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# Isotropic and anisotropic dipeptide films based on gas phase deposition

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## Abstract

We demonstrated the fabrication and application of well-ordered and vertically aligned dipeptide nanostructures based on a simple gas phase deposition. Deposited nanostructures exhibited the superhydrophobic property with a very low sliding angle. Highly reproducible SERS data have also been obtained after combining deposited films with a thin layer of gold. In addition to these, directional peptide films were, for the first time, successfully fabricated based on the oblique angle deposition technique. We believe that such bio-inspired materials would have a great impact in several technological applications involving catalysis, tissue engineering and biosensors.

[S] Online supplementary data available from [stacks.iop.org/Nano/23/225604/mmedia](http://stacks.iop.org/Nano/23/225604/mmedia)

(Some figures may appear in colour only in the online journal)

## 1. Introduction

Vertically aligned nanostructures have recently attracted increasing interest due to their unique properties and they have been exploited for a variety of applications including field emission devices, filter media, superhydrophobic surfaces, synthetic membranes, intracellular gene delivery devices, biosensors, composites, logic and memory devices, and numerous others [1–4]. Fabrication of well-ordered and aligned nanostructures may provide a number of advantages in any application where the surface area and the alignment of structures are considered key parameters. Besides synthetic materials, utilization of biological molecules for creating novel nanostructures is a promising approach due to their biocompatibility, extraordinary stability, functional flexibility and molecular recognition capability [5–12]. In particular, dipeptides, which can spontaneously form a variety of nanostructures within a certain solvent medium, serve as an excellent model for the possible applications in the biological and non-biological fields.

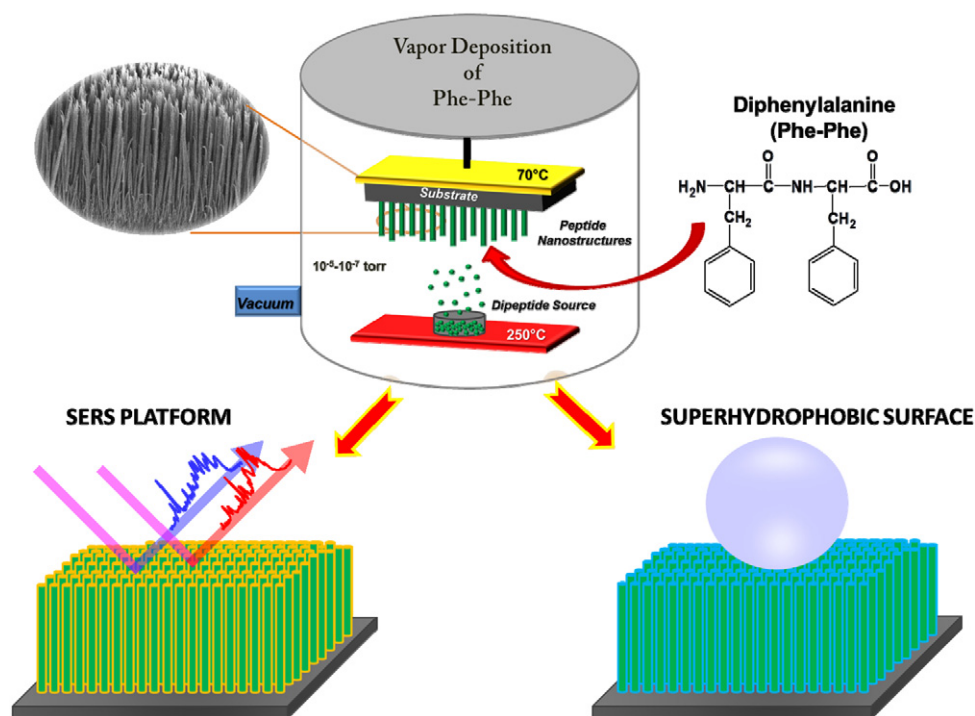
Recently, Abramovich *et al* reported that vertically aligned dipeptide-based nanostructures may be fabricated through a vapor deposition system [13]. Although they have demonstrated using such peptide structures in high

