Supporting Information

Conjugated Polmer-Peptide Hybrid System for Prostate-Specific Antigen (PSA) Detection

Kangwon Lee, Suparna Mandal, Jinga Morry, Onnop Srivannavit, Erdogan Gulari and Jinsang Kim*

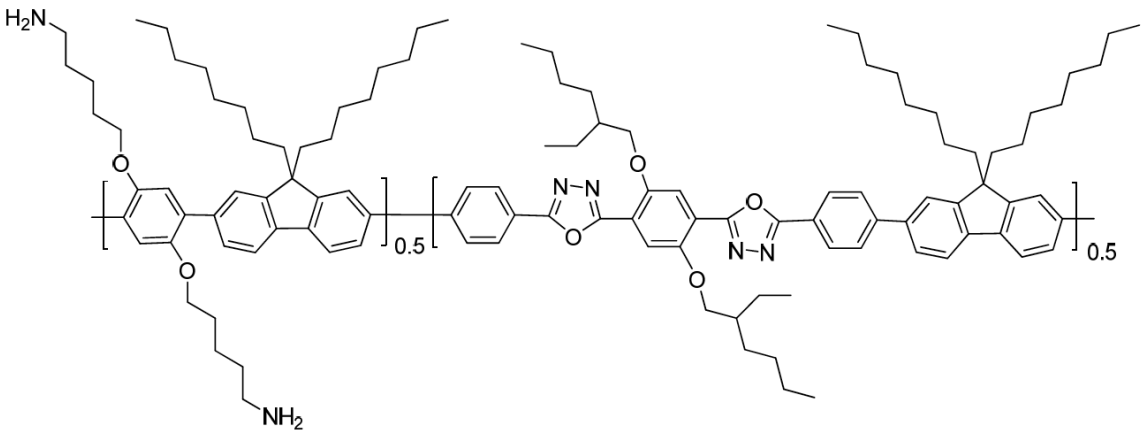


Figure S1. Chemical structure of P1.

Experimental

Materials and Methods All solvents and reagents for polymer (P1) preparation were used without further purification as received from Sigma-Aldrich Co. UV/Vis absorption spectra were recorded with a Varian Cary50 UV/Vis spectrophotometer. Photoluminescence spectra and quantum yield in solution and in the solid state were obtained by using PTI QuantaMasterTM spectrofluorometer equipped with an integrating sphere. The quencher, dimethylaminoazosulphonic acid (DABCYL), and the fluorescent dye, 4,5-carboxyfluorescein (FAM), used in labeling the peptide sequence were purchased from AnaSpec, Inc and Molecular Probes, Co. respectively. The detail synthetic route and characteristic data of the conjugated polymer, poly(oxadiazole-cophenylene-co-fluorene) (P1), has been previously reported (K. Lee, J.-M. Rouillard, T. Pham, E. Gulari and J. Kim, *Angew. Chemie. Int. Ed*. 2007, **46**, 4667).

Slides preparation Glass slides were cleaned in ammonium/hydrogen peroxide at 80 C for 30 min, followed by pirahna solution for 15 min. After drying, the slide was soaked in pirahna solution (H_2SO_4 : H_2O_2 = 35 ml : 15 ml) overnight, washed with DI water (30 ml) and dried with a stream of air. The glass slides were then transferred into a jar containing 97% aminopropyltrimethoxysilane (APTMS, 2 ml), DI water (2 ml) and high-purity methanol (48 ml) and sonicated for 30 minutes. Finally, the slides were washed with methanol, followed by water, and then dried under a stream of air. The slides were baked at 135 °C for 30 minutes before the addition of polyethylene glycol (PEG) linker.

Addition of PEG linker SCM−PEG−Fmoc was reacted with the hydroxyl group of the APTMS to form a reactive linker for P1. The glass slides were then washed with ethanol and DMF and placed in 20 % piperidine in dimethylformamide (DMF) for 2 hours.

P1 immobilization onto the glass slides The glass slides were reacted with 1,4-diphenylenediisothiocyanate (100 mg) in DMF (54 ml) in for 2 hours. The slides were then washed with dimethylformamide and dichloromethane before drying under a stream of air. Only one side of the slide was reacted with **P1** (2 mg) in pyridine (0.5 ml) and chloroform (9 ml). The slides were subsequently washed with chloroform, dichloromethane, and DI water. Further cleaning steps of the slide were achieved by sonication in chloroform for 5 min and drying in a vacuum oven.

