Supporting Information

Positively Charged Polymer Brushes–Functionalized Filter Paper for DNA Sequence Determination Following Dot Blot Hybridization Employing Pyrrolidinyl Peptide Nucleic Acid Probe

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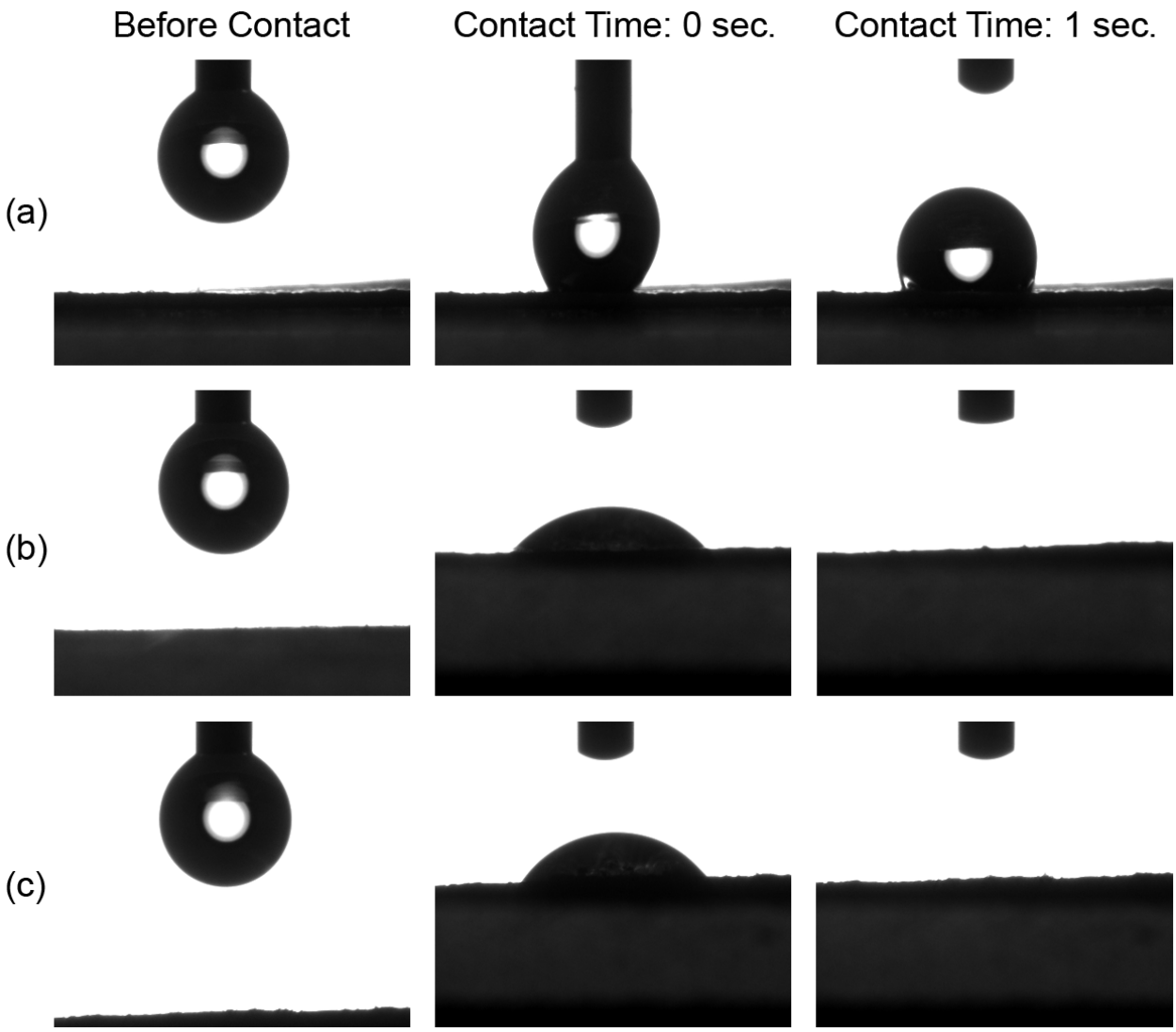

Fig. S1 Images of water droplet during water contact angle measurement on surface-modified filter paper: (a) immobilized with initiator, (b) grafted with PDMAEMA, and (c) grafted with QPDMAEMA.