surface area electrodes for energy storage applications and microfluidic chips, wetting properties of deposited films and their usage as a sensor platform have largely been unexplored. Herein, we demonstrated the fabrication of well-aligned and highly ordered peptide nanostructures onto the surfaces based on a traditional physical vapor deposition (PVD) system. The overall process is schematically depicted in scheme 1. The hydrophobic and oleophobic behaviors of deposited peptide films have also been examined via contact angle measurements. Furthermore, the deposited films were used as a template to fabricate surface-enhanced Raman spectroscopy (SERS) platforms that can manifest the SERS effect. Given their simplicity, efficiency and flexibility, the gas-phase-deposited peptide nanostructures may provide a new way to create advanced functional materials.

## 2. Experimental section

### 2.1. Deposition of dipeptides

In our work, we used diphenylalanine (Phe–Phe) dipeptides, which have no protecting group or any end functionalization, as a building block. In a typical experiment, the lyophilized



**Scheme 1.** Schematic illustration of gas phase deposition of dipeptides and their applications.

Phe–Phe powder (1–20 mg) was placed onto a tungsten boat and evaporated at about 250 °C under high vacuum ( $10^{-6}$  Torr) for varying deposition times (1–10 min) on different substrates (i.e. glass, silicon wafer). The distance between the peptide source and substrate was also altered, ranging from 1 to 5 cm. Directional peptide nanofilms were also deposited onto the surface by applying the same procedure. However, in this case, the solid substrates were oriented obliquely at varying deposition angles (i.e.  $\alpha = 20^\circ$  and  $10^\circ$ ) relative to the peptide source. As-deposited dipeptides were characterized by a JEOL JSM-6060 scanning electron microscope (SEM) with an acceleration voltage of 5 kV. The SEM images were then analyzed with the freeware IMAGEJ image analysis software.

## 2.2. Wetting properties

To improve wetting properties, isotropic and anisotropic peptide structures were first modified using 500  $\mu\text{l}$  of 1H,1H,2H,2H-perfluorooctyltriethoxysilane (PFS, Aldrich, Germany) at 180 °C for 2 h in a vacuum oven. The static contact angle measurements were done at 25 °C in ambient air using an automatic contact angle goniometer equipped with a flash camera (DSA 100 Krüss, Germany) applying a sessile drop method. The contact angles were calculated by using the software of the instrument. Note that the values reported here are the averages of at least three measurements taken at three different locations on each sample surface. Deionized water and olive oil were used for contact angle measurements.