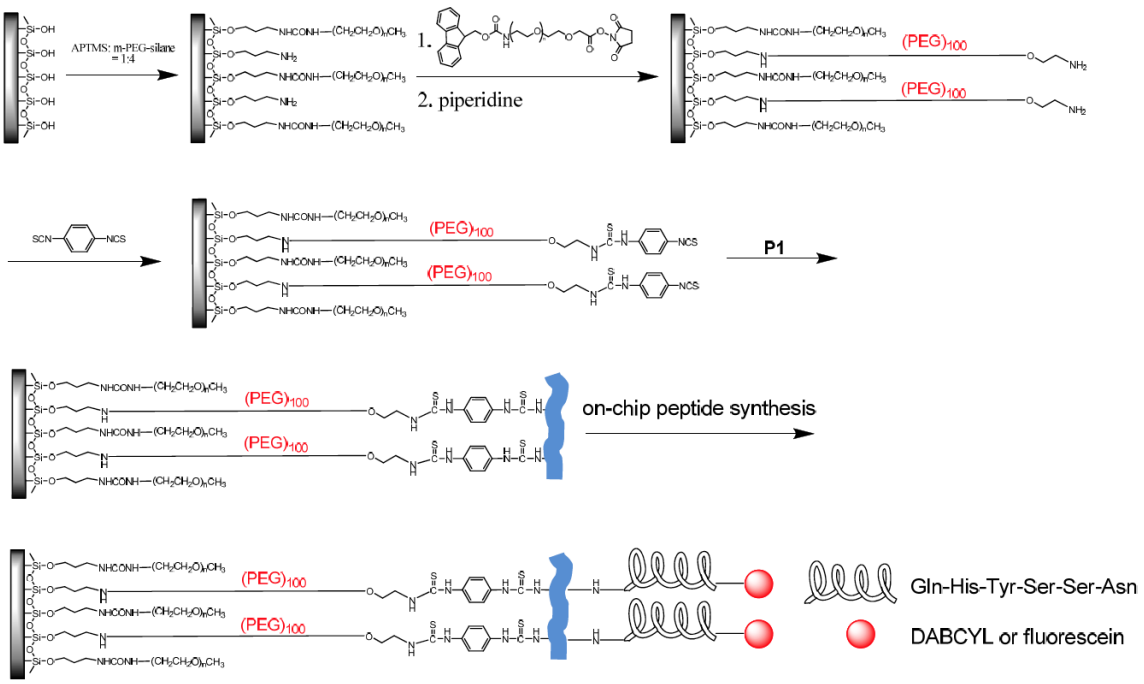
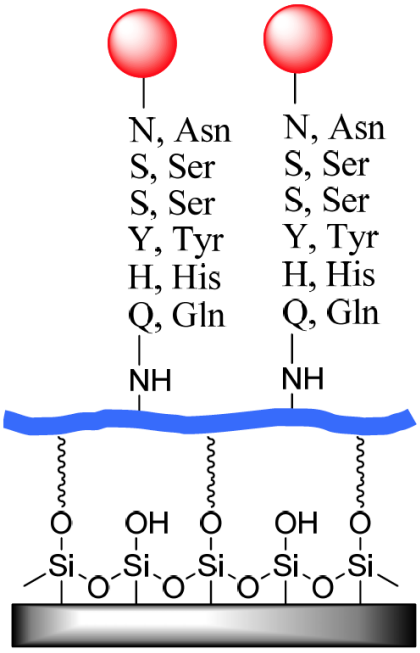


Figure S2. Schematic representation of polymer immobilization onto glass substrate and on-chip peptide synthesis.

Direct peptide synthesis onto the glass slides The glass slide was enclosed in a holder connected to a peptide synthesizer. Standard Fmoc (9-fluorenylmethyloxycarbonyl) was used for all steps. The glass slides were treated with a solution of 0.4 mmol of Fmoc protected amino acid, 0.4 mmol of 0.6 M solution of 2-(1h-7-azabenzotriazole-1-yl)-oxy-1,1,3,3,-tetramethyluroniumhexafluorophosphate (HATU) and 0.5 mmol of diisopropylethylamine (DIEA) in DMF. An activation time of 3 minutes and coupling time of 10 minutes was allowed for completion of coupling. Unsuccessful couplings were capped with a 20 % solution of acetic anhydride in DMF for 2 minutes. This was followed by extensive washing with DMF. Deprotection of Fmoc protecting group was done by flushing the glass substrate with a solution of piperidine in DMF for 20 minutes. The removal of side-chain protecting groups and the peptide from the resin at the end of the synthesis was achieved by incubating in a solution of trifluoromethanesulfonic acid, trifluoroacetic acid (TFA), dichloromethane, ethanedithiol, and thioanisole.



