Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Evaluation of High Sensitivity and Selectivity of M13 Bacteriophage-based SPR Sensor using Quantum Mechanics Calculation

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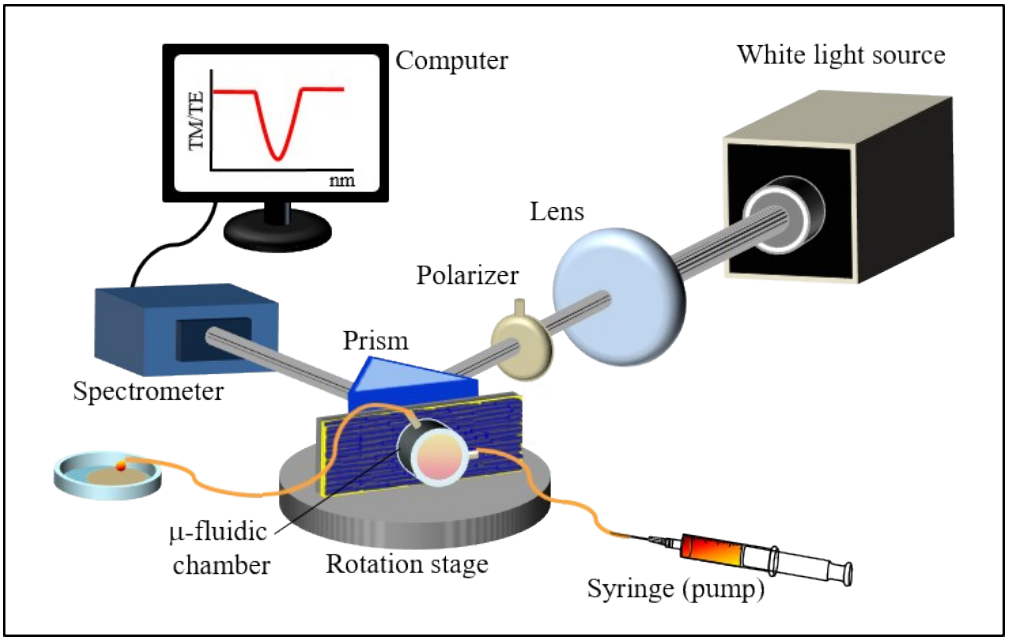

Figure S1 Schematic illustration of experimental set-up for WHW-phage-based SPR sensor.

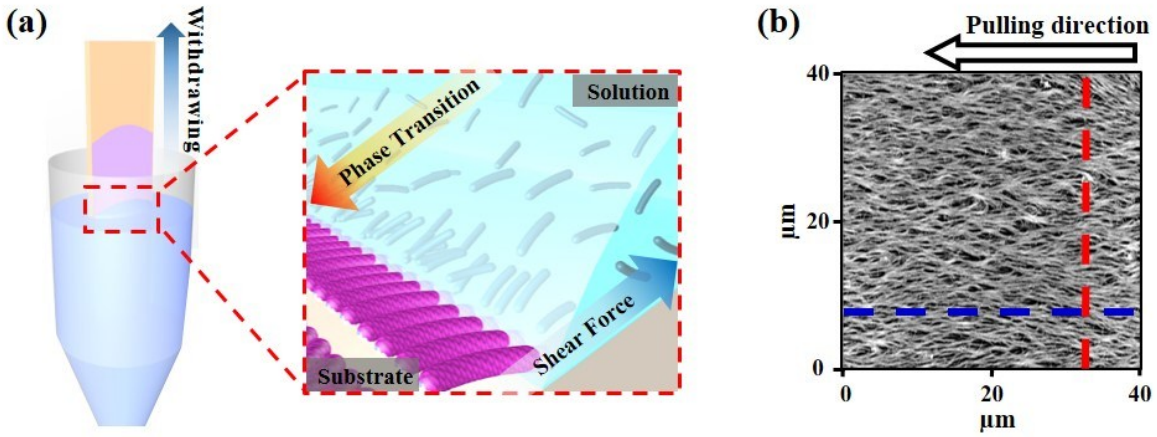

Figure S2 (a) Schematic illustration of self-templating process and (b) the AFM analysis well demonstrate the unidirectionally aligned WHW-phage matrices.