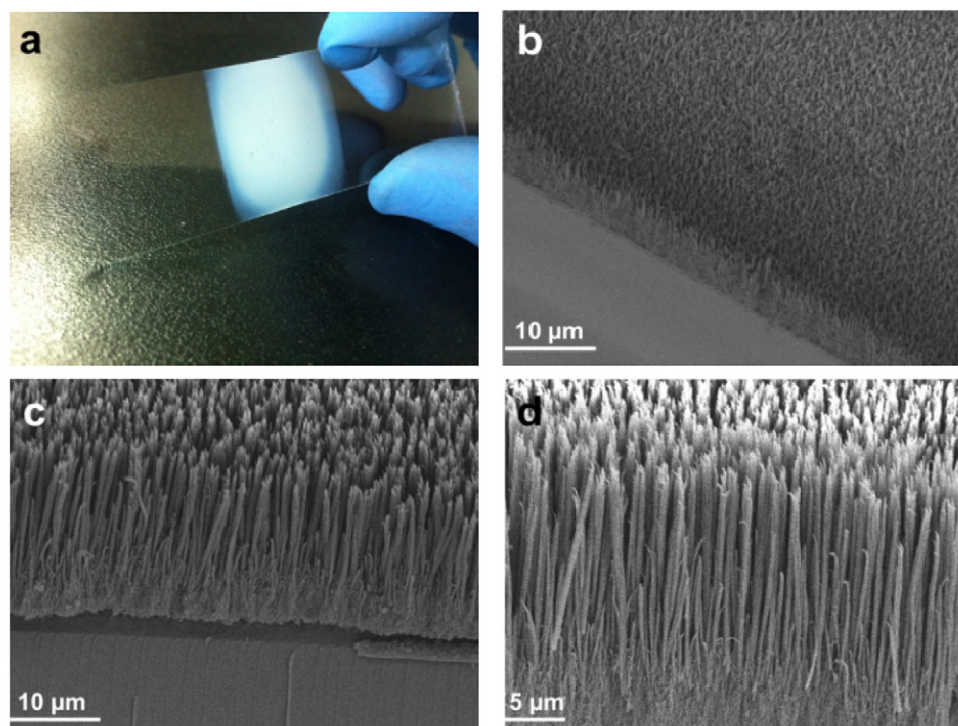
## 2.3. SERS experiments

The SERS platforms were fabricated using vertically aligned peptide films as a template. A thin layer of gold was then

thermally deposited from a heated tungsten boat onto the peptide films at about  $10^{-6}$  Torr base pressure in a deposition chamber. The thickness of gold was monitored *in situ* by a quartz crystal microbalance (QCM). The gold-coated peptide films were then held above the liquid in a sealed 1 ml vial containing 0.5 ml of 4-fluorobenzenethiol (FBT, Aldrich, Germany). The vial was held at 90 °C for 2 h in a vacuum oven, exposing the monolayer to the vapor phase. A Delta Nu Examiner Raman microscopy system was used for studying SERS platforms. The system consisted of a 785 nm laser source, a motorized microscope stage sample holder and a charge-coupled device (CCD) detector. Instrument parameters were as follows: 20 $\times$  objective, 30  $\mu\text{m}$  spot size, 10 s acquisition time and baseline correction was performed for all measurements.

## 3. Results and discussion

We first fabricated vertically aligned dipeptide-based nanostructures using Phe–Phe as a starting material in a conventional physical vapor deposition system (NANOVAK HV, Ankara, Turkey) which allows us to deposit peptide nanofibers on a large surface area (figure 1(a)). Figures 1(b)–(d) show the top and cross-section SEM images of the Phe–Phe nanostructures deposited on the glass surfaces. The SEM images clearly revealed that peptide nanostructures formed well-aligned, high aspect ratio and closely packed nanofibers with a density of  $6.2 \times 10^7$  nanofibers  $\text{cm}^{-2}$ . The average lengths of nanofibers were also found to be  $\sim 20$   $\mu\text{m}$ . Their diameters, however, showed a broad distribution, ranging from 50 to 600 nm. Notice that their lengths and diameters may be easily controlled by manipulation of the deposition



**Figure 1.** A photograph of vertically aligned peptide film on a glass surface (a), top (b) and side view (c)–(d) SEM images of gas phase growth peptide nanostructures.