Peptide synthesis

1. **Coupling:**
   - I. HATU(2-(1H-7-azabenzotriazole-1-yl)-oxy-1,1,3,3-tetramethyluroniumhexafluorophosphate)
   - II. DIEA (Diisopropylethylamine), Fmoc amino acid
2. **Capping:** Acetic anhydride in DMF
3. **Deprotection:** Piperidine in DMF
4. **Side chain removal:**
   - I. Trifluoromethanesulfonic acid
   - II. Trifluoroacetic acid
   - III. Dichloromethane
   - IV. Ethanidithiol
   - V. Thioanisole
5. **FAM labelling:**
   - I. 4, 5 carboxyfluorescein
   - II. HOBT
   - III. Diisopropylcarbodiimide.

Figure S3. The synthetic procedure of the on-chip peptide synthesis on the P1-coated substrate and the reagents used for the synthesis.

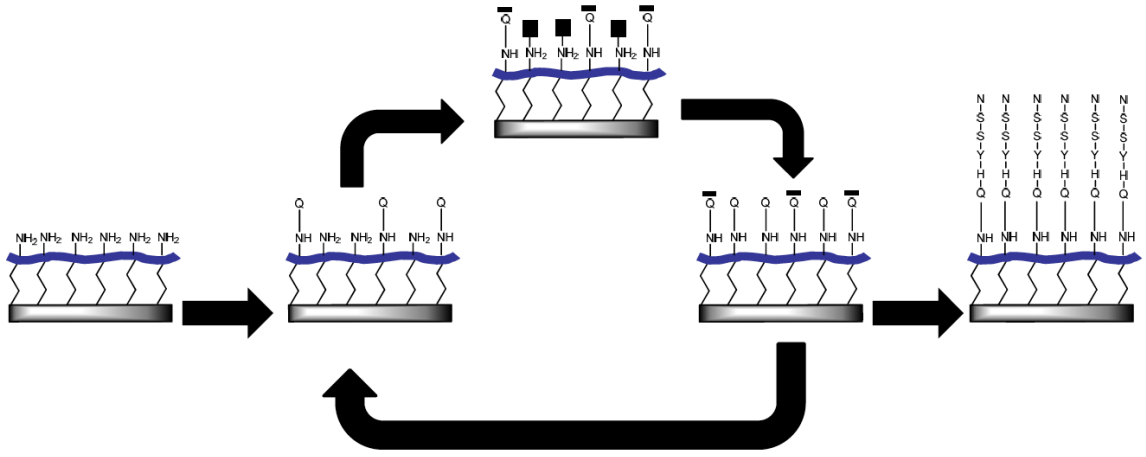


Figure S4. Fmoc solid phase peptide synthesis.

Fluorescent labeling with carboxyfluorescein The solution of FAM labeling was prepared by mixing 5 ml of FAM stock solution (0.5 mM in DMF) with 0.3 gm of hydroxybenzotriazole (HOBT) and 500 µl of diisopropylcarbodiimide (DIC). The glass substrates were incubated in this

solution for 2 hours. Finally, the slides were washed with DMF and ethanol consecutively. DABCYL labeling was done in a same manner as fluorescein labeling.

PSA cleavage test 10 μL of PSA was added onto the peptide coated region of the glass slides and incubated with 90 μL of PSA buffer (100 mM Tris, 0.5 M NaCl) in a petridish at 37 °C for 4 hours. After washing the glass with the buffer and DI water, the photoluminescence spectra of each glass slide were examined by using PTI QuantaMasterTM spectrofluorometer before and after the incubation.

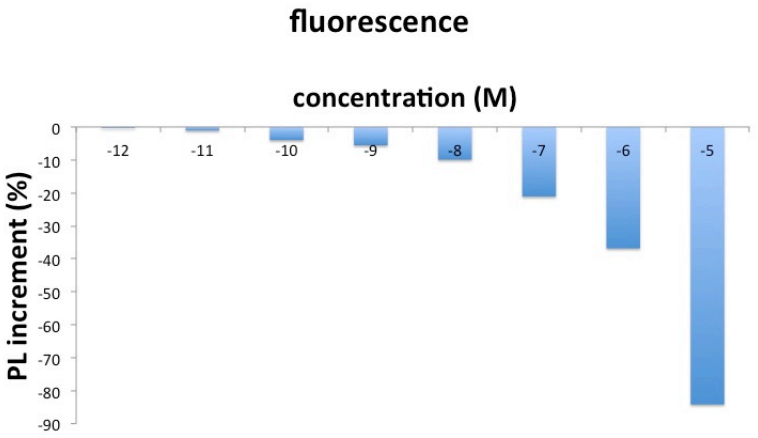


Figure S5. The change of the dye emission intensity at 525 nm after incubating with various concentrations of PSA. The decrease was calculated after normalizing the PL spectra at the P1 emission at 425 nm.