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Electronic Supplementary Information (ESI)

Recognition of Diamond with Phage Displayed Peptides

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Reagents and Materials

SiO_2/Si wafers were purchased from University Wafer, Inc (Boston, MA). The diamond wafer (1.0 μm thick on SiO_2/Si) was purchased from Advanced Diamond Technologies, Inc (Romeoville, IL). The bacteriophage Ph.D.-12 library was purchased from New England Biolabs, Inc. (Lpswich, MA). Luria-Bertani's broth (LB), polyethylene glycol (PEG, weight-averaged molecular mass Mw = 8000 g/mol), sodium bicarbonate ($NaHCO_3$), agarose, sodium chloride (NaCl), sodium iodide, Tween-20, isopropyl α D-thiogalactoside (IPTG), agar, biotin-conjugated M13 phage antibody, hydrochloric acid (HCl), and glycine were purchased from Fisher Scientific (Pittsburg, PA). Bovine serum albumin (BSA), Tris(hydroxymethyl)aminomethane (Tris), and avidin-FITC were from Sigma-Aldrich (St Louis, MO). Ethylenediaminetetraacetic acid (EDTA) was obtained from MP Biomedicals (Solon, OH). Sodium chloride was purchased from Avantor Performance Materials. Inc (Phillipsburg, NJ). Ethanol was purchased from Pharmco-Aaper (Brookfield, CT). 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal) was purchased from American Bionanlytical (Natick, MA). Transmission electron microscope grid, 600 square mesh (Ni) was from Ted Pella Inc (Redding, CA). TEM grid, 400 lines mesh (Cu), was from Electron Microscopy sciences (Hatfield, PA). Deionized water (18.2 MΩ-cm) was obtained from a Nanopore Diamond filtration system (Barnstead, NH).

Phage Display Selection

A volume of 10 μl of phage display library (New England BioLabs, Ph.D. 12, 10^{13} pfu ml^{-1}) in 1.5 ml Tris-HCl (50 mM, pH 7.5) and NaCl (150 mM) buffer solution (TBS) with 0.1% tween-20, was incubated with diamond on SiO_2/Si chip (1cm x 1cm) for 1 h in a 35 mm petri dish, then

eluted from the substrate with 1.5 ml glycine-HCl, 1% BSA (0.2 M, pH 2.2) and neutralized with 1 M Tris-HCl buffer at pH 9.1. The eluted phages were amplified in 20 ml of a 1:100 dilution of log phase *Escherichia coli* (ER 2738)[1] grown in sterile LB media at 37 °C for 4.5 h. Bacteria was pelleted via centrifugation and the supernatant was retained. Phage particles were precipitated from the supernatant through the addition of 1/5 of the volume 20% (w/v) PEG-8000, 2.5 M NaCl. After centrifugation at 14000 rpm for 15 min, the phage pellet was resuspended in 1 ml TBS. This purification and precipitation processes were repeated and finally the phage molecules were suspended in 0.2 ml TBS. The eluted phages were incubated with a SiO_2/Si substrate for 1 hour, to screen out the phages which bind to the SiO_2/Si substrate. The entire "biopanning" process was repeated a total of three times, using TBST buffer with tween-20 ranging from 0.1%, 0.3% to 0.5% in every rotation of selection. After the third round of panning, the refined libraries were prepared for DNA sequencing to identify the peptides specific to diamond.

DNA Purification and Sequencing

Following the third round of panning, *E.coli* was mixed with different dilutions of eluted phages, and melt agarose in LB medium. After this, the agarose containing phages and *E.coli* was poured onto the agar plates containing 40 mg ml^{-1} X-gal, 50 mg ml^{-1} IPTG. Individual blue plaques were selected and separately amplified in 10 µl of overnight *E.coli* in 1 ml of sterile LB culture medium at 37 °C for 4.5 h. After amplification, phage particles were selectively precipitated as described above and the single stranded viral DNA was obtained using the manufacturer's instruction. This was accomplished by the addition of a mixture of 100 µl of iodide buffer (10 mM Tris-HCl, 1 mM EDTA, 4 M NaI) and 250 µl of 100% ethanol, which selectively precipitated single-stranded phage DNA, followed by washing with 70 % ethanol and centrifugation at 14000 rpm for 10 min. DNA was resuspended in TE buffer (10 mM Tris-HCl,

1 mM EDTA). The sequencing of the DNA was performed by Eton Bioscience, Inc. (San Diego, CA).