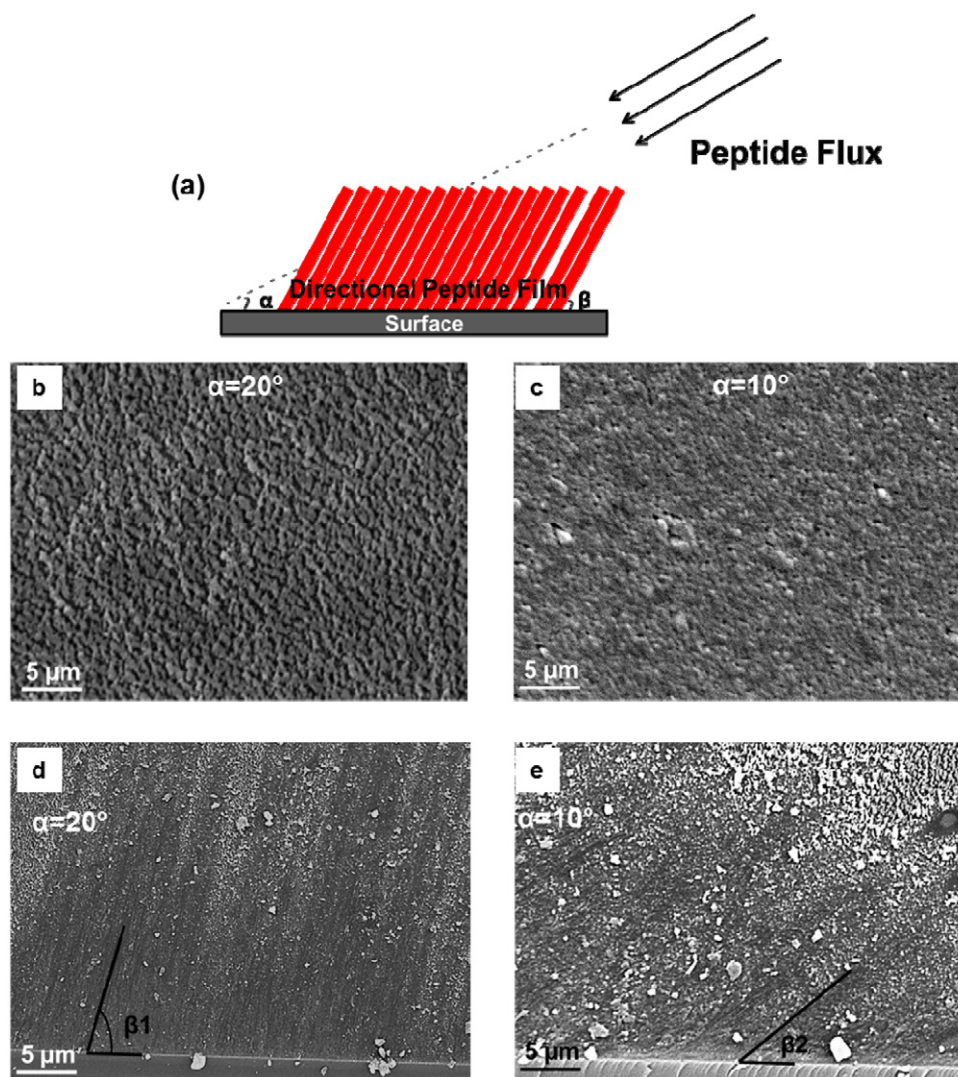
parameters (i.e. deposition time and rate, distance between the substrate and source, and amount of precursor material). Meanwhile, we did not observe any tubular Phe–Phe structure as reported previously [13, 14]. Abramovich *et al* reported that the morphology of deposited Phe–Phe in the vacuum phase was mainly hollow tubes, similar to the morphology of peptide nanotubes that self-assemble in the solution phase [13]. In another work, Park and co-workers fabricated peptide nanostructures through a vapor-transport process [14] and they observed only well-faced single-crystalline peptide nanowires. These apparent diversities in morphology may arise from a number of factors. It is known that the interactions between the surface and peptides, peptide protecting groups (i.e. Boc, Fmoc) and temperature play an important role during the self-assembly process in the solution phase. Although factors such as temperature, differences in protecting group of peptides and surface pre-treatment have been demonstrated to affect the structures observed, the self-assembly process and alignment of the structure in the vacuum phase still remains a mystery. Thus the mechanism of these processes requires further investigation and it would be interesting to use temperature, surface hydrophobicity via different surface moieties and the protecting group of the peptide as tuning agents to form different peptide structures.

In addition to the fabrication of vertically aligned peptide structures, we have tried to fabricate directional peptide films based on the oblique angle deposition (OAD) technique. Similar to isotropic peptide films, lyophilized Phe–Phe powder ( $\sim 10$  mg) was first placed onto a boat and evaporated at different deposition angles of  $\alpha = 20^\circ$  and  $10^\circ$  (figure 2(a)). Due to the surface diffusion and self-shadowing growth

