Supporting Information

Denaturation methods for reusable magnetic biosensors

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The criteria for screening orthogonal DNA sequences

- Hairpin formation

  To avoid hairpin structures in both probes and targets, we excluded the sequences with a hairpin whose melting temperature (T_M) is above 25 °C and Gibbs free energy (ΔG) is negative.

- Homodimer formation

  To avoid homodimer formation of both probes and targets, we selected the sequences that cannot form homodimers with T_M below 15 °C and ΔG above -9 kcal mol^{-1}.

- Undesired heterodimer formation

  Given that each probe is immobilized on different sensors, heterodimers between different probes are reasonably exclusive and thus heterodimers between non-complementary probes and targets or different targets are the main concern. To avoid the formation of these heterodimers, the sequences that cannot form heterodimers with T_M below 20 °C and ΔG above -9 kcal mol^{-1} were selected.

- Hybridization of complementary pairs

  For the complementary target-probe pairs, the sequences with T_M above 60 °C and ΔG below -30 kcal mol^{-1} were selected.

Table S1. Sequences of the target and probe DNAs and their melting temperatures (T_M)

	Probe and Target Sequences (5' to 3')		T_M* (℃)
P1-1	Probe	/5AmMC6/ TTTTT TTTTT GTTCG TATGC CTAAT TCTAC CAGTA	61.5
	Target	/5Biosg/ TTTTT TTTTT TACTG GTAGA ATTAG GCATA CGAAC	
P1-2	Probe	/5AmMC6/ TTTTT TTTTT TAGTT GATTC GTGAT GGATT GTGCA	63.5
	Target	/5Biosg/ TTTTT TTTTT TGCAC AATCC ATCAC GAATC AACTA	
P1-3	Probe	/5AmMC6/ TTTTT TTTTT CACGA CCCTC TTATA TCCCA GACAG	64.5
	Target	/5Biosg/ TTTTT TTTTT CTGTC TGGGA TATAA GAGGG TCGTG	
P1-4	Probe	/5AmMC6/ TTTTT TTTTT GGCAT CTTTA TCCGA GGAAT TAGCT	63.2
	Target	/5Biosg/ TTTTT TTTTT AGCTA ATTCC TCGGA TAAAG ATGCC	
P2-1	Probe	/5AmMC6/ TTTTT TTTTT TCAAC ATCGG AGGAG TGGGT AGATC	66.0
	Target	/5Biosg/ TTTTT TTTTT GATCT ACCCA CTCCT CCGAT GTTGA	
P2-2	Probe	/5AmMC6/ TTTTT TTTTT TTCGG TTACT TGGGA ATGAT CTGAC	62.9
	Target	/5Biosg/ TTTTT TTTTT GTCAG ATCAT TCCCA AGTAA CCGAA	
P2-3	Probe	/5AmMC6/ TTTTT TTTTT TTCCT TGGTA CTAAT TCTGT GCTCT	61.8
	Target	/5Biosg/ TTTTT TTTTT AGAGC ACAGA ATTAG TACCA AGGAA	
P2-4	Probe	/5AmMC6/ TTTTT TTTTT CCGAC CAAAT TTTCC ACCAA GAGAT	64.1
	Target	/5Biosg/ TTTTT TTTTT ATCTC TTGGT GGAAA ATTTG GTCGG	
P3-1	Probe	/5AmMC6/ TTTTT TTTTT TTGTG ACATT CTAAG TACGT GCCTC	63.0
	Target	/5Biosg/ TTTTT TTTTT GAGGC ACGTA CTTAG AATGT CACAA	
P3-2	Probe	/5AmMC6/ TTTTT TTTTT GTTGG AGTAA ATCGG ATAGG TGAGC	63.6
	Target	/5Biosg/ TTTTT TTTTT GCTCA CCTAT CCGAT TTACT CCAAC	
P3-3	Probe	/5AmMC6/ TTTTT TTTTT GTCTT ATTTG TCGTC AGGTT ACACT	61.3
	Target	/5Biosg/ TTTTT TTTTT AGTGT AACCT GACGA CAAAT AAGAC	
P3-4	Probe	/5AmMC6/ TTTTT TTTTT CCTGA TTAGA CTAGT AGATT CCGCT	61.7
	Target	/5Biosg/ TTTTT TTTTT AGCGG AATCT ACTAG TCTAA TCAGG	
P4-1	Probe	/5AmMC6/ TTTTT TTTTT AGTCC TAGTT TGTGT TTTAG TGCTG	61.7
	Target	/5Biosg/ TTTTT TTTTT CAGCA CTAAA ACACA AACTA GGACT	
P4-2	Probe	/5AmMC6/ TTTTT TTTTT CCAAC TTCCC CTGTA TAATT CCCAA	62.9
	Target	/5Biosg/ TTTTT TTTTT TTGGG AATTA TACAG GGGAA GTTGG	

*Melting temperatures were calculated at 25 ℃ with 150 mM of Na^+, and 0.5 μM of DNA.