Table S1 PNA and DNA sequences used in this study

DNA targets

Code	Sequence	Description
b-DNA SLE2	d(5′-b-TTGGGAAGGGGAA-3′)	Positive control
DNA SLE1	d(5′-TTGGGAGGGGGAA-3′)	Complementary to b-PNA SLE1
		Single mismatch to b-PNA SLE2
DNA SLE2	d(5′-TTGGGAAGGGGAA-3′)	Complementary to b-PNA SLE2
		Single mismatch to b-PNA SLE1
DNA (AG)	d(5′-TGTGGATAGTGA-3′)	Non-complementary to b-PNA (TG)
DNA (AC)	d(5′-GTCATAGCATCA-3′)	Complementary to b-PNA (TG)

PNA probes

Code	Sequence	Description
b-PNA SLE1	b-$(egl)_2$-TTCCCCCTCCCAA-LysNH_2	Complementary to DNA SLE1
		Single mismatch to DNA SLE2
b-PNA SLE2	b-$(egl)_2$-TTCCCCTTCCCAA-LysNH_2	Complementary to DNA SLE2
		Single mismatch to DNA SLE1
b-PNA (TG)	b-$(egl)_2$-TGATGCTATGAC-LysNH_2	Complementary to DNA (AC)
		Non-complementary to DNA (AG)

Table S2 Description of DNA and PNA sequences used for each spot of the test results shown in Figs. 3, 5-6.

Spot position	DNA	PNA	Remark
1	b-DNA SLE2	-	positive (+)
2	DNA SLE1	b-PNA SLE2	negative (-)
3	DNA SLE2	-	negative (-)
4	DNA SLE2	b-PNA SLE2	positive (+)
5	-	b-PNA SLE2	negative (-)
6	-	-	negative (-)

Table S3 Characteristic of PDMAEMA synthesized by ARGET ATRP.

Entry	$[Sn(EH)_2]_0/[CuBr_2]_0$[a]	solvent	time (h)	% conv.[b]	M_n(theo) [c]	M_n(exp)[d]	PDI[d]
1	250	anisole	19	99	31,086	53,488	1.30
2	250	acetone	24	72	22,608	21,670	1.38
3	400	acetone	24	90	28,260	28,495	1.28
4	667	acetone	24	90	28,260	23,983	1.35

[a]DMAEMA/EBiB/$CuBr_2$/Me_6TREN = 200:1:0.006:0.25. $[DMAEMA]_0$ = 4.12 M, The polymerization was conducted in a scintillation vial with volume of free space = 7.7 mL. [b]Determined by ^{1}H NMR in $CDCl_3$. [c]M_n(theo) = ($[DMAEMA]_0/[EBiB]_0$) × conversion. [d]Determined by GPC in THF based on polystyrene standards.

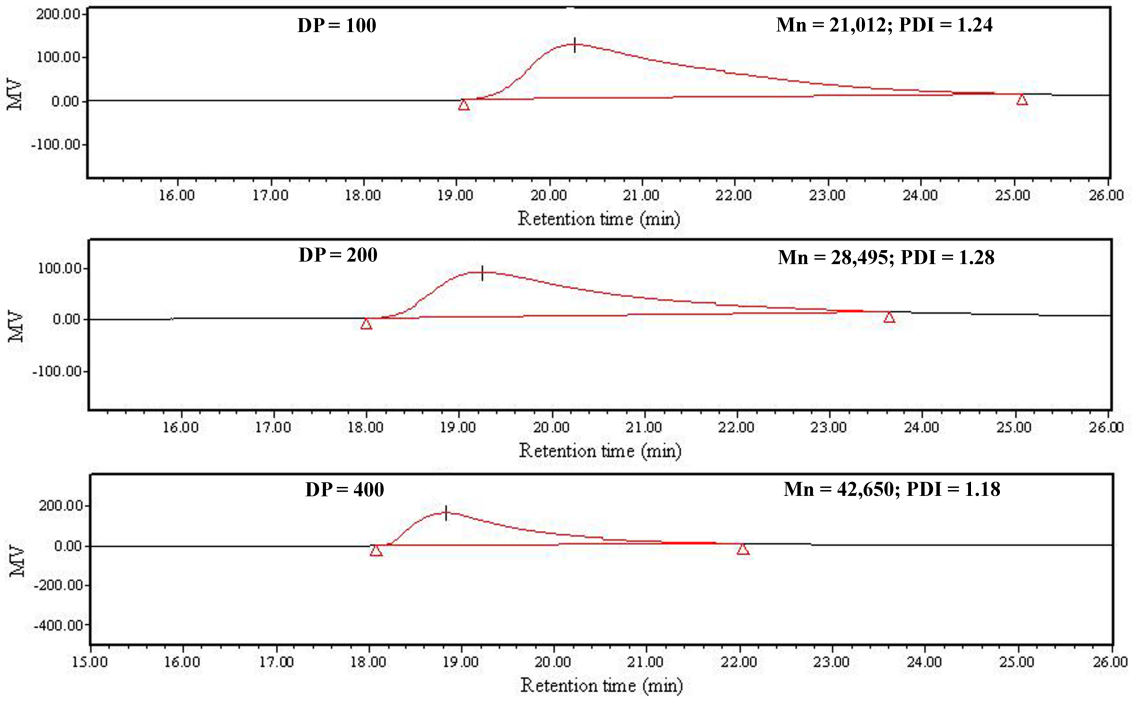

Fig. S2 GPC traces of PDMAEMA synthesized by ARGET ATRP having target DP of 100, 200, and 400 obtained using a Waters E600 column, THF as a mobile phase with a flow rate of 1.0 $mL.min^{-1}$ at 35 °C and narrow polystyrene standards.

