Electronic Supplementary Material (ESI) for Chemical Communications

9G DNAChip: A Platform for the Efficient Detection of the Proteins

Keum-Soo Song[§], Satish Balasaheb Nimse[†], Junbae Kim,[†] Junghoon Kim[§], Van-Thao Ta[†],

Van-Thuan Nguyen[†], Taisun Kim[†]*

[†]Institute for Applied Chemistry and Department of Chemistry, Hallym University, Chuncheon, 200-702, Korea

[§]Biometrix Technology, Inc. 202 BioVenture Plaza, Chuncheon, 200-161, Korea

Fax: +82-33-256-3421

E-mail: tskim@hallym.ac.kr

Supporting Information

1. Materials

All chemicals were purchased from Sigma-Aldrich Chemicals, Korea. All the oligonucleotides were purchased from Bioneer, Korea. For labeling of the oligonucleotides and antibodies with Cy5, the Cy5Dye mono-reactive NHS ester (PA25001) was purchased from GE Healthcare UK Limited, Buckinghamshire, UK. C-Reactive Protein Antigen (CRPAg) (Catalog #. 30-AC05), CRP monoclonal antibody (CRPAb) (Catalog #. 10-C33C), CRP monoclonal secondary antibody (CRPAB) (Catalog #. 10-C33B), Prostate Specific Antigen (PSAAg) (Catalog #. 30-AP-15), PSA monoclonal antibody (PSAAb) (Catalog #.10-P20E, Clone- M701042), PSA monoclonal secondary antibody (PSAAB) (Catalog #.10-P20E, Clone- M701041) were purchased from the Fitzgerald Industries International, USA. Goat anti-Mouse IgG (IgG) (Catalog #. BGA001) was purchased from Bore Da Biotech Co. Ltd., South Korea. Glass slides (2.5x7.5 cm) were purchased from Paul Marienfeld GmbH & Co. KG, Germany**.** All washing solvents for the substrates are of HPLC grade from SK Chemicals, Korea. Ultrapure water (18 M Ω/cm) was obtained from a Milli-Q purification system (Millipore). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as an internal standard. Chemical shifts (δ) are reported in ppm, and spin-spin coupling constants (*J*) are in Hz.

2. Instruments

Oligonucleotides were spotted using Qarray2 microarrayer (Genetix Technologies, Inc.) Hybridization was done at 25^{0}C using the commercial incubator and then the slides were dried using the commercial centrifuge (1000rpm). The fluorescence signal of the microarray was measured on ScanArrayLite (GSI Lumonics), and the images were analyzed by Quant Array software (Packard Bioscience).

3. Abbreviations:

CRP: C-reactive protein; PSA: Prostate-specific antigen; CRPAg: C-reactive protein antigen; PSAAg: Prostate-specific antigen; CRPAb: CRP monoclonal antibody; PSAAb: PSA monoclonal antibody; CRPAB: CRP monoclonal secondary antibody; PSAAB: PSA monoclonal secondary antibody; IgG: Immunoglobulin G; IgGAb: IgG monoclonal antibody; T (T1-T4): Target oligonucleotide complementary to the probe oligonucleotides; Probe (Probe 1-11): Probe oligonucleotide immobilized on the DNA chip; IgGAb-T1: antibody-DNA conjugate of IgG and target oligonucleotide T1; CRPAb-T2: antibody-DNA conjugate of CRPAb and target oligonucleotide T2; PSAAb-T3: antibody-DNA conjugate of PSAAb and target oligonucleotide T3; Cy5-CRPAB: Cy-5 dye labeled CRP monoclonal secondary antibody; Cy5-PSAAB: Cy-5 dye labeled PSA monoclonal secondary antibody; Cy5-CRPAB-CRPAg-CRPAb-T2: biomolecular complex of Cy5-CRPAB, CRPAg, and CRPAb-T2; Cy5-PSAAB-PSAAg-PSAAb-T3: biomolecular complex of Cy5-PSAAB, PSAAg, and PSAAb-T3.