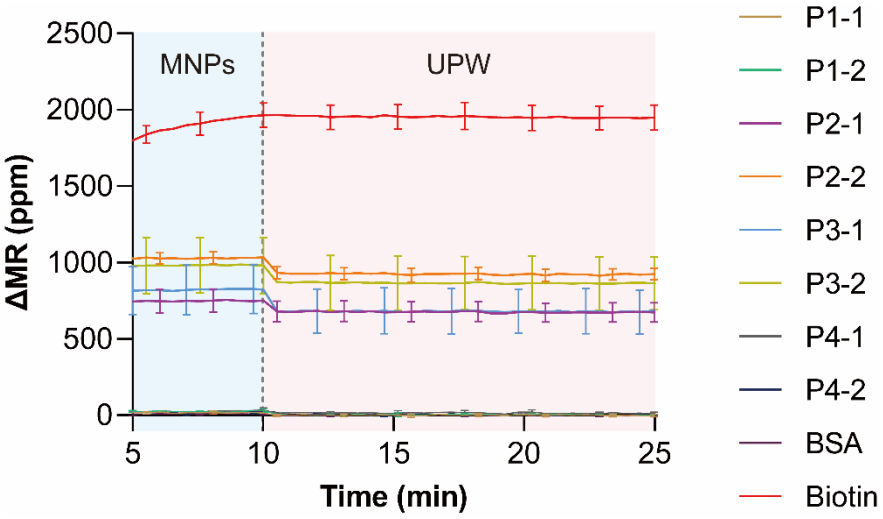

Fig. S1. Real-time signals from sensors treated with UPW. The sensor temperature was maintained at 25 °C, and UPW was added to the chip at 10 min. The error bars represent the SD of four identical sensors (n = 4).

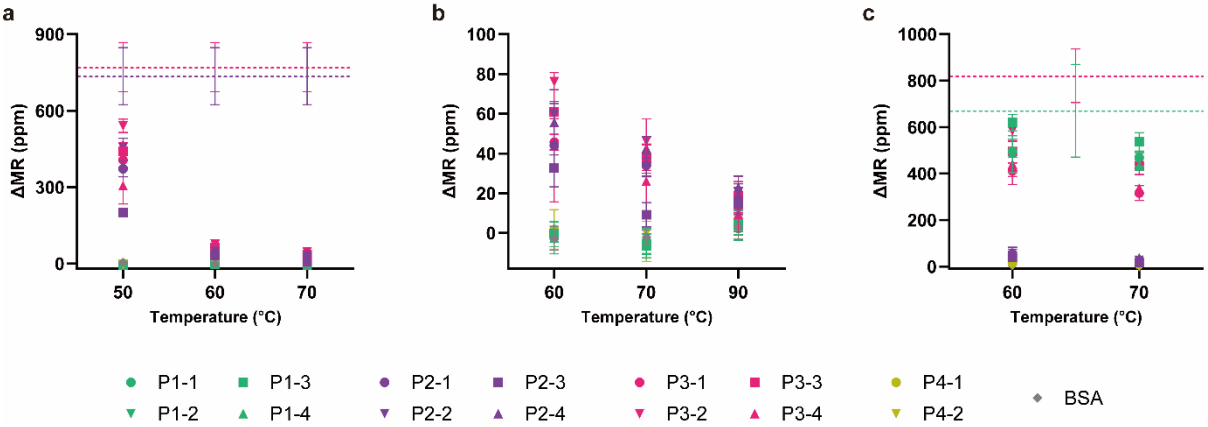

Fig. S2. (a) Remaining GMR biosensor signals after denaturation with TE buffer at 50, 60, and 70 °C for 10 min. Dashed lines indicate the average hybridization signals from sensors in each quadrant before denaturation (purple: P2 and red: P3). (b) Comparison of remaining signals after denaturation with TE buffer at 60, 70, and 90 °C. (c) Hybridization signals (target groups P1 and P3) from the chips regenerated using TE buffer at 60 and 70 °C. Dashed lines represent the average hybridization signals from fresh chips without prior regeneration (green: P1 and red: P3). Error bars indicate the SD from four identical sensors (n = 4).