mechanism, the OAD deposition results in tilted peptide films on the surface. The morphologies of as-deposited samples were shown in figures 2(b)–(e). The directional nanostructured films are inclined an assemble at an angle  $\beta_1 = 74^\circ$  for  $\alpha = 20^\circ$  and  $\beta_2 = 37^\circ$  for  $\alpha = 10^\circ$ , respectively (figures 2(d) and (e)). As expected, the deposited film becomes more porous with the increase of deposition angle (figures 2(b) and (c)). Although the deposited peptides formed unidirectional films, we could not observe any distinct fiber-like structure similar to fabricated vertically aligned peptides. This obvious contradiction in morphology is possibly due to the mechanism of OAD. At the beginning of the deposition, peptide molecules diffuse onto the surface and formed a multilayer film. Beyond a critical layer thickness which depends on the strain and chemical potential of the peptide film, growth continues through some nucleation sites. Afterwards, the shadowing effect will play a greater role in the peptide film growth. We found that well-distributed nucleation sites were formed at the beginning of the deposition process (SI, figure S1 available at [stacks.iop.org/Nano/23/225604/mmedia](http://stacks.iop.org/Nano/23/225604/mmedia)). However, during the growing process, peptide molecules formed much more closely packed and thin fibrillar structures, possibly due to the self-shadowing mechanism and their high affinity to each other.

Wetting properties of solid surfaces have recently attracted increasing interest due to their scientific significance and potential applications including self-cleaning surfaces, prevention of snow adhesion, anticorrosion and antifouling [15–17]. Thus, we further investigated the hydrophobic and oleophobic behaviors of peptide nanofibers grown by PVD. The contact angle with water and oil for as-deposited





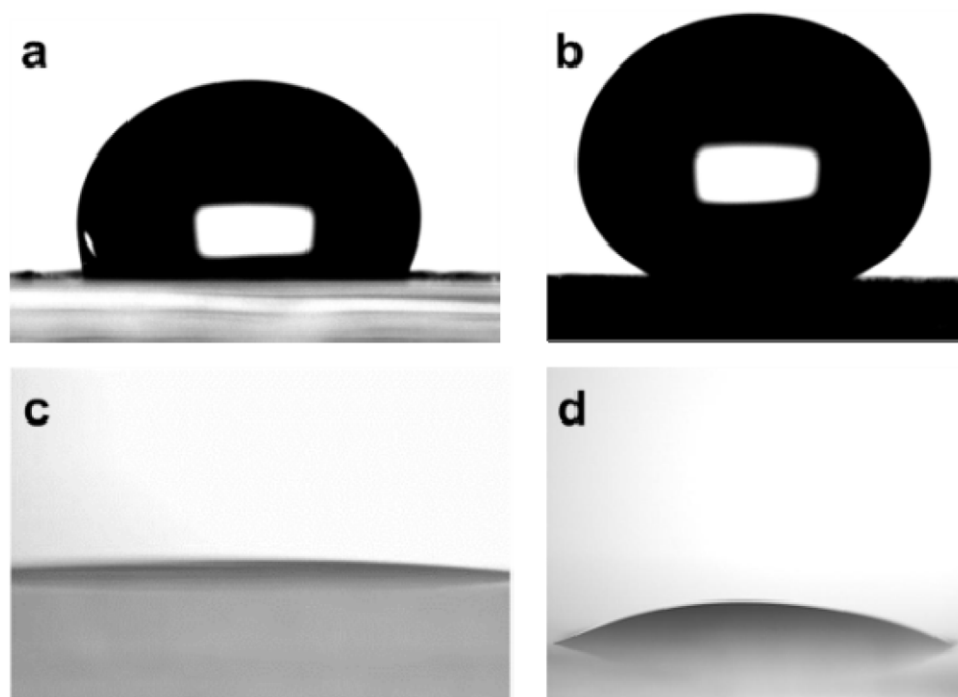
**Figure 2.** Schematic representation of fabrication process for directional films and SEM images of deposited structures: Top (b)–(c) and side view (d)–(e) of directional peptide structures.

