Detection of Genetic Markers Related to High Pathogenicity in Influenza by SERS

Supporting Information

Pierre Negri and Richard A. Dluhy*

Department of Chemistry, University of Georgia, Athens, GA 30602

*Author to whom correspondence should be sent

tel: +1-706-542-1950

fax: +1-706-542-9454

dluhy@uga.edu

Experimental Methods

SERS Substrates Fabrication and Patterning. Aligned Ag nanorod substrates were fabricated using an oblique-angle vapor deposition (OAD) method according to previously published procedures.[1, 2] In brief, standard 2.54 × 7.62 cm glass slides (Gold Seal®, Becton Dickinson Company, Franklin Lakes, NJ) were first cleaned with heated Piranha solution (4:1 conc. H_2SO_4: 30% H_2O_2) for 15 minutes, thoroughly rinsed with copious amounts of deionized water and dried under a gentle stream of N_2. The glass slides were loaded into a custom made e-beam deposition system with background pressure maintained at less than 5×10^{-6} Torr. First, a 20 nm adhesion layer of Ti (99.99%, Kurt J. Lesker Company, Clairton, PA) was deposited onto the glass slide at a rate no greater than 0.2nm/s. Next, a 500 nm layer of Ag (>99.99%, Kurt J. Lesker Company) was deposited at a rate of approximately 0.3-0.4 nm/s. Finally, the substrates were rotated to 86° relative to the incident vapor source so that their surface forms a 4° angle with the incident vapor, allowing the growth of the Ag nanorods at a constant rate of 2.0-3.0 Å/s until a quartz crystal microbalance (QCM) registered a final nominal thickness of 2000 nm. We have established a calibration relationship between nanorod length and the mass of Ag deposited on the quartz crystal where 2000 nm nominal thickness results in tilted nanorods of ~900 nm length.[2] All deposition rates and thicknesses were monitored by a Quartz Crystal Microbalance (QCM) located inside the deposition chamber and positioned at normal incidence to the vapor source. After deposition, the substrates were allowed to cool down to room temperature in vacuum inside the deposition chamber for 2 hours. The substrates were then removed from the deposition chamber and stored in a nitrogen purge type glove box inside a Petri dish to avoid any surface contamination. Prior to their use, the Ag nanorod substrates were cleaned for 4 minutes

in an Ar^+ plasma using a plasma cleaner (Model PDC-32G, Harrick Plasma, Ithaca, NY) to remove any surface contamination.[3]

Following nanofabrication and cleaning of the nanorod substrates, a stainless steel mold was used in conjunction with PDMS to produce a 4 x 10 well PDMS-pattern SERS-active microwell substrate. In brief, liquid polydimethylsiloxane (PDMS, Sylgard® 184 Silicone Elastomer Kit, Dow Corning, Midland, MI) was added to a mold and cured by low temperature heating to produce the patterned substrate according to previously published procedures[4, 5] (with slight modifications as follows. A stainless-steel mold/substrate assembly was pre-heated in a bench top oven (Fisher Scientific) at 55°C for 10 minutes prior to the addition of a mixture of PDMS base, curing agent, and accelerator (20:2:1, w/w) through the opening of the substrate/mold assembly. The mixture was allowed to cure on the SERS-active substrate at 55°C for approximately 45 minutes, and cool down to room temperature for about 10 minutes. Finally, the well patterning plate was de-attached gently from the patterned substrate, creating a uniform, 4 × 10 well PDMS-pattern SERS-active microwell array substrate.

Preparation of Synthetic DNA and RNA Samples. The 5' C6 thiolated ss-DNA probes were received lyophilized and dissolved in molecular biology grade water down to a concentration of 1000 nM for the SERS experiments, and 10 μg/ mL (~1000 nM) for the ELISA experiments, respectively. The synthetic RNA target sequences were received lyophilized and prepared in the binding buffer spanning the concentration range of 10^3-10^{-4} nM at 1:10 increments for the SERS experiment. The TAMRA labeled RNA target sequences were dissolved in the binding buffer for the ELISA experiment at varying concentration (1000 nM to 0.1 nM).