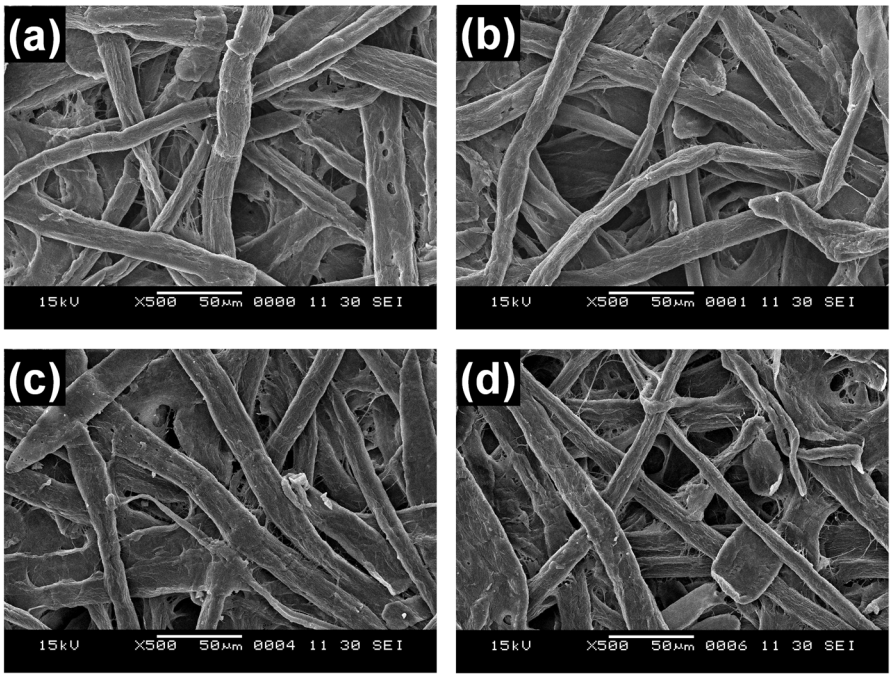

Fig. S3 SEM images of filter paper: (a) unmodified, (b) immobilized with initiator, (c) grafted with PDMAEMA (target DP =200), and (d) grafted with QPDMAEMA (target DP =200).

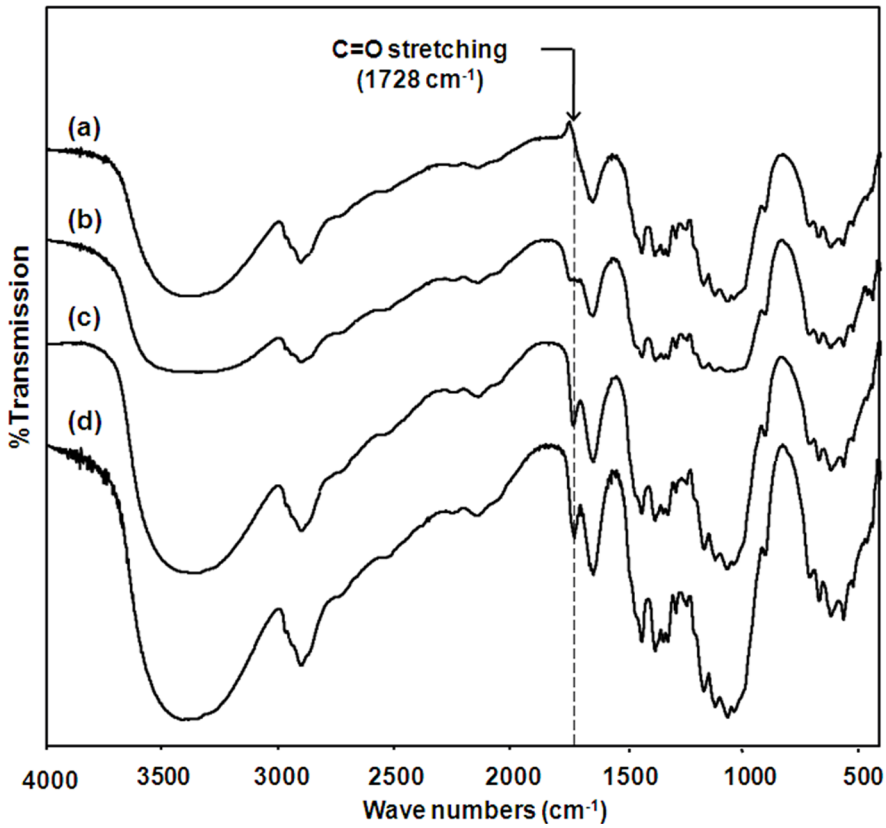

Fig. S4 FT-IR spectra of material scraped from the surface-modified filter paper: (a) unmodified, (b) immobilized with initiator, (c) grafted with PDMAEMA, and (d) grafted with QPDMAEMA.