structures without any treatment was observed as  $98^\circ \pm 5^\circ$  and  $5^\circ \pm 3^\circ$ , respectively (figures 3(a) and (c)). In order to improve their wetting properties, they were then modified by the vapor of PFS, which is a low surface free energy compound, at  $180^\circ\text{C}$  for 2 h in a vacuum oven. After modification, both the water and oil contact angles increase to  $158^\circ \pm 4^\circ$  and  $19^\circ \pm 3^\circ$  (figures 3(b) and (d)), whereas the water contact angle of the PFS-modified bare silicon surface was  $107^\circ \pm 6^\circ$  (SI, figure S2(a) available at [stacks.iop.org/Nano/23/225604/mmedia](http://stacks.iop.org/Nano/23/225604/mmedia)). The results showed that the wetting behavior of the deposited peptide film originated from the increased surface roughness, caused upon the formation of nanofibers. These nanofibers allow the air to be trapped beneath liquid droplets and decrease the surface free energy via the incorporation of fluorosilanes [9]. The sliding angle on the vertically aligned peptide-modified surface was also found to be  $\sim 3^\circ$ , which means water droplets could roll off easily on these surfaces (see the movie in SI available at [stacks.iop.org/Nano/23/225604/mmedia](http://stacks.iop.org/Nano/23/225604/mmedia)). With respect to the stability of deposited peptide films for prolonged times, there was no change in

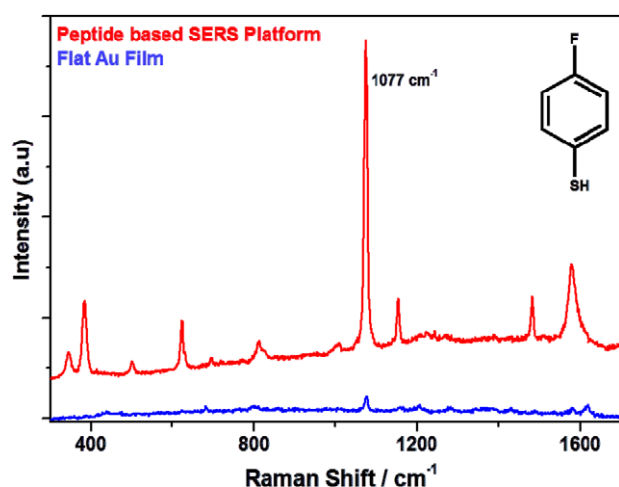
its wetting property nor in morphology even after a week of incubation.

In the case of directional peptide films, the water contact angles were also found as  $116^\circ \pm 4^\circ$  for  $\alpha = 10^\circ$  and  $127^\circ \pm 6^\circ$  for  $\alpha = 20^\circ$ , respectively (SI, figures S2(b)–(c) available at [stacks.iop.org/Nano/23/225604/mmedia](http://stacks.iop.org/Nano/23/225604/mmedia)). It is clear that isotropic films exhibited better hydrophobicity compared to oblique films. These apparent diversities may arise from the surface roughness and wire-to-wire distance which allows the air to be trapped. The SEM images clearly revealed that, for isotropic films, surface roughness and gaps between fibers are bigger than in the oblique films. In addition to these, we investigated the unidirectional wetting using our oblique films. However, we could not observe any anisotropic wetting behavior, possibly due to the dense fiber morphology in oblique films.

One of the main aims of the present study is to fabricate SERS platforms that can enhance the Raman signal of the molecules adsorbed on them. We thought that deposited peptide structures based on PVD may provide significant



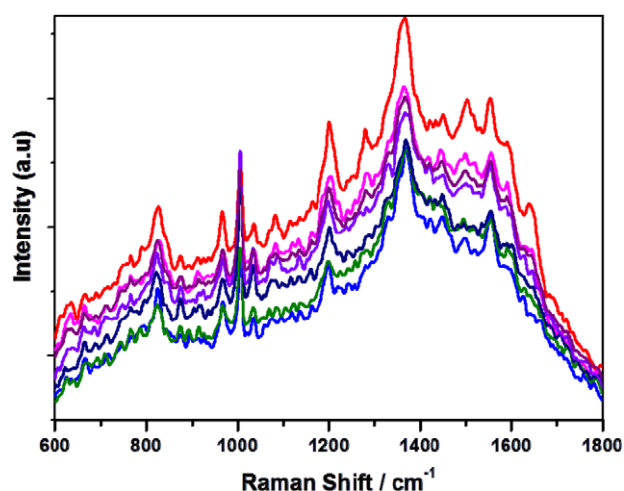
**Figure 3.** Photographs of droplet applied onto the deposited peptide films before and after PFS modification. The CAs with water are  $98^\circ \pm 5^\circ$  (a), and  $158^\circ \pm 4^\circ$  (b); the CAs with oil are  $5^\circ \pm 3^\circ$  (c) and  $19^\circ \pm 3^\circ$  (d).



