were selected to reduce the native fluorescence background from microorganisms and to minimize the autofluorescence of proteins [12]. AgFON substrates for SERS measurements under NIR excitations were optimized by measuring the dependence of the LSPR spectral position on nanosphere diameter. The plasmon peaks of AgFON substrates can be tuned by controlling the size of the spheres used in the underlying nanosphere mask (Fig. 1). Fig. 2 shows the LSPR reflectance spectra of AgFON substrates with nanospheres having diameters of 390, 510, and 600 nm. A parallel measurement was also performed on an AgFON sample with 720 nm diameter spheres. The spectrum, however, is not shown because the reflectance minimum is shifted beyond the red limit (∼900 nm) of the CCD detector. In Fig. 2C, the reflectance spectrum of the AgFON substrate with the nanosphere diameter, $D = 600$ nm, and the mass thickness of Ag film ($d_m = 200$ nm) shows a reflectivity minimum near 753 nm, which is close to the NIR excitations used in the SERS measurement. Therefore, 600 nm nanospheres were selected to fabricate AgFON samples used for the CaDPA detection which were conducted in air.

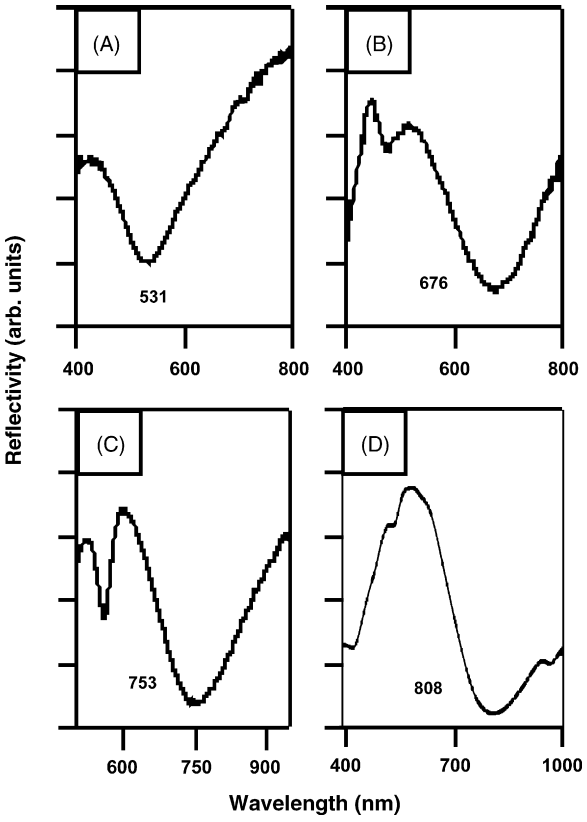

Fig. 2. LSPR reflectance spectra of different AgFON substrates ($d_m = 200$ nm). (A) $D = 390$ nm, in air, (B) $D = 510$ nm, in air, (C) $D = 600$ nm, in air, and (D) DT/MH functionalized AgFON substrate in aqueous environment, $D = 390$ nm.

When the external dielectric media around AgFON substrates change from air to an aqueous environment, the LSPR peaks shifts to longer wavelengths. Since the SERS detection of glucose in bovine plasma has to be done in an aqueous environment, it is imperative to evaluate the LSPR of AgFON substrates in an aqueous dielectric media. As illustrated in Fig. 2D, the AgFON substrate fabricated using 390 nm nanospheres, exhibiting LSPR reflectivity minimum ~808 nm, was determined to be the optimal substrate for the glucose sensing experiments.

2.2. *Bacillus* spore detection based on SERS

The rapid and accurate identification of bioagents is a vital task for first-responders in a biological attack. *B. anthracis*, a spore-forming bacterium and a dangerous pathogen for the disease anthrax, is an important example. A bacillus spore structurally consists of several protective layers and a core cell. CaDPA exists in these protective layers and contributes ~5–10% to the dry weight of spores. CaDPA has been widely used as the spore biomarker because other potentially interfering species lack this particular molecule in such high proportions [8,9,11,13]. In this section, we describe a rapid detection protocol of bacillus spores biomarkers based on SERS.

