# SUPPORTING INFORMATION

## *Sub-nanomolar Detection of Prostate Specific Membrane Antigen in Synthetic Urine by Synergistic, Dual Ligand Phage*

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**Materials and Methods**

All chemicals and reagents were purchased from Sigma-Aldrich, and used as received unless otherwise noted. KO7 helper phage, horseradish peroxidase conjugated to α-M13 monoclonal antibody were purchased from GE Healthcare Life Sciences. PSMA was a generous gift from Drs. William Ernst and Gary Fuji (Molecular Express). 4-Azido butanoic acid was a generous gift from Dr. Ting-Bin Yu and Professor Zhibin Guan (UC, Irvine). 4-Pentynoic acid (GFS Chemicals, Inc.), O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate, HBTU (GL Biochem Ltd.), Tris-(benzyltriazolylmethyl) amine (Anaspec Inc.), triethyammonium acetate buffer (Fluka Biochemika) and sorbitan monolaurate, Tween-20 (EMD Science) were used as received. Milli-Q UV water was used for the preparation of solutions.

**M13 bacteriophage propagation**

The M13 phage display vectors (phagemids) containing the genes encoding P8 fused to either peptide **1** or **2** were used to transform $CaCl_2$ competent *E. coli* XL-1 Blue cells, before plating onto LB agar plates supplemented with 50 μg/mL carbenicilin. The cells were grown at 37 °C in 2 mL 2YT media supplemented with carbenicilin (50 μg/mL) until the culture reached log-phase growth. The culture was then infected with KO7 helper phage ($10^{10}$ phage/mL) and shaken at 250 rpm for 1 h at 37 °C. The starting culture was then transferred to 75 mL 2YT/carbenicilin media supplemented with kanamycin (10 μg/mL). The culture was shaken at 250 rpm for 16-18 h at 37 °C. To isolate the phage from the cells, the culture was centrifuged for 10 min at 10 krpm at 4 °C. The supernatant was decanted into separate tubes, and the phage was precipitated by addition of $1/5^{th}$ volume of PEG-NaCl (2.5 M NaCl, 20% PEG-8000). The solution was placed on ice for 1 h. Next, the phage were recovered by centrifugation for 20 min at 10 krpm. The supernatant was discarded, and the phage pellet was resuspended in phosphate-buffered saline (PBS, 135 mM NaCl, 2.50 mM KCl, 8.00 mM $Na_2HPO_4$, 30.0 mM $KH_2PO_4$, pH 7.2) with added 0.05% Tween-20. After additional centrifugation for 10 min at 15 krpm, the phage precipitation step was repeated as described above. Phage concentration was determined by UV absorbance at 268 nm ($OD_{268}$ = 8.31 nM).

For incorporation of phage in the virus-PEDOT films, the phage pellet obtained after the above protocol was re-suspended in aqueous $LiClO_4$ (12 mM) solution.

**Solid Phase Peptide Synthesis**

The peptides were synthesized at 0.40 mmol scale following the standard procedures for solid-phase peptide synthesis with Fmoc-protected amino acids on Rink-amide resin (Novabiochem). The last coupling step was performed with 4-azido butanoic acid or 4-pentynoic acid to yield the azide- or alkyne-functionalized peptides respectively. The synthesized peptides with a carboxamide C-terminus were cleaved from the resin by treatment with 9.5 mL trifluoroacetic acid, 250 μL triisopropylsilane and 250 μL of water in a $N_2$ atmosphere for 3 h. The cleavage mixture was filtered from the resin, and the peptides were precipitated by addition of ice-cold diethyl ether. The peptides were recovered by centrifugation at 3 krpm for 20 min at 4 °C, and resuspended in water before lyophilization. The peptides were characterized by MALDI-TOF mass spectrometry, followed by reverse phase HPLC purification with a $C_{18}$ column. Fractions containing the purified peptides were combined and concentrated using rotary evaporation, followed by lyophilization. The purified peptides were further characterized by MALDI-TOF mass spectrometry. The calcd *m*/*z* for peptide-**1** $[M^+]$ 1349.67, found 1350.1. The calcd *m*/*z* for peptide-**2** $[M^+]$ 2040.28, found 2040.23. The calcd *m*/*z* for alkyne-functionalized $K_{14}$ peptide $[M+Na]^+$ 1914.37, found 1914.18 .