Hydrophobic/Hydrophilic Analysis of Diamond-Binding Peptides

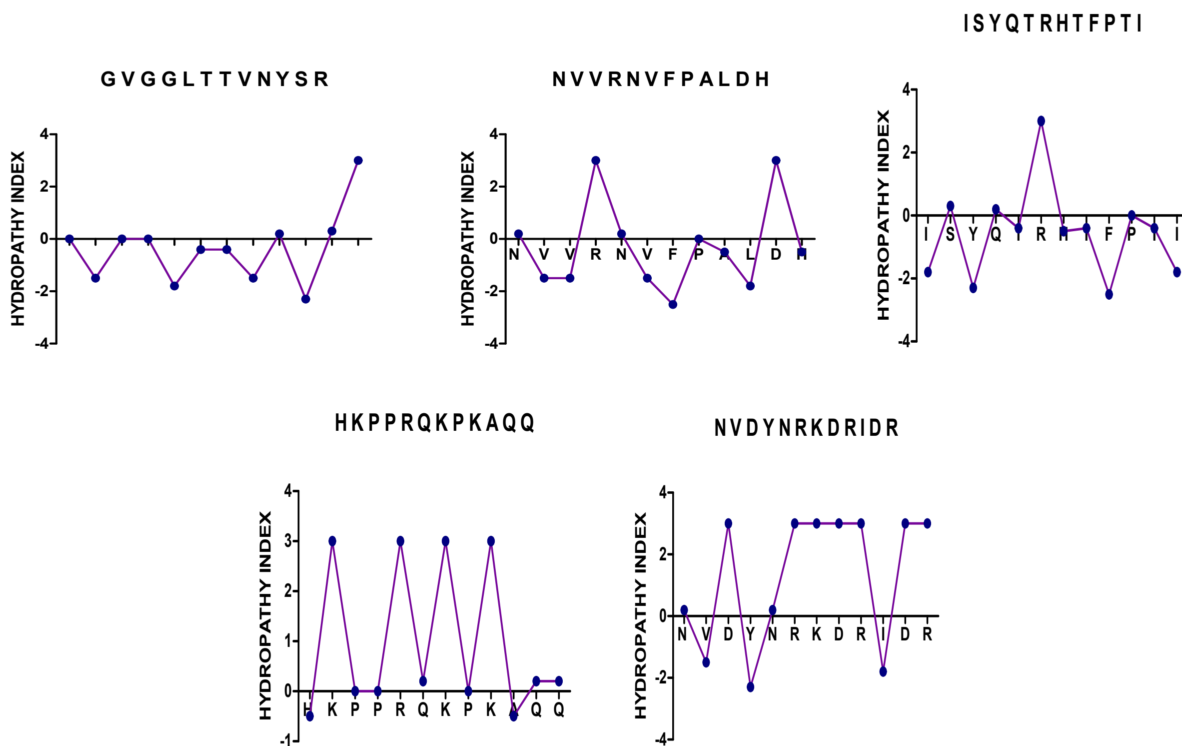

Fig. S1 Hydropathy indices of diamond binding peptides, determined based on the Hopp-Woods scale.

Fig. S1 shows the hydropathy indices of different diamond binding peptides obtained from three rounds of biopanning. A mixed hydropathy response is obtained from the analysis of the diamond binding phage displayed peptides. The amino acid residues of the peptides show different properties of hydrophilicity and hydrophobicity, indicating the complexity of the nature of peptide recognition on diamond surface.