CaDPA was successfully extracted from *B. subtilis* spores, harmless simulants for *B. anthracis* spores, by sonicating in 0.02 M HNO_3 solution for 10 min. Fig. 3A shows a high signal-to-noise ratio SERS spectrum of a 3.1×10^{-13} M spore suspension (3.7×10^4 spores in 0.2 μL, 0.02 M HNO_3) on a AgFON substrate (D = 600 nm, d_m = 200 nm). A parallel SERS experiment was conducted using 5.0×10^{-4} M CaDPA (Fig. 3B) for comparison. Clearly, the SERS spectrum of *B. subtilis* spores is dominated by the bands associated with CaDPA. The SERS spectra in Fig. 3, however, display noticeable differences at ~1595 cm^{-1}, which are from the acid form of dipicolinate. The peak at 1050 cm^{-1} in Fig. 3A is from the symmetrical stretching vibration of NO_3^-, which is used as an internal standard to reduce sample to sample deviations.

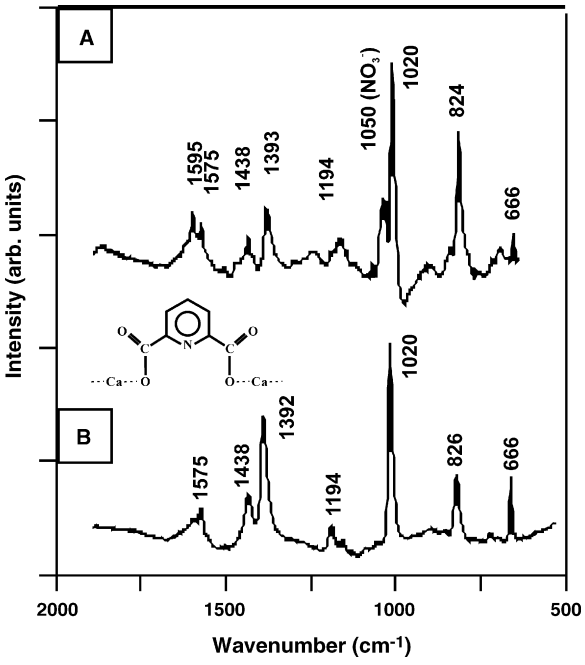

Fig. 3. (A) SERS spectrum of 3.1×10^{-13} M spore suspension (3.7×10^4 spores in 0.2 μL, 0.02 M HNO_3) on a AgFON substrate. (B) SERS spectrum of 5.0×10^{-4} M CaDPA. λ_{ex} = 750 nm, P_{ex} = 50 mW, acquisition time t = 1 min, D = 600 nm, and d_m = 200 nm.

Furthermore, the SERS signal from extracted CaDPA was measured over the spore concentration range 10^{-14}–10^{-12} M to determine the saturation binding capacity of the AgFON surface and calculate the adsorption constant ($K_{spore} = 1.7 \times 10^{13}$ M^{-1}). In Fig. 4, each data point represents the average intensity of the 1020 cm^{-1} peak from three spectra. The standard deviation is shown by the error bars. At low spore concentrations, the peak intensity increases linearly with concentration (Fig. 4, inset). At higher spore concentrations, the response levels off as the adsorption sites on the AgFON substrate become fully occupied. Saturation occurs when the spore concentrations exceed ~2.0×10^{-13} M (2.4×10^4 spores in 0.2 μL, 0.02 M HNO_3).

The inset in Fig. 4 was used to determine the limit of detection. In this study, the LOD is defined as the concentration of spores for which the strongest SERS signal of CaDPA at 1020 cm^{-1} is equal to three times the background SERS signal within a 1-min acquisition period. The background signal refers to the SERS intensity from a sample with a spore concentration equal to zero, which is calculated to be the intercept of the low concentration end of the spore adsorption isotherm. Obviously, lower detection limits can be achieved using longer acquisition times and/or higher laser excitation power, however, these parameters are reasonable for high throughput, real-time, and on-site analysis of potentially harmful species. The LOD for *B. subtilis* spores, evaluated by extrapolation of the linear concentration range of the adsorption isotherms (Fig. 4, inset),