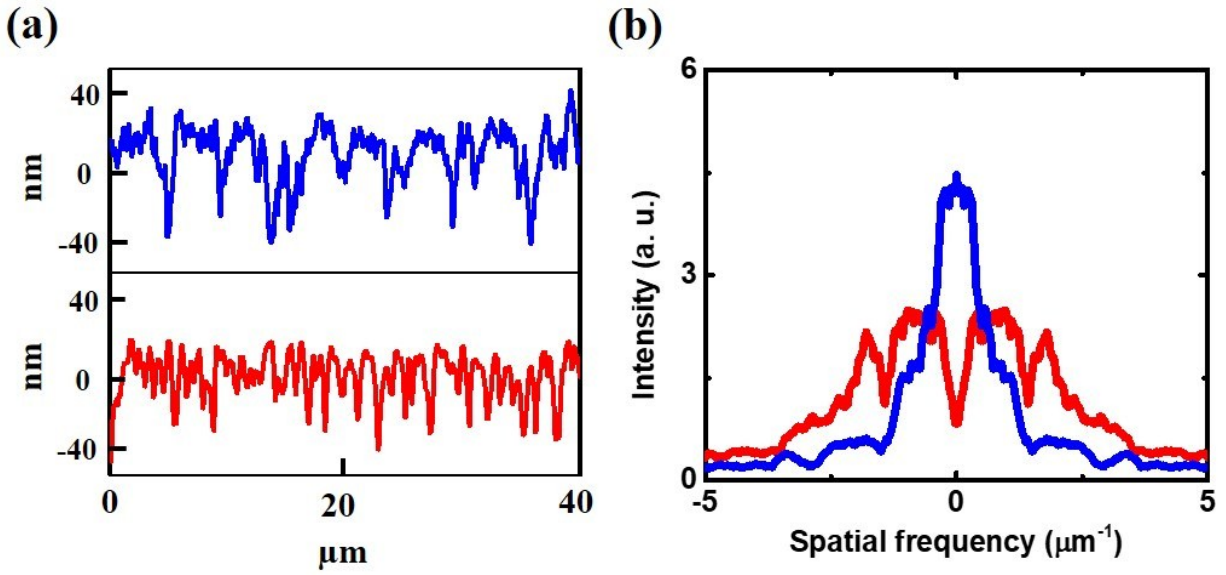

Figure S3 (a) The comparison of height profile of the AFM image in the direction that parallel with and orthogonal to the withdrawing direction shows more clear evidence. The height profile along with the line that orthogonal to the withdrawing direction shows fluctuating shape, whereas the other line shows relatively smooth height profile. (b) A fast fourier transform (FFT) analysis was performed for quantitative demonstration of unidirectionally aligned WHW-phage matrices. A FFT power spectrum of the line that orthogonal to the withdrawing direction shows broad distribution of spatial frequency compared with the other line; which means WHW-phage matrices are composed of the bundle structure that aligned along with withdrawing direction.

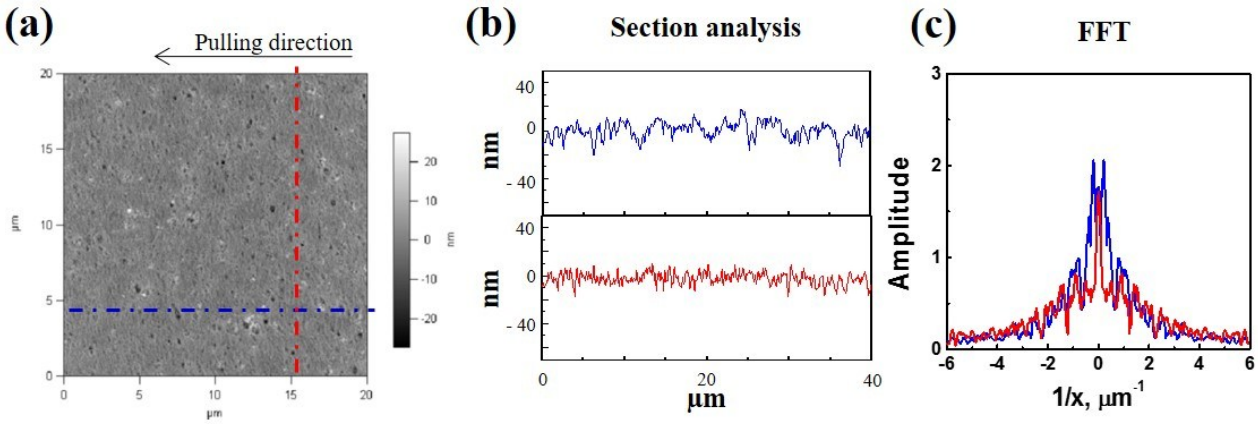

Figure S4 (a) AFM image of self-assembled nanostructure consisted of wild-type phage. (b)The height profile analysis and (c)fast fourier transform analysis reveals planar structure due to the lack of self-assembly time.