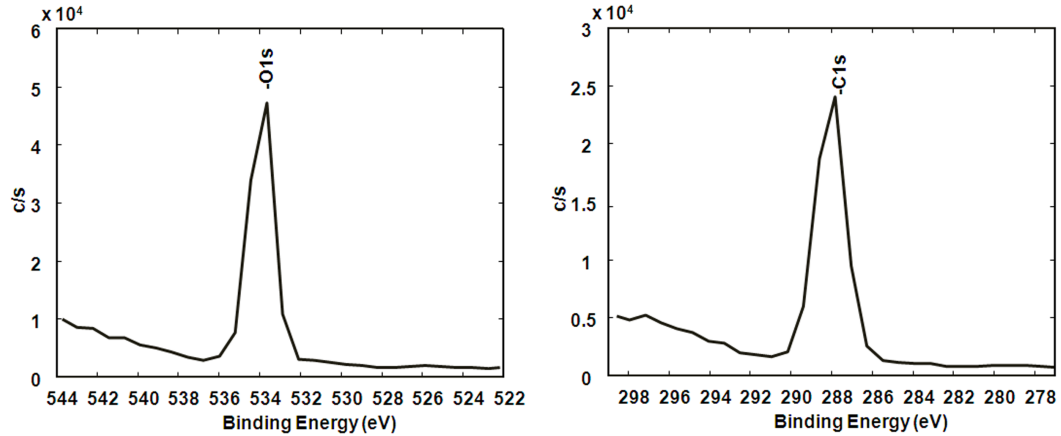

Fig. S5 XPS spectra of O1s and C1s regions of unmodified filter paper.

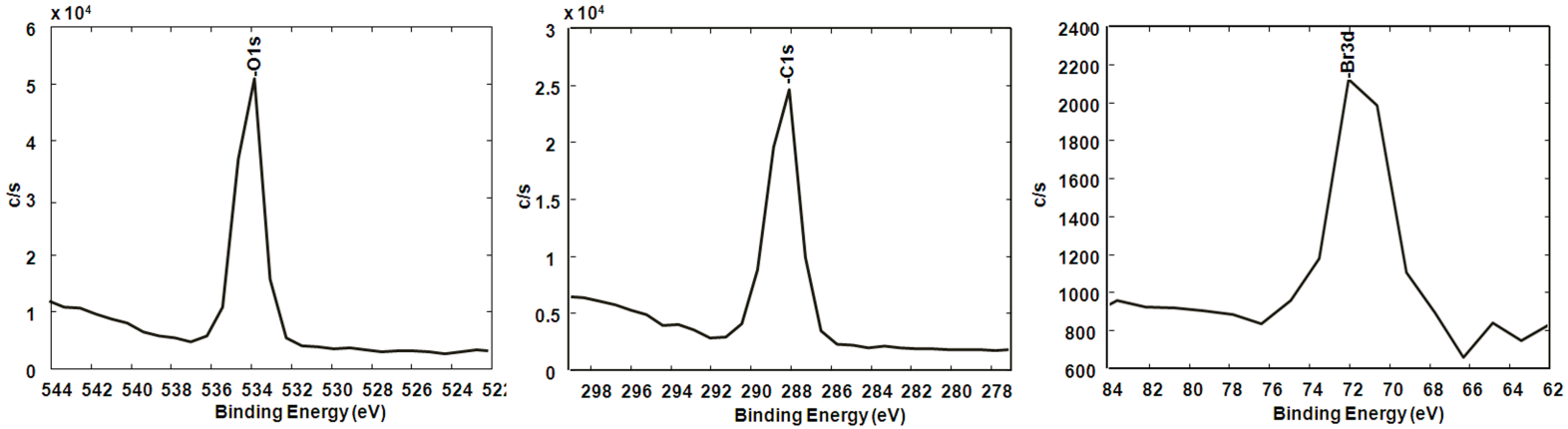

Fig. S6 XPS spectra of O1s, C1s, and Br3d regions of filter paper immobilized with initiator.