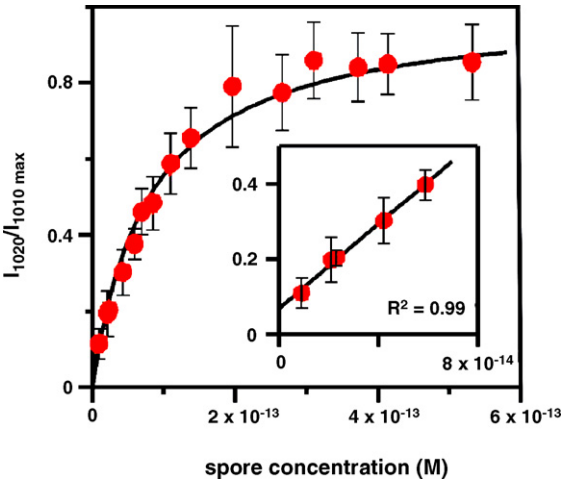

Fig. 4. Adsorption isotherm for *B. subtilis* spore suspension onto a AgFON substrate. I_{1020} was taken from SERS spectra that correspond to varying spore concentrations in 0.2 μL, 0.02 M HNO_3 on AgFON substrates. λ_{ex} = 750 nm, P_{ex} = 50 mW, acquisition time t = 1 min, D = 600 nm, and d_m = 200 nm. The inset shows the linear range that is used to determine the LOD. Each data point represents the average value from three SERS spectra. Error bars show the standard deviations.

is found to be 2.1×10^{-14} M (2.6×10^3 spores in 0.2 μL, 0.02 M HNO_3), well below the anthrax infectious dose of 10^4 spores.

The speed and sensitivity of this SERS sensor indicate that this technology can be used as a viable option for the field analysis of potentially harmful environmental samples. Increasing the affinity binding constant by developing an appropriate capture layer on AgFON to preconcentrate CaDPA is an important future goal.

2.3. Glucose detection based on SERS

For glucose detection, different SAMs have been explored to determine the most effective partition layer. Previously, decanethiol [10] and (1-mercaptoundeca-11-yl)tri(ethyleneglycol), EG3 [2,5], were found to effectively partition glucose. Although DT successfully partitions glucose in the physiologically relevant range (0–25 mM), it is hydrophobic and therefore, cannot be used in vivo. EG3 was then chosen as a partition layer because it is biocompatible and able to resist the non-specific binding of proteins [14,15]. EG3 effectively partitions glucose and allows for the quantitative detection of glucose even in the presence of serum albumin [2]. However, due to the intricate synthesis method, EG3 is not readily available. Thus, a new mixed SAM that consists of decanethiol and mercaptohexanol has been explored. Previously, the SERS-based sensor was also optimized for NIR excitation wavelength [5] so it can eventually be placed under the skin and reduce photodamage of tissue. NIR excitation was also used for the new DT/MH-functionalized AgFON. Reversibility experiments have confirmed that the DT/MH SAM has better partitioning capabilities than the DT and EG3 layers used previously.

Reversibility is an important parameter because an implantable glucose sensor must successfully monitor the fluctuation of glucose continuously throughout the day. This can only be accomplished if the sensor can completely partition and departition glucose. To simulate real-time sensing, 0 and 100 mM aqueous glucose solutions (pH ~7) were alternately introduced into a flow cell containing the DT/MH-modified AgFON in 20-min intervals without flushing the sensor between measurements (Fig. 5, inset). Fig. 5A shows the normal Raman spectrum of an aqueous saturated glucose solution for comparison to the SERS spectra. Peaks at 1462, 1365, 1268, 1126, 915, and 850 cm^{-1} correspond to crystalline glucose peaks [16]. The raw SERS spectra prior to the spectral subtraction agree with our previous studies [2,5,10,17]. Fig. 5B and C are the difference spectra that demonstrate partitioning of glucose into the DT/MH functionalized SAM with glucose features at 1461, 1371, 1269, 1131, 916, and 864 cm^{-1} (λ_{ex} = 532 nm, P = 10 mW, t = 20 min). Literature has shown that SERS bands can shift up to 25 cm^{-1} compared to normal Raman bands of the same compound [18]. Therefore, the features in the difference spectra correspond to the glucose peaks in the normal Raman spectrum of glucose (Fig. 5A). Departitioning of glucose is demonstrated by the difference spectra shown in Fig. 5D and E. The absence of glucose spectral