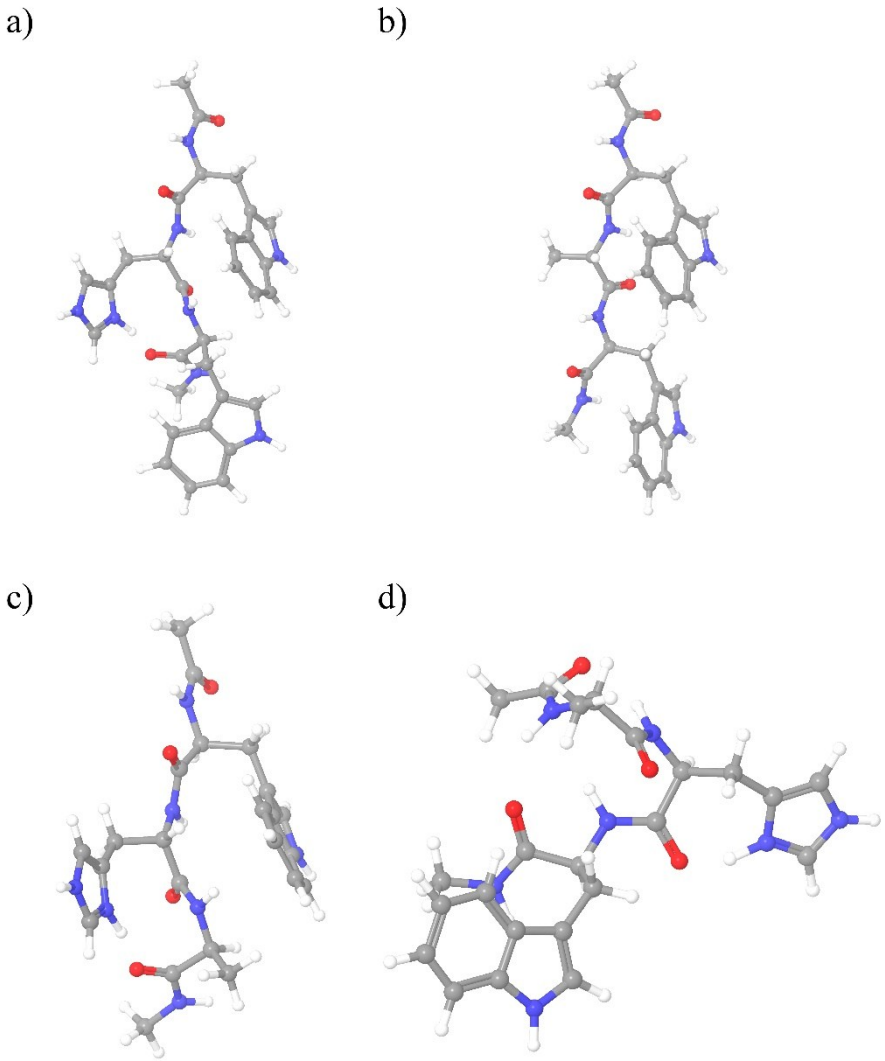

Figure S5 The most stable conformations of free, unbound peptides (a) WHW, (b) WAW, (c)WHA and (d) AHW.

Table S1. Energy and absolute energy of the WHW peptide.

	protonated[a]		protonated Me[b]		protonated acetylated[c]	
Conformers	ΔE (kcal/mol) [d]	energy (hartree) [e]	ΔE (kcal/mol) [d]	energy (hartree) [e]	ΔE (kcal/mol) [d]	energy (hartree) [e]
Extended	0.0	-1787.8723	0.0	-1827.1588	0.0	-1940.4818
Alpha helix	5.7	-1787.8639	6.2	-1827.1496	2.5	-1940.4780
3(10) helix	5.7	-1787.8639	6.4	-1827.1494	1.8	-1940.4791
Collagen helix	0.0	-1787.8723	0.0	-1827.1587	0.1	-1940.4817
Left handed a helix	2.5	-1787.8687	2.3	-1827.1554	3.6	-1940.4764
Phi helix	8.6	-1787.8596	9.7	-1827.1443	6.6	-1940.4720
Beta sheet	5.8	-1787.8638	3.1	-1827.1543	4.8	-1940.4747
Parallel b sheet	13.3	-1787.8526	13.4	-1827.1388	7.9	-1940.4701