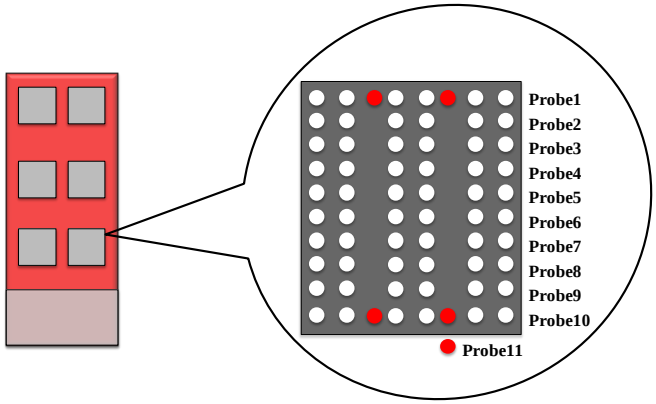
4. Probes and target oligonucleotides:

Table S1. Sequences and the nomenclature of probes and target oligonucleotides

Capture Probes	Sequence
Probe1	5'-GGGGGGGGG CTT TAT TTT CC TAC GAC TTG GGG AGG-3'
Probe2	5'-GGGGGGGGG CTT TAT TTT CC ATC GTG TAG CGG TGG-3'
Probe3	5'-GGGGGGGGG CTT TAT TTT CG TAG GAC ATG GCC ACC-3'
Probe4	5'-GGGGGGGGG CTT TAT TTT GC TAC CTG TTG CCG TGG-3'
Probe5	5'-GGGGGGGGG CTT TAT TTT CC ACT GTT CTC GGC ACG-3'
Probe6	5'-GGGGGGGGG CTT TAT TTT CC CAT CAC TGG TGG AGG-3'
Probe7	5'-GGGGGGGGG CTT TAT TTT CT TGC GAC AAG CCC AGG-3'
Probe8	5'-GGGGGGGGG CTT TAT TTT CA CAC GCT ATC GGG TGG-3'
Probe9	5'-GGGGGGGGG CTT TAT TTT TA CAC GAC CTG CGG AGC-3'
Probe10	5'-GGGGGGGGG CTT TAT TTT CC CAT ACC TTG GGA GGG-3'
Probe11	5'-GGGGGGGGG TTT CCT AGT GGC TCT ATG GTA AC-3'
Target DNA	Sequence
T1	3'-GG ATG CTG AAC CCC TCC TTT TTT TTT TTT-NH_2-5'
T2	3'- GC ATC CTG TAC CGG TGG TTT TTT TTT TTT-NH_2-5'
T3	3'- GG TGA CAA GAG CCG TGC TTT TTT TTT TTT- NH_2-5'
T4	3'- GGA TCA CCG AGA TAC CAT TG GAG ACT GCG-5'
Cy5-IgG-T1	3'-GG ATG CTG AAC CCC TCC TTT TTT TTT TTT-IgG-Cy5-5'
Cy5-CRPAB-CRPAg-CRPAb-T2	3'-AA ATT CCG TTC CAC TTC TTT TTT TTT TTT- CRPAb-CRPAg-CRPAB-Cy5-5'
Cy5-PSAAB-PSAAg-PSAAb-T3	3'-GG GTA GTG ACC ACC TCC TTT TTT TTT TTT-PSAAb-PSAAg-PSAAB-Cy5-5'
Cy5-T4	3'- GGA TCA CCG AGA TAC CAT TG GAG ACT GCG -Cy5-5'

The GGG GGG GGG sequence in the oligonucleotide probes (Probe1-Probe11) is used for the immobilization of the oligonucleotide on the 9G DNAChip. Whereas the CTT TAT TTT in the oligonucleotide probe is used as a vertical spacer. The TTT TTT TTT TTT and GAG ACT GCG sequence in the target oligonucleotides T1-T3 and T4 respectively, are also used as a vertical spacer.