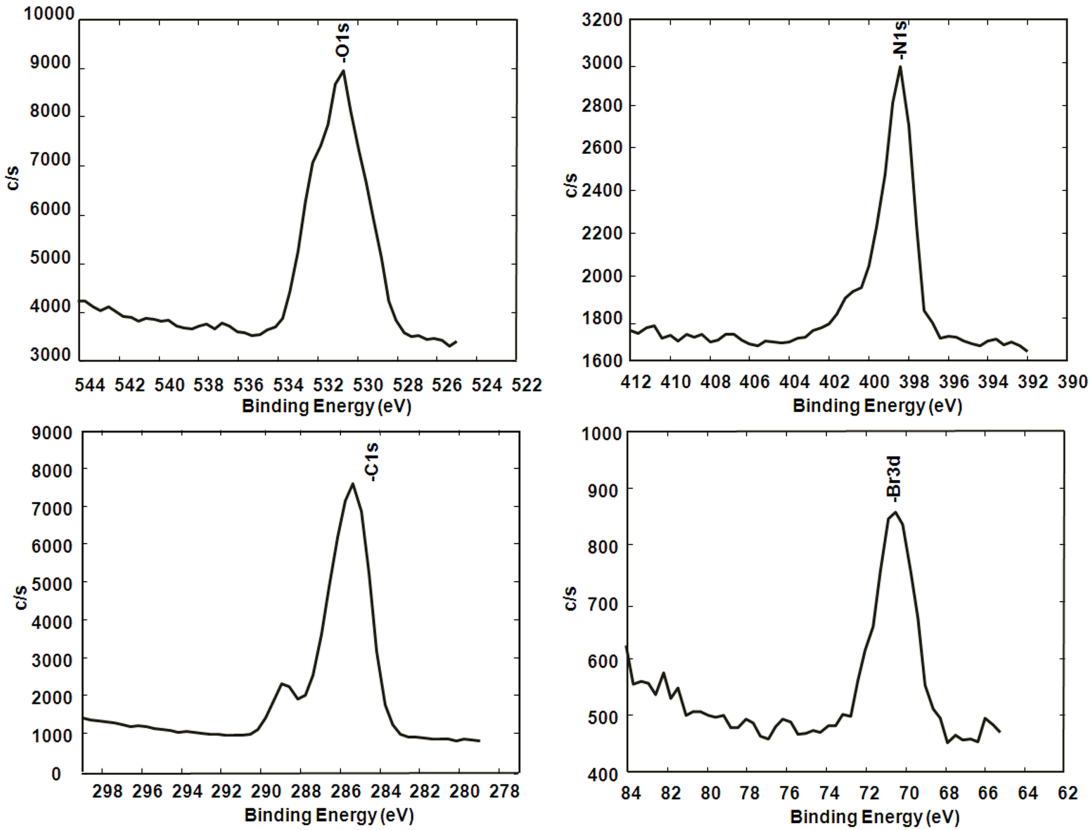

Fig. S7 XPS spectra of O1s, N1s, C1s, and Br3d regions of PDMAEMA-grafted filter paper.