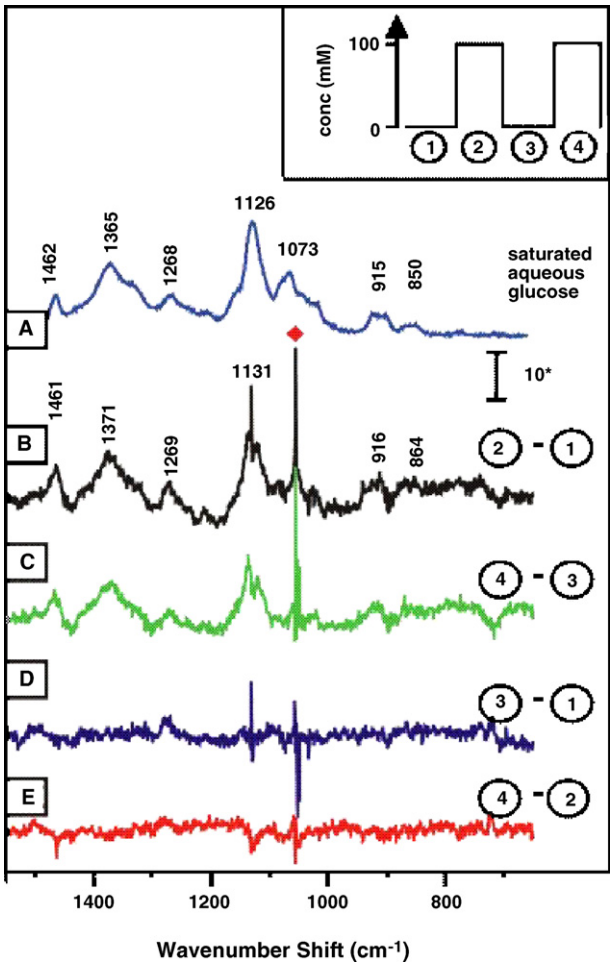

Fig. 5. SERS spectra demonstrating reversibility of DT/MH functionalized SAM. Inset shows glucose cycling sequence of pulse experiment, λ_{ex} = 532 nm, P_{ex} = 10 mW, t = 20 min, pH ~7. The normal Raman spectrum of aqueous saturated glucose solution is shown in (A). Difference spectra demonstrating (B and C) partitioning and (D and E) departitioning.

features clearly demonstrates complete departitioning of glucose. The spectra were normalized by using nitrate as an internal standard to minimize laser power fluctuations. Specifically, the symmetric stretching vibration of NO_3^- at 1053 cm^{-1} was used for normalization. The sharp peak seen in all the difference spectra at 1053 cm^{-1} represents imperfect subtraction of the nitrate internal standard. These experiments clearly demonstrate that the DT/MH-functionalized AgFON is a reversible sensing surface that can completely partition and departition glucose.

In addition to reversibility, an implantable glucose sensor must also partition and departition in a reasonable time scale in order to be used continuously. The real-time response was evaluated by calculating the 1/e time constants for partitioning and departitioning. The experiments were conducted in bovine plasma to simulate the in vivo environment.

A DT/MH-functionalized AgFON was first incubated in bovine plasma for ~5 h. The AgFON substrate was then placed in a flow cell and a solution containing 50 mM glucose in bovine plasma was introduced at t = 0 to observe partitioning. Departitioning was evaluated by injecting 0 mM glucose in