**Click chemistry reaction for the synthesis of $K_{CS}$-2**

As a representative protocol for the click chemistry reaction, the synthesis of $K_{CS}$-**2** was adapted from Lumiprobe Corporation's protocol for application to peptides with the following exceptions.[1] First, the reaction was performed at 200 μM azide-derivatized peptide concentration in 75% acetonitrile and 25% water. Second, water was used as the

solvent, in place of DMSO. Third, a final concentration of 1 mM $CuSO_4$ was used, and the acetone precipitation step was skipped. Product formation was confirmed by MALDI-TOF mass spectrometry before purification. For purification, the reaction mixture was first concentrated using 2 kD MW cut-off micro concentrators, and then further concentrated under high vacuum to approximately 100 μL. The concentrated reaction mixture was purified using reverse-phase analytical HPLC. Purified product was then subjected to reverse-phase analytical HPLC and MALDI-TOF, to verify purity and confirm identity, respectively. The calcd *m*/*z* for $K_{CS}$-**1** [$M^+$] 3240.05, found 3240.08. The calcd *m*/*z* for $K_{CS}$-**2** [$M^+$] 3930.31, found 3930.35.

**PSMA Targeting Enzyme-Linked Immunosorbent Assay (ELISA)**

In a phage-based ELISA, specific wells of a 96-well microtiter plate (Nunc Maxisorp) were coated with 100 μL/well of a solution of PSMA (5.56 or 11.1 nM) monomer or dimer protein diluted in PBS. The plate was incubated for 1 h on a shaker at room temperature. The coating solution was removed, and the wells were blocked with 320 μL/well of 0.2% w/v solution of BSA in PBS for 30 min, and washed three times with 300 μL/well of wash buffer PT (0.05% Tween-20 in PBS). Separately, phage-displayed PSMA-binding peptides were serially diluted along with a negative control (KO7) in phage dilution buffer, PBT (0.1% w/v BSA, 0.1% Tween-20 in PBS). The wells on the ELISA plate were then incubated with the phage samples (100 μL/well) for 1 h. The wells were washed five times with PT and then incubated with horseradish-peroxidase-conjugated anti-M13 antibody (100 μL/well, 1:5000 dilution in PBT) for 30 min. The wells were washed three times with PT, and two times with PBS. The plate was then developed by incubating with

HRP substrate solution (100 μL/well; 1 mg/mL *o*-phenylenediamine dihydrochloride and 0.02% w/v $H_2O_2$) in citric acid buffer (50 mM citric acid, 50 mM $Na_2HPO_4$, pH 5.0). Following an appropriate incubation time, HRP activity was measured spectrophotometrically at 450 nm using a microtiter plate reader (Bio-Tek).

**Phage-based wrapping ELISA:** Phage (5 nM in 225 μL) and 2 μL of the purified cycloaddition reaction product, $K_{CS}$-**1/2** (308.7 μM) in water: acetonitrile (60:40), were mixed and serially diluted in PBT. The solution were shaken at room temperature for 15 min on an orbital shaker. After blocking the ELISA plate, the wells were washed three times with PT and once with 300 μL/well of PBS-NaCl (PBS supplemented with 0.2 M NaCl). The wrapped-phage solutions (100 μL/well) were then transferred to the appropriate wells of an ELISA plate, prepared as described above. The wells were washed four times with PT and two times with PBS, followed by incubation with horseradish-peroxidase-conjugated anti-M13 antibody (1:15000 dilution). The wells were then washed six times with wash buffer PT, and two times with PBS. Levels of phage binding were quantified as described above.