5. Scheme for immobilization of the Probes on the 9G DNAChip



Scheme S1. Scheme for immobilization of the Probes on the 9G DNAChip

6. Labelling of oligonucleotides, antibodies and preparation of Protein-DNA conjugates

6a. Labelling of oligonucleotides and proteins with Cy5 dye

Briefly, the Cy5-T1, Cy5-T2, Cy5-T4, the Cy5 labeled Goat anti-Mouse IgG (Cy5-IgG), and the Cy5 labeled PSA monoclonal secondary antibody (Cy5-PSAAB) were obtained by the reaction of the amine functions in the amine modified oligonucleotides (T1, T2, T4) and amine function in the antibodies (IgG, PSAAB, CRPAB) with the Cy5Dye mono-reactive NHS ester, respectively, by following the standard protocol provided by the manufacture with the mono-reactive Cy5Dye^{TM} (GE Healthcare UK Limited, Buckinghamshire, UK).

6b. Synthesis and purification of the Protein-DNA conjugates

The Cy5-IgG-T1 and the PSAAb-T3 were synthesized by the reaction of Cy5-IgG, PSAAb with the slfo-SMCC activated oligonucletides T1 and T3, respectively following the reported method.[1]

6c. Preparation of a biomolecular complex (Cy5-PSAAB-PSAAg-PSAAb-T3)

The biomolecular complex Cy5-PSAAB-PSAAg-PSAAb-T3 was obtained by mixing the 5µl of the PSAAb-T3 conjugate (16µg/ml), 5µl of the Cy5-PSAAB (25µg/ml), and 5µl of the PSA-Ag (1ng/ml) in the 30µl of the hybridization solution for 5min and used immediately.

7. Composition of used solutions:

1. Immobilization solution (pH = 7.4): 15% glycerol, 50mM butyl amine, 600mM NH_4Cl
2. Blocking buffer solution (pH = 7.4): 0.5% milk casein in 4xSSC
3. Hybridization buffer (pH = 7.4): 25% Formamide, 0.1% Triton X-100, 6xSSC
4. Washing buffer solution A (pH = 7.4): 0.1% SDS in 4xSSC
5. Washing buffer solution B (pH = 7.4): 4xSSC

8. Hybridization

8a. Hybridization mixture

5µl each of the IgGAb-T1 (0.2µg/ml), CRPAb-T2 (g/ml), Cy5-CRPAB (25µg/ml), PSAAb-T3 conjugate (25µg/ml), Cy5-PSAAB (25µg/ml), and Cy5-T4 (40fmol/µl) were mixed in the hybridization solution to make 50µl of final hybridization mixture.

Table S2. Final composition of the hybridization mixture before loading on the chip

Constituents	Individual Detection			Simultaneous Detection		
	CRPAg	PSAAg	NC	CRPAg	PSAAg	NC
IgGAb-T1(0.2µg/ml)	5µl	5µl	5µl	5µl	5µl	5µl
CRPAb-T2 (5µg/ml)	5µl	5µl	5µl	5µl	5µl	5µl
PSAAb-T3 (5µg/ml)	5µl	5µl	5µl	5µl	5µl	5µl
Cy5-CRPAB (25µg/ml)	5µl	5µl	5µl	5µl	5µl	5µl
Cy5-PSAAB (25µg/ml)	5µl	5µl	5µl	5µl	5µl	5µl
Cy5-T4 (40fmol/ml)	5µl	5µl	5µl	5µl	5µl	5µl
Hybridization solution (12xSSC, 0.1%BSA, 5mM EDTA, 0.05%NaN_3)	15µl	15µl	20µl	-	-	20µl
Hybridization solution (18xSSC, 0.15%BSA, 5mM EDTA, 0.05%NaN_3)	-	-	-	10µl	10µl	-
CRPAg (0.001ng/ml-10ng/ml)	5µl	-	-	5µl	-	-
PSAAg (0.001ng/ml-10ng/ml)	-	5µl	-	-	5µl	-

8b. General Hybridization Procedure

Each hybridization chamber of 9G DNAChip was covered with the mixture of 40μl of the hybridization buffer solution and 5μl of target DNA (Cy5-T1 or Cy5-T2) and then incubated at 25^0C for 30 min. Then 9G DNAChip was rinsed with washing buffer solutions A and B successively for 2 min each in order to remove the excess target DNA and dried with commercial centrifuge (1000 rpm). The fluorescence signal of the microarray was measured on ScanArrayLite, and the images were analyzed by Quant Array software.

9. References:

1. Y. Jung, J. M. Lee, H. Jung, B. H. Chung, *Anal. Chem.* **2007**, *79*, 6534-6541.