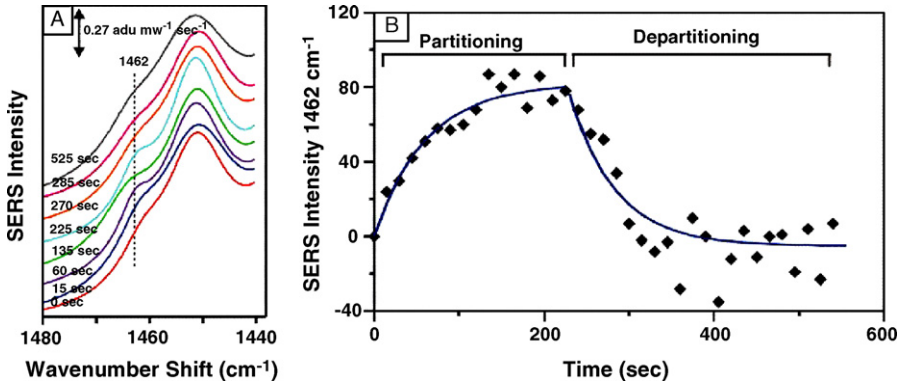

Fig. 6. Real-time response to step change in glucose concentration. (A) SER spectra of SAM at various times. Glucose peak is at 1462 cm^{-1} and SAM peaks are at 1451 and 1428 cm^{-1}. Fifty micromolars glucose was injected at $t = 0$ and flushed with bovine plasma at $t = 225$ s. Partitioning and departitioning of glucose is demonstrated in (B) with $\lambda_{ex} = 785$ nm, $P_{ex} = 100$ mW, $t = 15$ s. The $1/e$ time constants were calculated to be 28 s for partitioning and 25 s for departitioning.

bovine plasma into the flow cell at $t = 225$ s. Throughout the experiment, SERS spectra were collected continuously at 15 s intervals.

Fig. 6 shows the real-time amplitude changes of the 1462 cm^{-1} peak over the course of 600 s. The amplitude of the 1462 cm^{-1} peak was obtained by fitting the spectra to the superposition of three Lorentzian lineshapes using PeakFit. Then, the amplitude was plotted versus time (Fig. 6B). The plot was finally fitted with an exponential curve, yielding the $1/e$ time constants. The $1/e$ time constants were calculated to be 28 s for partitioning and 25 s for departitioning (Fig. 6B). These experiments demonstrate that partitioning and departitioning occur in less than 1 min, making the SERS-based sensor a potential candidate for implantable, continuous sensing.

Futhermore, an ideal glucose sensor must also be able to detect glucose in the clinically relevant range 10–450 mg/dL (0.56–25 mM), under physiological pH and in complex media.

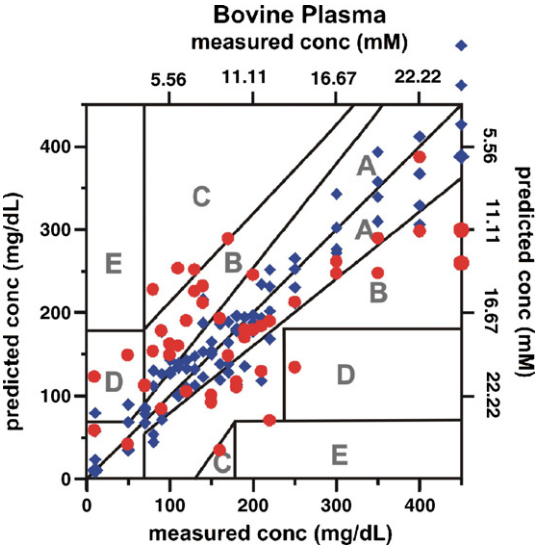

Fig. 7. Calibration (◆) and validation (●) plots of glucose in bovine plasma on Clarke error grid. Calibration plot was constructed using 92 data points and validation plot was constructed using 46 data points. Sample was incubated for 2 min in glucose concentrations ranging from 10 to 450 mg/dl with $\lambda_{ex} = 785$ nm, $P_{ex} = 10$–30 mW, $t = 2$ min. RMSEC = 34.3 mg/dL (1.9 mM) and RMSEP = 83.16 mg/dL (4.62 mM).

DT/MH-functionalized SAM was placed in a flow cell and incubated for 2 min with glucose (10–450 mg/dl) in filtered bovine plasma. Bovine plasma was used to simulate the in vivo environment that the sensor will be exposed to when it is implanted under the skin in interstitial fluid. SERS spectra were collected using multiple samples and multiple spots with a near-infrared laser source ($\lambda_{ex} = 785$ nm, $P = 8.4$ mW, $t = 2$ min). Partial least-squares leave-one-out (PLS–LOO) analysis was used to construct a calibration model with 92 independent spectral measurements of known glucose concentrations based on 7 latent variables. The resulting calibration model and concentration predictions are presented on a Clarke error grid (Fig. 7).