**Figure 4.** SERS spectra of FBT on Au-coated peptide nanofibers and Au flat surfaces.

advantages over traditional SERS surfaces due to their high surface roughness, which may easily be controlled depending on the deposition conditions. Besides, gas phase deposition of peptides does not require any template or lithographic approaches, thus making it a simple yet versatile method to fabricate a new generation of nanostructured SERS platforms. To obtain the SERS signal, we first coated our deposited peptide films with gold ( $\sim 33$  nm) through PVD (SI, figure S3 available at [stacks.iop.org/Nano/23/225604/mmedia](http://stacks.iop.org/Nano/23/225604/mmedia)). 4-fluorobenzenethiol (FBT) molecules were then adsorbed onto the gold-coated films in the gas phase. Figure 4 shows the SERS spectra of FBT on a smooth gold surface

and gold-coated peptide film under a 785 nm laser as an excitation source. The spectra are consistent with most of the reports for FBT. It is also found that the Raman signal is significantly enhanced on the gold-coated peptide film compared to the signal on a smooth surface. Note that both Au surfaces have the same film thickness. Meanwhile, we have not observed any background signal from peptide nanostructures interfering with the FBT spectra. The surface enhancement factor (EF) of the modifying molecules for the gold-coated peptide nanostructures have also been calculated using C–F stretch mode intensity ( $\sim 1077$   $\text{cm}^{-1}$ ) as reported in the literature [18]. The resultant EF was found as  $3.7 \times 10^5$ . It is well known that SERS enhancement is generally originated by a long-range electromagnetic effect and short-range chemical effect. The nanoscale roughness, which was provided by our vertically aligned peptide nanowires, stimulates the excitations of localized surface plasmon resonance with enhanced local electromagnetic fields. In addition to electromagnetic enhancement, chemical interactions between the underlying gold-coated peptide film and molecules, which were detected, contributes to SERS intensity enhancement due to the coupling of their electronic polarizabilities. In the literature, numerous works have been done to enhance SERS intensity based on different techniques. The Li group reported that gold-coated dipeptide-based flower-like mesocrystals provided  $6.6 \times 10^4$  SERS enhancement [19]. Demirel and co-workers also observed a  $\sim 1 \times 10^6$  enhancement factor when they used anisotropic poly(p-xylylene) films [20]. The Whitesides group fabricated gold nanotubes based on shadow evaporation and found that their substrate gave an enhancement factor of  $\sim 5 \times 10^5$  [21]. We believe that our vertically aligned peptide



**Figure 5.** Reproducible SERS spectra for Rhodamine B molecules on peptide-based SERS platforms.

nanofilms may serve as reliable and effective SERS substrates for biosensor applications.

SERS has remarkable selectivity and analytical sensitivity but the difficulty in easy preparation of robust and uniform SERS substrates with surface morphologies, which can deliver maximum SERS enhancement with high reproducibility, is a major problem [20]. Thus, we tested the reproducibility of SERS spectra that were obtained from gold-coated peptide nanostructures using Rhodamine B as a model molecule. The data show highly reproducible spectra across a surface with a little deviation for Rhodamine B spectra with seven samples (figure 5). Reproducibility was also maintained from surface to surface with the control of preparation (data not shown). These results clearly revealed that peptide-based films may provide a simple and quick fabrication method to achieve highly sensitive and uniform SERS substrates.

#### 4. Conclusions

In summary, we successfully fabricated peptide-based nanostructures through PVD and OAD. The superhydrophobic surface with a very low sliding angle was obtained from modifying the vertically aligned peptide nanofibers by a fluorosilane molecule. These structures may provide a simple and flexible way to create new generations of bio-inspired superhydrophobic surfaces. By combining a thin layer of

gold film with deposited peptide structure, highly sensitive and reproducible SERS platforms were also obtained. Future works will focus on investigating possible usage of peptide films, both isotropic and anisotropic, in digital microfluidic platforms and catalysis applications.

#### Acknowledgments

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