Procedures for DNA Probes Immobilization and Binding of RNA Target. The 5' C6 thiolated ss-DNA probes were immobilized onto the Ag nanorod substrates by addition of 20 μL of 1000 nM of the oligonucleotide solution to a patterned microwell overnight at room temperature to yield a self-assembled monolayer (SAM). After incubation, the DNA solution was removed from the substrate surface and the microwell was rinsed three times with molecular biology grade water to remove any unbound material. The surface was then blow dried with N_2 before addition of the spacer molecule. 20 μL of a 100 nM solution of the spacer molecule 6-mercapto-1-hexanol (MCH) was then added to the microwell to promote the correct oligonucleotide conformation and minimize non-specific binding of DNA or RNA molecules to the Ag surface. The spacer molecule was incubated for 6 hours at room temperature before rinsing with molecular biology grade water and blow drying with N_2. Following formation of the ss-DNA-spacer complex, hybridization of the RNA samples to the oligonucleotide-functionalized Ag nanorod surface was accomplished by adding 20 μL of a 1 nM synthetic RNA solution diluted in the binding buffer. The substrate was then incubated at 37°C for 2 hours in a humid environment to prevent dehydration of the samples on the substrate surface. Following incubation, the RNA solution was removed from the substrate, and any non-specifically adsorbed RNA molecules were removed by thoroughly rinsing the microwell twice with the binding buffer followed by a final wash with molecular biology grade water to remove the salts present in the buffer. The substrate was then dried under a gentle stream of N_2 prior to analysis.

Table and Figure Caption

Table S.1. Observed SERS vibrational bands with assignments in the spectrum of the DNA probe-spacer complexes.

Wavenumber, cm^{-1}	**Assignment**
623	C-C-C ring in-plane bending of A[6]
687	C-S stretching vibration of the thiolated aptamer[7]
731	Ring breathing mode of A[6, 8, 9]
793	Ring breathing mode of both C and T[10]
1023	Amino group vibration of C[9]
1045	Asymmetric out-of-plane deformation of NH_2 in C[8]
1089	Backbone phosphate stretching mode of nucleic acids[11]
1275	Ring stretching and C-H bending of T[6, 8, 12]
1332	Ring stretching mode of A[12]
1454	Ring breathing vibration of A[9]
1496	C8=N7 vibration of the G ring[12]
1506	External phenyl ring stretching of A[13]
1558	Ring stretching mode of G and A[7]
1631	External phenyl ring stretching of C[13]

Figure S.1. Schematic diagram of the overall strategy for DNA probe immobilization onto the Ag nanorod array, addition of the spacer molecule 6-mercapto-1-hexanol, and detection strategy. Immobilization of the thiolated DNA probe onto the surface of the Ag nanorod array (i), addition of -mercapto-1-hexanol as the spacer molecule (ii), and incubation with the RNA samples (iii).

References

1. S. B. Chaney, S. Shanmukh, Y.-P. Zhao and R. A. Dluhy, *Appl Phys Lett*, 2005, **87**, 31908-31910.
2. J. D. Driskell, S. Shanmukh, Y. Liu, S. B. Chaney, X. J. Tang, Y. P. Zhao and R. A. Dluhy, *J Phys Chem C*, 2008, **112**, 895-901.
3. P. Negri, N. E. Marotta, L. A. Bottomley and R. A. Dluhy, *Appl Spectrosc*, 2011, **65**, 66-74.
4. J. L. Abell, J. D. Driskel, R. A. Dluhy, R. A. Tripp and Y.-P. Zhao, *Proc SPIE*, 2009, **7321**, 732103-732111.
5. J. L. Abell, J. D. Driskell, R. A. Dluhy, R. A. Tripp and Y.-P. Zhao, *Biosens Bioelec*, 2009.
6. C. Otto, T. J. J. Vandentweel, F. F. M. Demul and J. Greve, *J Raman Spectrosc*, 1986, **17**, 289-298.
7. M. Rycenga, J. M. McLellan and Y. N. Xia, *Chem Phys Lett*, 2008, **463**, 166-171.
8. J. S. Suh and M. Moskovits, *J Am Chem Soc*, 1986, **108**, 4711-4718.
9. J. Kundu, O. Neumann, B. G. Janesko, D. Zhang, S. Lal, A. Barhoumi, G. E. Scuseria and N. J. Halas, *J Phys Chem C*, 2009, **113**, 14390-14397.
10. K. Kneipp and J. Flemming, *J Mol Struc*, 1986, **145**, 173-179.
11. A. Barhoumi, D. Zhang, F. Tam and N. J. Halas, *J Am Chem Soc*, 2008, **130**, 5523-5529.
12. C. V. Pagba, S. M. Lane and S. Wachsmann-Hogiu, *J Raman Spectrosc*, 2010, **41**, 241-247.
13. S. Basu, S. Jana, S. Pande and T. Pal, *J Coll Inter Sci*, 2008, **321**, 288-293.