Generation of Diamond Micropatterns

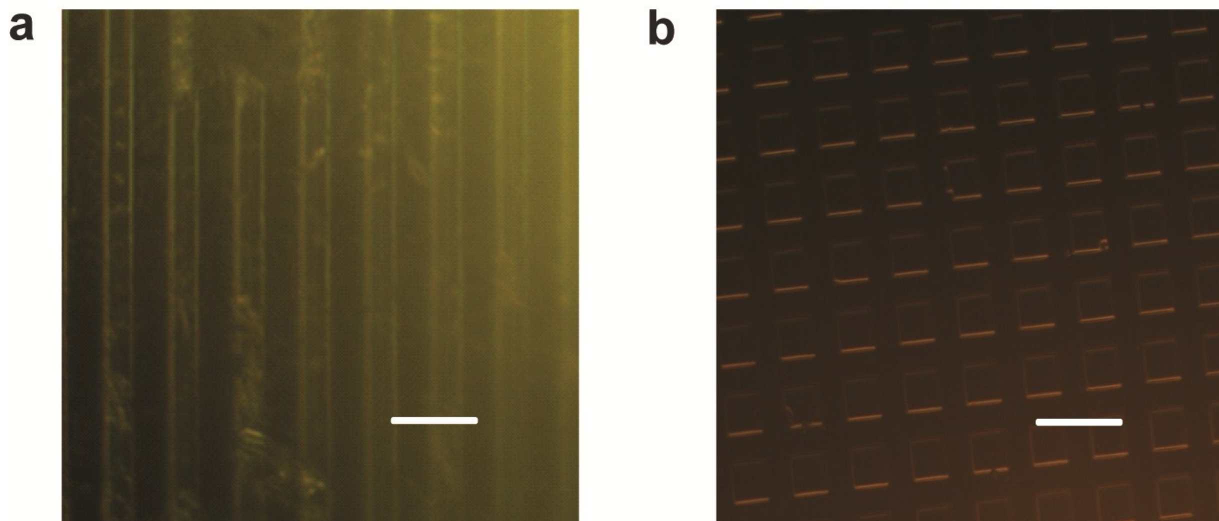

Fig. S2 Optical images of diamond micropatterns on SiO_2/Si surface, after etching by oxygen plasma with **a.** 400 mesh lines/bar TEM grid, and **b.** 600 mesh square TEM grid. All scale bars: 40 μm.

Different types of TEM grids were used as shadow masks for the micropattering of diamond. The TEM grids (400 mesh line, and 600 mesh square) were covered on the surface of diamond (1 μm thickness of diamond on SiO_2/Si substrate), followed by plasma etching at 100 W for one hour with a plasma etcher (PlasmaEtch, Carson city, Nv, USA). Through etching, the diamond exposed to plasma was removed, and the diamond covered with TEM grids were protected from etching. After removing the TEM grids on the diamond chips, diamond patterns can be revealed. As shown in Figure S2, different diamond patterns can be formed with different TEM grids after plasma etching, which were characterized with a bright field microscope.

Fluorescent Characterization with Diamond-Binding Phage Displayed Peptides

Fluorescent characterization with diamond-binding phage displayed peptides was conducted on diamond surface (Fig. 2a left image), SiO_2/Si surface (Fig. 2a right image), and diamond micropatterns (Fig. 3). The diamond surface was exposed to 10 µl of diamond binding phage displayed peptides (GVGGLTTVNYSR) in TBS buffer for 0.5 h, followed by washing with TBS buffer, and incubated with 0.1 M $NaHCO_3$, 1% BSA, at pH 8.6 for 0.5 h in order to reduce the non-specific adsorptions of antibody and FITC on the substrate. Then, the surface was exposed to biotin conjugated anti-M13 phage antibody[2] (1 µg in 1.5 ml buffer) for 0.5 h with gently shaking, and then rinsed with TBS to remove the unconjugated antibody. Finally, 10 µl of avidin–FITC (2.0 unit ml^{-1}) was applied to the biotin conjugated phage through a biotin–avidin interaction, and the surface was exposed to the FITC label for 0.5 h, and then rinsed several times with TBS to remove the unconjugated avidin–FITC label. The color intensity of the surface was characterized with a fluorescence microscope. The same procedure was applied for the fluorescent characterization of diamond-binding peptides for SiO_2/Si substrate and diamond micropatterns.

Fluorescent Characterization with M13 Phage

Fluorescent characterization with M13 phage (without displayed peptides) was conducted on diamond surface, SiO_2/Si surface (Figure 2b). The diamond surface and SiO_2/Si substrate was exposed to 10 µl of the M13 phage without displayed peptides in TBS buffer for 0.5 h, followed by washing with TBS buffer, and incubated with 0.1 M $NaHCO_3$, 1% BSA, at pH 8.6 for 0.5 h in order to reduce the non-specific adsorptions of antibody and FITC on the substrate. Then, the surface was exposed to biotin conjugated anti-M13 phage antibody (1 µg in 1.5 mL) for 0.5 h with gently shaking, and then rinsed with TBS to remove the unconjugated antibody. Finally, 10 µl of avidin–FITC label (2.0 unit ml^{-1}) was applied to the biotin conjugated phage through a

biotin–avidin interaction, and the surface was exposed to the FITC label for 0.5 h, and then rinsed several times with TBS to remove the unconjugated avidin–FITC label. The color intensity of the surface was characterized by a fluorescence microscope.

References

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