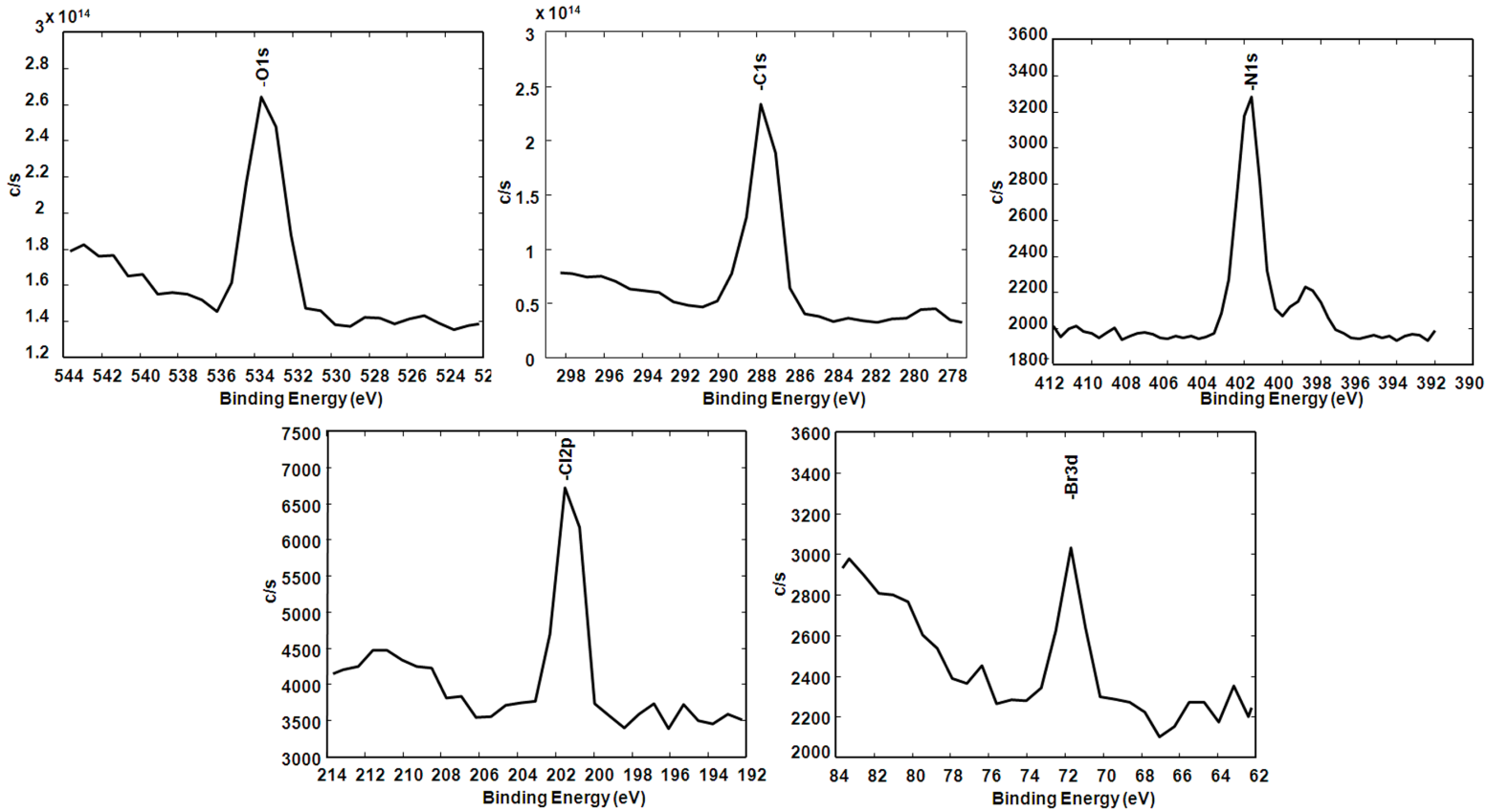

Fig. S8 XPS spectra of O1s, C1s, N1s, Cl2p, and Br3d regions of QPDMAEMA-grafted filter paper.