**Synthesis of Phage-PEDOT films**

A circular gold electrode (CH instruments) was manually polished with three diamond polishing pastes (Ted Pella) having particle sizes of 1, 0.5 and 0.25 μm on a polishing microcloth (Buehler), and sonicated in nanopure water for 10 min. A flame-cleaned platinum film electrode was used as the counter electrode. The electrode was placed in the virus-PEDOT plating solution (12.5 mM $LiClO_4$, 2 mM EDOT and 3 nM phage).

Electropolymerization occurred by cycling between 0.2 and 1.15 V vs Ag/AgCl reference electrode for 10 cycles using a PARSTAT 2273 controlled with POWERCV software (Princeton Applied Research, Oak Ridge, TN), at a scan rate of 20 mV/s. Synthesized films were rinsed with Milli-Q water and transferred to the run buffer, 0.1% Tween 20 in phosphate buffered fluoride (PBF, 4.2 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$ and 140 mM NaF, pH 7.2).

**SEM Analysis**

Scanning electron microscopy was performed using a Philips XL30 FEG SEM at a 10 kV operating voltage. The samples were coated with a thin layer of Ir by sputter coating prior to SEM analysis.

**Impedance Measurements**

Freshly synthesized virus-PEDOT films were rinsed with Milli-Q water and then equilibrated in PBF-Tween buffer for 10 min. Then five consecutive EIS scans were acquired using a PARSTAT 2273 potentiostat controlled by POWERSine software (Princeton Applied Research, Oak Ridge, TN), at 50 frequency data points spanning 0.1 Hz to 1 MHz with a 10 mV voltage modulation amplitude. The electrode was then rinsed with water, and incubated with PSMA for 30 min followed by rinsing with water and PBF-Tween. The electrode was returned to the run buffer and equilibrated for 10 min before acquisition of five consecutive EIS scans. The data was fit to the Hill equation using GraphPad Prism.[2]

For biosensing with wrapped-viruses, the synthesized virus-PEDOT films were

rinsed with Milli-Q water, and then incubated with $K_{CS}$-**1** (308.7 μM) for 15 min. The electrode was then rinsed with water and PBF-Tween, followed by incubation with PSMA. EIS scans were acquired as described above. For synthetic urine runs, the electrode with the bio-affinity matrix was incubated with PSMA in synthetic urine to achieve the desired concentration. The EIS scans were acquired as described above.