The Clarke error grid is a standard for evaluating the reliability of glucose sensors in the clinically relevant concentration range (0–450 mg/dL) [19]. Data points that fall in the A and B range are acceptable values. Values outside the A and B range indicate potential failure to detect blood glucose levels properly, usually resulting in erroneous and even fatal diagnosis.

The PLS analysis yielded a root mean square error of calibration (RMSEC) of 34.3 mg/dL (1.90 mM) with 98% of the data falling in the A and B region of the Clarke error grid. The root mean square error of prediction (RMSEP) was calculated to be 83.16 mg/dL (4.62 mM) with 85% of the validation points falling in the A and B range of the Clarke error grid. The RMSEP can be improved by increasing the number of data points in the calibration set. Overall, the results show that the DT/MH-modified AgFON glucose sensor is capable of making accurate glucose measurements in the presence of interfering analytes. An important future goal is testing the DT/MH-functionalized AgFON in vivo.

Acknowledgments

The authors acknowledge Dr. Haitao Ji at Northwestern University for the technical assistance with the *B. subtilis* spore culture. The research was supported by the National Science Foundation (DMR-0076097, CHE0414554), the Air Force Office of Scientific Research MURI program (F49620-02-1-0381), the National Institutes of Health (DK066990-01A1), the

U.S. Army Medical Research and Material Command (W81XWH-04-1-0630).

Reference

[1] S. Nie, S.R. Emory, Science 275 (1997) 1102 (Washington, DC).
[2] C.R. Yonzon, C.L. Haynes, X. Zhang, J.T. Walsh Jr., R.P. Van Duyne, Anal. Chem. 76 (2004) 78.
[3] X. Zhang, M.A. Young, O. Lyandres, R.P. Van Duyne, J. Am. Chem. Soc. 127 (2005) 4484.
[4] O. Lyandres, N.C. Shah, C.R. Yonzon, J.T. Walsh Jr., M.R. Glucksberg, R.P. Van Duyne, Anal. Chem. 77 (2005) 6134.
[5] D.A. Stuart, C.R. Yonzon, X. Zhang, O. Lyandres, N. Shah, M.R. Glucksberg, J.Y. Walsh, R.P. Van Duyne, Anal. Chem. 77 (2005) 4013.
[6] C.L. Haynes, R.P. Van Duyne, J. Phys. Chem. B 107 (2003) 7426.
[7] A.D. McFarland, M.A. Young, J.A. Dieringer, R.P. Van Duyne, J. Phys. Chem. B 109 (2005) 11279.
[8] S. Farquharson, A.D. Gift, P. Maksymiuk, F.E. Inscore, Appl. Spectrosc. 58 (2004) 351.
[9] S. Farquharson, L. Grigely, V. Khitrov, W. Smith, J.F. Sperry, G. Fenerty, J. Raman Spectrosc. 35 (2004) 82.
[10] K.E. Shafer-Peltier, C.L. Haynes, M.R. Glucksberg, R.P. Van Duyne, J. Am. Chem. Soc. 125 (2003) 588.
[11] G.F. Bailey, S. Karp, L.E. Sacks, J. Bacteriol. 89 (1965) 984.
[12] R. Weissleder, Nat. Biotechnol. 19 (2001) 316.
[13] R. Goodacre, B. Shann, R.J. Gilbert, E.M. Timmins, A.C. McGovern, B.K. Alsberg, D.B. Kell, N.A. Logan, Anal. Chem. 72 (2000) 119.
[14] K.L. Prime, G.M. Whitesides, J. Am. Chem. Soc. 115 (1993) 10714.
[15] C. Dicke, G. Hahner, J. Am. Chem. Soc. 124 (2002) 12619.
[16] S. Soderholm, Y.H. Roos, N. Meinander, M. Hotokka, J. Raman Spectrosc. 30 (1999) 1009.
[17] M.F. Mrozek, M.J. Weaver, Anal. Chem. 74 (2002) 4069.
[18] A.M. Stacy, R.P. Van Duyne, Chem. Phys. Lett. 102 (1983) 365.
[19] W.L. Clarke, D. Cox, L.A. Gonder-Frederick, W. Carter, S.L. Pohl, Diab. Care 10 (1987) 622.