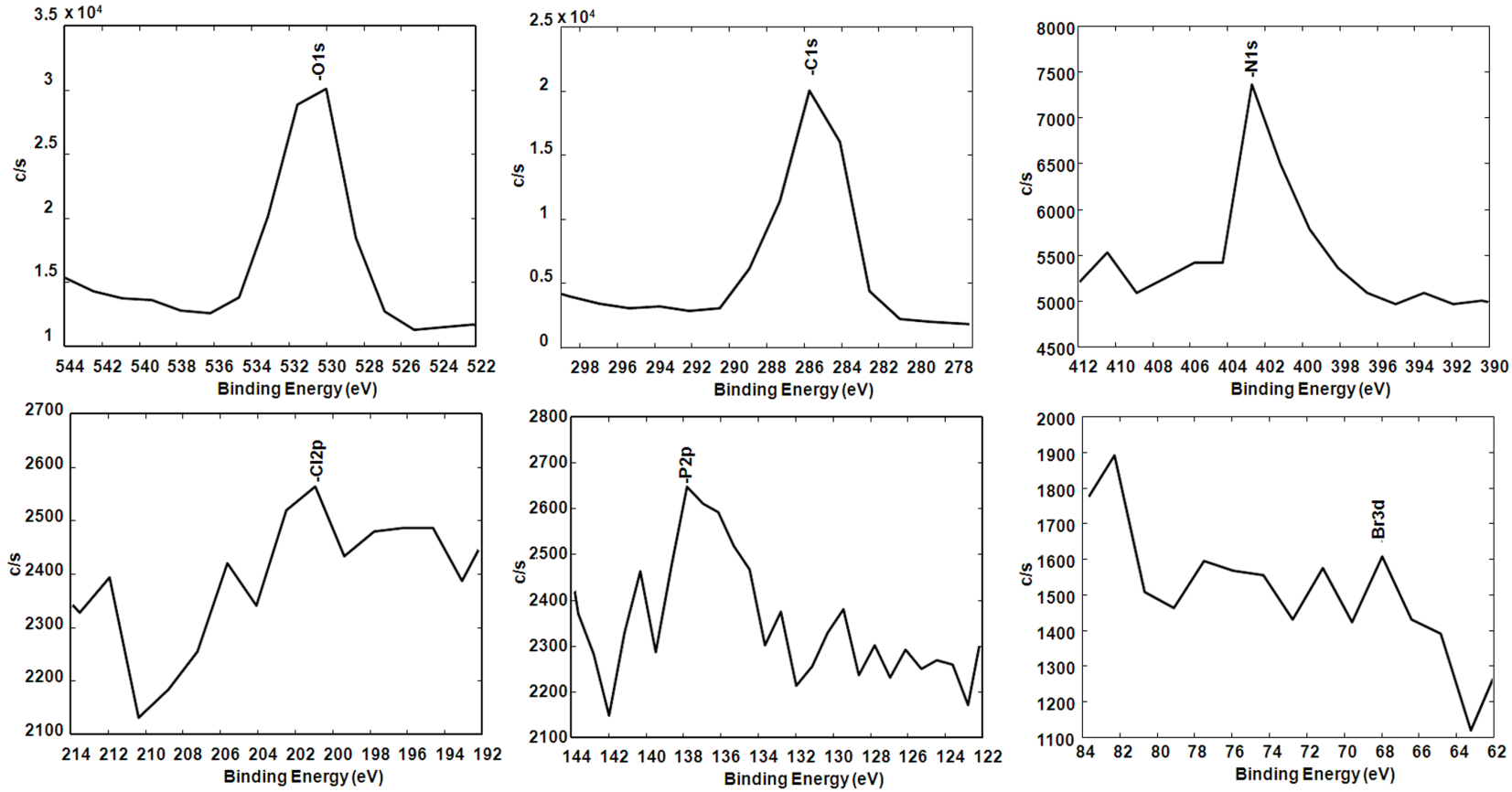

Fig. S9 XPS spectra of O1s, C1s, N1s, Cl2p, P2p, and Br3d regions of QPDMAEMA-grafted filter paper after DNA adsorption.

Table S4 Atomic composition of surface-modified filter paper as determined by XPS analysis.

Sample	Atomic composition (%)						C/N
	C	O	Br	N	Cl	P	
Unmodified	58.2	41.7	N/D	N/D	N/D	N/D	-
Immobilized with initiator	57.7	41.5	0.8	N/D	N/D	N/D	-
Grafted with PDMAEMA	65.0	26.8	0.2	8.0	N/D	N/D	8.1
Grafted with QPDMAEMA[a]	69.2	24.9	0.2	5.8	<0.1	N/D	11.9
Grafted with QPDMAEMA after DNA adsorption	60.4	33.6	0.1	4.6	0.15	1.2	13.1

N/D = Not detected, [a] the analysis was done after chlorine exchange

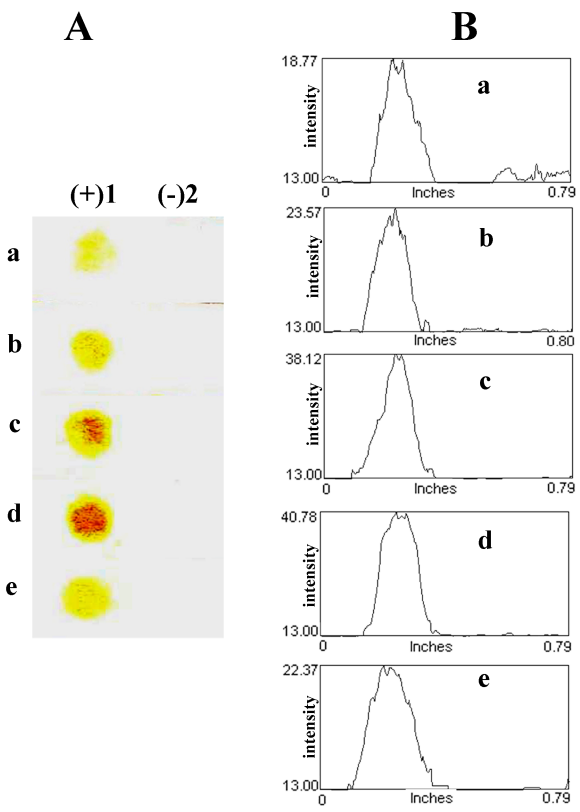

Fig. S10 Representative images (column A: scanned image and column B: profile image) of the test results demonstrating the effect of amount and type of HRP enzyme ((+)1= w/ b-DNA SLE2 and (-)2 = w/o b-DNA SLE2): a) 20 ng avidin-HRP (2 μL of 10 μg/mL), b) 50 ng avidin-HRP (2 μL of 25 μg/mL), c) 30 ng SA-HRP (3 μL of 10 μg/mL), d) 20 ng SA-HRP (2 μL of 10 μg/mL), and e) 10 ng SA-HRP (1 μL of 10 μg/mL).