**Supplementary Data**

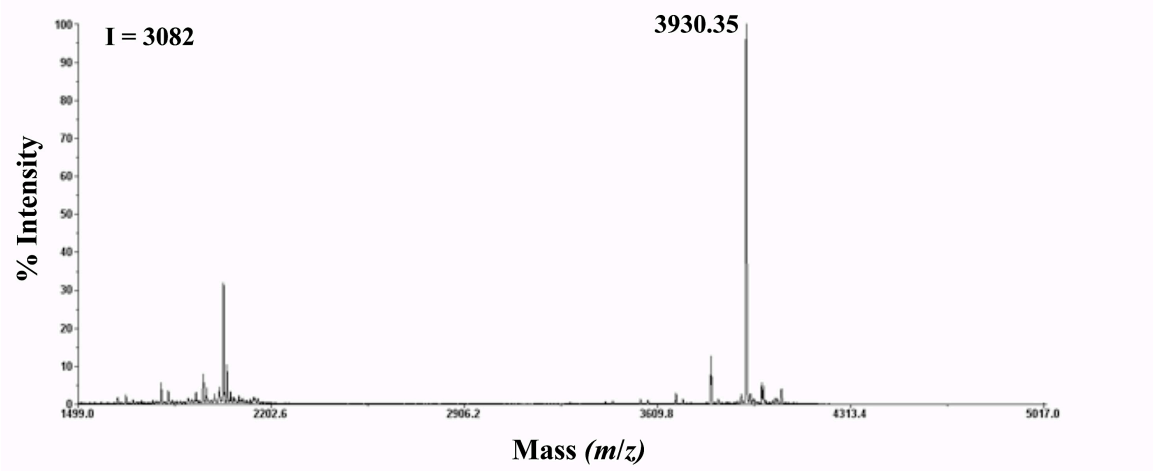


**Figure S1.** A representative MALDI-TOF of the purified product from the azide-alkyne cycloaddtion reaction. The azide-functionalized peptide-**2** and alkyne-functionalized oligolysine yields $K_{CS}$-**2**. The calcd *m*/*z* for $K_{CS}$-**2** [$M^+$] 3930.31, found 3930.35.

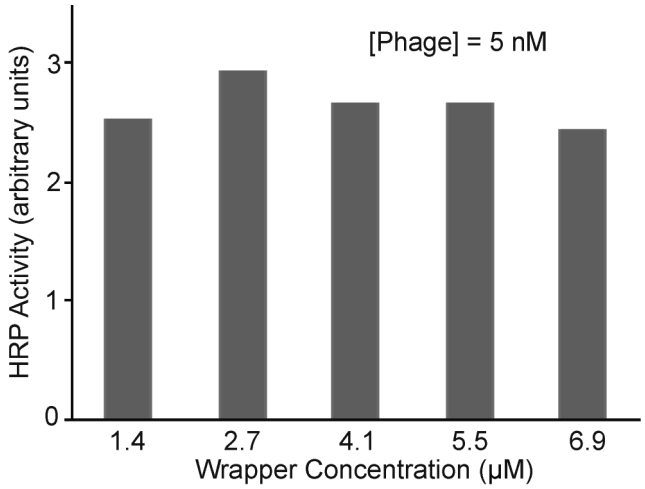


**Figure S2.** Phage-based ELISA illustrating optimization of $K_{CS}$-**1** levels. A concentration of 2.7 μM for $K_{CS}$-**1**, offers an approximate 15% increase in apparent affinity to PSMA, versus other concentrations.

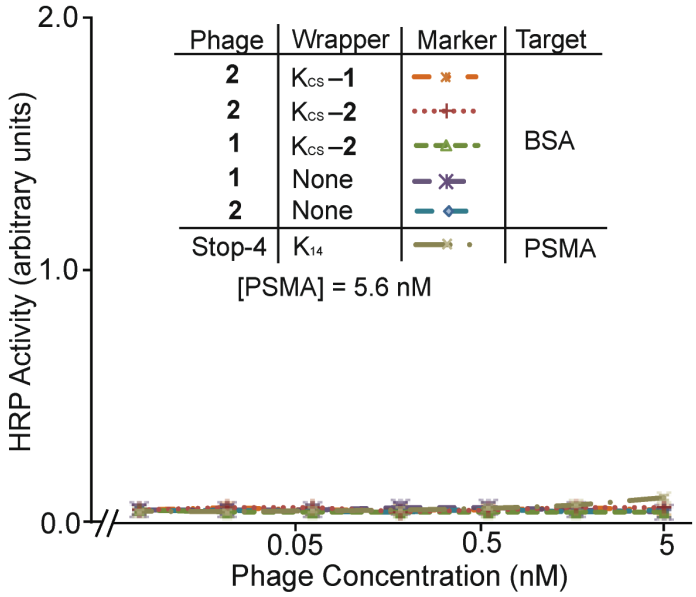


**Figure S3.** Phage-based ELISA illustrating additional negative controls, the PSMA binding ligands targeting BSA and Stop-4 phage wrapped with $\mathbf{K_{14}}$ targeting PSMA.

**Additional References:**

(1) "Protocol: Click-Chemistry Labeling of Oligonucleotides and DNA" to be found under http://www.lumiprobe.com/protocols/click-chemistry-dna-labeling.

(2) H.J. Motulsky, "Analyzing Data with GraphPad Prism, GraphPad Software Inc., San Diego CA" to be found under http://www.graphpad.com, **1999**.