[a] N terminal was represented by NH_2 and His was pronated. [b] N terminal was represented by $NHCH_3$ and His was pronated. [c] N terminal was acetylated and His was pronated. [d] Energy of the conformation relative to the most stable conformation (in kcal/mol) [e] Absolute energy of the conformation from M06-2X/6-31G**

Table S2. Energy and absolute energy of the WAW peptide.

	protonated[a]		protonated Me[b]		protonated acetylated[c]	
Conformers	ΔE (kcal/mol) [d]	energy (hartree) [e]	ΔE (kcal/mol) [d]	energy (hartree) [e]	ΔE (kcal/mol) [d]	energy (hartree) [e]
Extended	2.0	-1562.5010	2.0	-1601.7871	0.0	-1715.1131
Alpha helix	5.5	-1562.4958	5.6	-1601.7817	3.0	-1715.1086
3(10) helix	5.4	-1562.4959	5.5	-1601.7819	3.2	-1715.108348
Collagen helix	2.0	-1562.5010	1.9	-1601.7871	0.2	-1715.112771
Left handed a helix	0.0	-1562.5040	0.0	-1601.7900	0.6	-1715.1122
Phi helix	3.8	-1562.4983	4.4	-1601.7834	1.7	-1715.110561
Beta sheet	5.1	-1562.4965	5.1	-1601.7824	3.6	-1715.107672
Parallel b sheet	3.6	-1562.4986	1.3	-1601.7880	5.1	-1715.1055

[a] N terminal was represented by NH_2 and His was pronated. [b] N terminal was represented by $NHCH_3$ and His was pronated. [c] N terminal was acetylated and His was pronated. [d] Energy of the conformation relative to the most stable conformation (in kcal/mol) [e] Absolute energy of the conformation from M06-2X/6-31G**

Table S3. Energy and absolute energy of the WHA peptide.

	protonated[a]		protonated Me[b]		protonated acetylated[c]	
Conformers	ΔE (kcal/mol) [d]	energy (hartree) [e]	ΔE (kcal/mol) [d]	energy (hartree) [e]	ΔE (kcal/mol) [d]	energy (hartree) [e]
Extended	3.2	-1425.3707	0.0	-1464.6620	0.0	-1577.9849
Alpha helix	4.9	-1425.3681	5.5	-1464.6539	1.6	-1577.9826
3(10) helix	5.1	-1425.3679	5.6	-1464.6537	1.9	-1577.9821
Collagen helix	0.0	-1425.3755	0.0	-1464.6620	0.0	-1577.9849
Left handed a helix	3.7	-1425.3699	3.7	-1464.6565	2.2	-1577.9817
Phi helix	8.1	-1425.3635	9.3	-1464.6483	6.5	-1577.97535
Beta sheet	3.1	-1425.3709	1.9	-1464.6593	0.0	-1577.9850
Parallel b sheet	10.7	-1425.3596	10.9	-1464.6458	5.3	-1577.9770

[a] N terminal was represented by NH_2 and His was pronated. [b] N terminal was represented by $NHCH_3$ and His was pronated. [c] N terminal was acetylated and His was pronated. [d] Energy of the conformation relative to the most stable conformation (in kcal/mol) [e] Absolute energy of the conformation from M06-2X/6-31G**

Table S4. Energy and absolute energy of the AHW peptide.

	protonated[a]		protonated Me[b]		protonated acetylated[c]	
Conformers	ΔE (kcal/mol) [d]	energy (hartree) [e]	ΔE (kcal/mol) [d]	energy (hartree) [e]	ΔE (kcal/mol) [d]	energy (hartree) [e]
Extended	6.3	-1425.3658	8.9	-1464.6482	8.8	-1577.9752
Alpha helix	3.1	-1425.3705	4.2	-1464.6551	4.5	-1577.9816
3(10) helix	3.1	-1425.3704	4.2	-1464.6551	4.6	-1577.9815
Collagen helix	5.6	-1425.3668	5.5	-1464.6533	8.8	-1577.9752
Left handed a helix	0.0	-1425.3751	0.0	-1464.6615	0.0	-1577.9883
Phi helix	3.1	-1425.3705	4.2	-1464.6552	4.5	-1577.9816
Beta sheet	5.5	-1425.3669	5.5	-1464.6534	8.8	-1577.9752
Parallel b sheet	4.4	-1425.3685	4.4	-1464.6550	5.5	-1577.9801