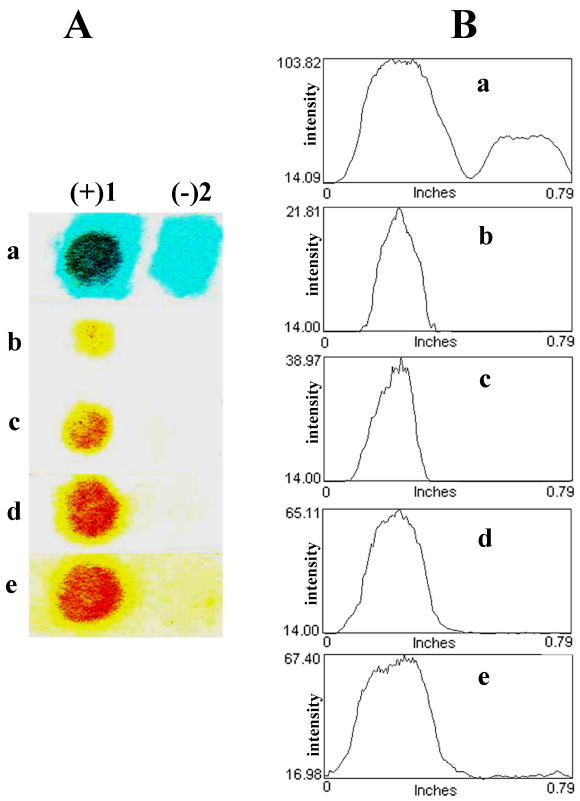

Fig. S11 Representative images (column A: scanned image and column B: profile image) of the test results demonstrating the effect of type and concentration of substrate and reaction time used in the step of enzymatic signal amplification ((+)1= w/ b-DNA and (-)2 = w/o b-DNA) using 20 ng SA-HRP (2 μL of 10 μg/mL): a) 250 μL of 1.6 mg/mL of TMB in DMF/ H_2O (1/9) and 250 μL of 1.6 mg/mL of urea-H_2O_2 for 1 min, b) 250 μL of 0.8 mg/mL OPD and 250 μL of 0.8 mg/mL urea-H_2O_2 for 1 min, and c-e) 250 μL of 1.6 mg/mL OPD and 250 μL of 1.6 mg/mL urea-H_2O_2 for 1, 2 and 5 min, respectively.

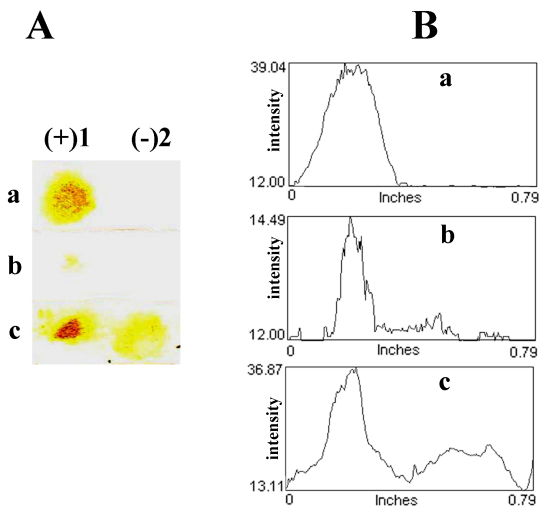

Fig. S12 Representative images (column A: scanned image and column B: profile image) of the test results demonstrating the effect of blocking solution before SA-HRP addition ((+)1 = w/ b-DNA and (-)2 = w/o b-DNA): a) 1% BSA in 0.1 M PBS pH 7.4 containing 0.1 M NaCl, b) 1% skim milk in 0.1 M PBS pH 7.4 containing 0.1 M NaCl, and c) w/o blocking. The tests were performed using the mixture of 250 μL of 1.6 mg/ mg/mL OPD and 250 μL of 1.6 mg/mL urea-H_2O_2 as substrate mixture to activate color for 1 min.

Table S5 Description of DNA and PNA sequences used for each spot of the test results shown in **Fig. 4**.

Entry	Sequence	Spot position					
		1	**2**	**3**	**4**	**5**	**6**
a	DNA	b-DNA SLE2	DNA SLE1	DNA SLE2	DNA SLE2	-	-
	PNA	-	b-PNA SLE2	-	b-PNA SLE2	b-PNA SLE2	-
	Remark	positive (+)	negative (-)	negative (-)	positive (+)	negative (-)	negative (-)
b	DNA	b-DNA SLE2	DNA SLE1	DNA SLE2	DNA SLE2	-	-
	PNA	-	b-PNA SLE1	-	b-PNA SLE1	b-PNA SLE1	-
	Remark	positive (+)	positive (+)	negative (-)	negative (-)	negative (-)	negative (-)
c	DNA	b-DNA SLE2	DNA (AG)	DNA (AC)	DNA (AC)	-	-
	PNA		b-PNA (TG)	-	b-PNA (TG)	-	-
	Remark	positive (+)	negative (-)	negative (-)	positive (+)	negative (-)	negative (-)