[a] N terminal was represented by NH_2 and His was pronated. [b] N terminal was represented by $NHCH_3$ and His was pronated. [c] N terminal was acetylated and His was pronated. [d] Energy of the conformation relative to the most stable conformation (in kcal/mol) [e] Absolute energy of the conformation from M06-2X/6-31G**

Experimental section

1. Genetic Engineering of the M13 Bacteriophage

In order to develop major coat protein engineered phage for 2,4,6-trinitrotoluene (TNT) explosives, we previously identified the TNT binding peptide using phage display with a commercially available 12mer linear peptide library (Ph.D.™-12, New England Biolab, Ipswich, MA). We identified the consensus TNT binding peptide (Trp-His-Trp: WHW) and confirmed it's specificity. We also incorporated the consensus TNT binding peptide (WHW) on the major coat proteins (pVIII) of the M13 phages. In order to compare the specificity of WHW engineered phage, we constructed alanine-substituted control phage (WAW, AHW, WHA) using site-directed mutagenesis of the WHW-phage. The constructed phages were amplified using bacterial cultures and purified through standard polyethylene glycol precipitation. The phage solution was further purified by filtration through 0.45 μm pore size membranes. To verify phage stability, DNA sequences were confirmed at each step of the amplification at the DNA Sequencing Facility at University of California, Berkeley (Berkeley, CA).

2. Phage Film Fabrication Using Self-templating Process

We constructed a phage deposited film using home-made phage film development appartus using a KD Scientific syringe pump motor (KD Scientific Inc, Holliston, MA). Before film deposition, the gold coated glass (Platypus Tech.) was treated with 3 mM cysteamine in DI water for 30 min to increase the adhesion of the phage film and then washed with DI water and ethanol. We fabricated a unidirectionally aligned WHW phage matrices on gold coated glass at a concentration of 3.0 mg/mL, with a significantly high withdrawing speed (300μm/min).

To ensure the stability of phage matrices during the sensing experiments, the phage films deposited on the substrates were crosslinked in a sealed chamber filled with glutaraldehyde vapor (3 days). Any remaining aldehydes were blocked with 2-aminoethanol solution (100 mM) for 30 min.

3. Atomic Force Microscopy (AFM) Analysis

The AFM images were collected using an MFP3D AFM (Asylum Research, Santa Barbara, CA) and analyzed using Igor software 6.0 (WaveMetrics, Inc. Lake Oswego, OR) and the Asylum software package (Asylum Research, Santa Barbara, CA). All AFM images were taken in air using contact mode AFM by utilizing the nanoprobe tips which were made of silicon and with ~8 nm of tip radius of curvature. (PPP-CONTSCR, NANOSENSORS, Neuchatel, Switzerland)

4. SPR Measurements

SPR analysis was performed using the Kretschmann optical configuration (Figure S1). Briefly, a tungsten halogen lamp with a multi-wavelength light source was used for illumination with a polarizer positioned on the input path of the light for transverse magnetic fields. The prism coupler and the phage film were mounted on an *x-y-z* stage. We made an enclosed cell of ~100 μl using PDMS molds using a simlar method reported previously (Langmuir, OJW 2012). Flow of solution into the cell was implemented using a 1 mm internal diameter tube. We injected 1 ml of solution into the cell at a flow rate of 50 μl/min. The outflow from the cell was carried through to a reservoir. The reflected spectrum was measured by a fiber optic spectrometer (USB4000-UV-Vis, Ocean Optics, Dunedin, FL), and data acquisition was performed using a homemade LabVIEW program (LabVIEW 2009, National Instrument,

Austin, TX). The SPR spectrum was calculated from linearly polarized light parallel/perpendicular to the incidence plane (TM/TE configuration).

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5. Quantum Mechanical Calculations

All QM calculations used the Jaguar v8.4 quantum chemistry software (Schrödinger, Inc., New York, NY, USA). To obtain the geometries and energies of the tripeptides and their complexes with TNT, we used the M06-2X/6-31G**39 level of density functional theory (DFT) calculations. Tripeptides, WHW and its alanine(A) analogues, WAW, WHA, and AHW were used to model the TNT binding properties. Various choices were used to simulated the N- and C-terminal of the tripeptides, and the following choice was found to be best to represent the conformations of the tripeptides: N-terminal of the peptides were acetylated and C-terminal of the peptides were terminated by N-methylamide. Imidazole ring of the His residue were protonated to reflect the pH condition. In order to generate several conformers of the peptides, initial values of Ramachandran angles (φ, and ψ) corresponding to the various secondary structures were chosen, and the resultant structures were optimized.