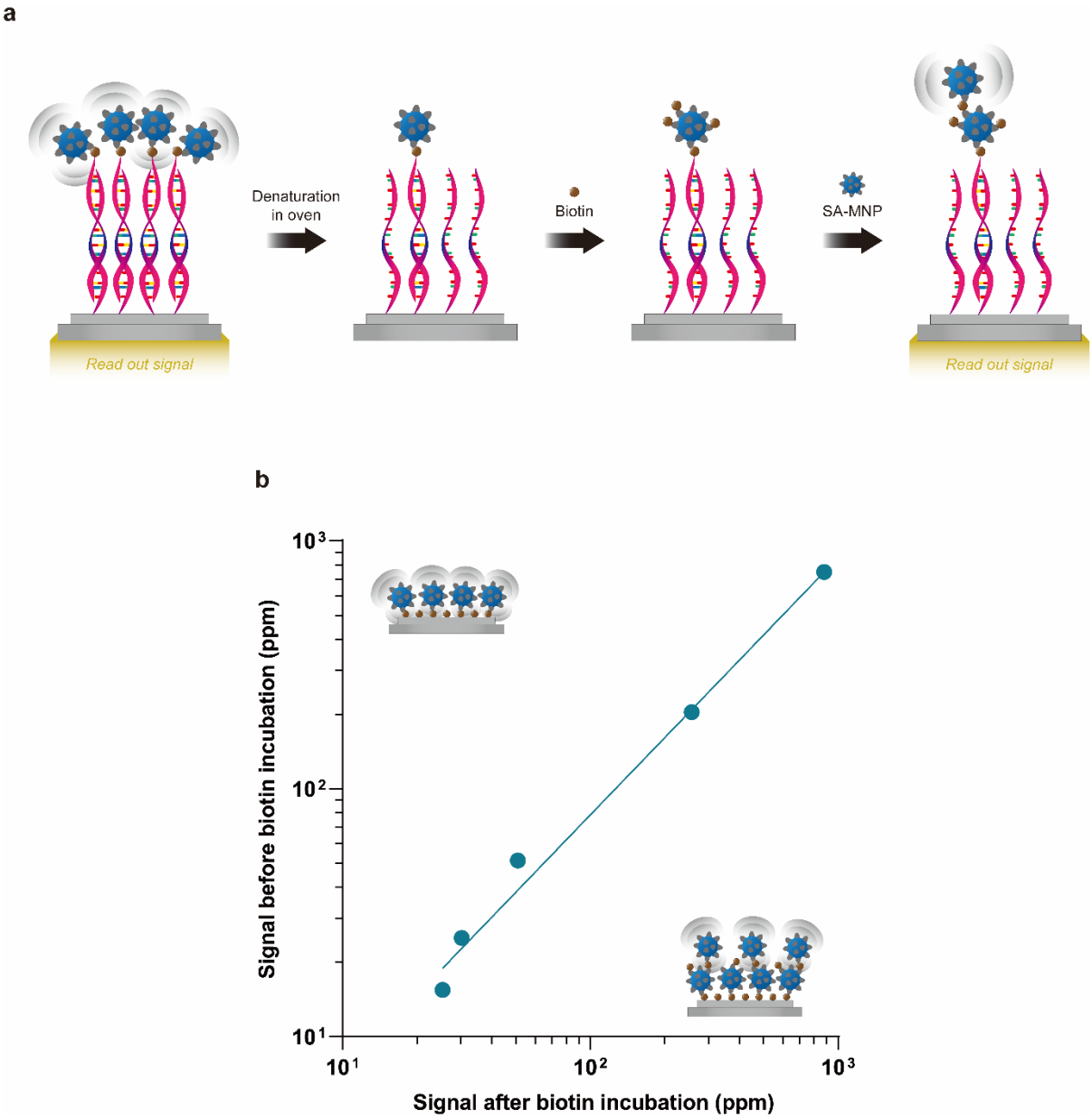

Fig. S3. (a) Schematic of enumerating remaining MNPs (or DNA hybrids conjugated with MNPs) after 90 °C denaturation process by attaching additional MNPs to the remaining MNPs on the sensor surface using biotinylated BSA. First, after obtaining the saturated signals, the cartridges with each denaturant were placed in an oven for different durations: 10, 30, and 60 min. Then, the chips were rinsed outside the oven and incubated with biotinylated BSA. After insertion into the reader stations, streptavidin-coated MNPs were added and the amount of remaining MNPs was estimated. (b) The relation between the signals before (corresponding to the remaining MNPs) and after biotinylated BSA incubation.

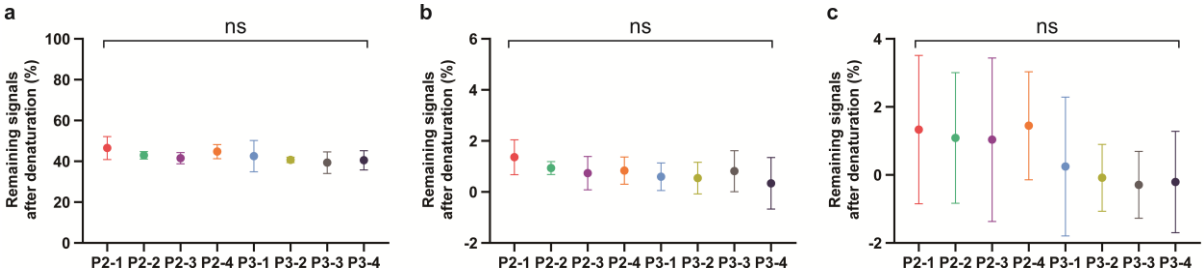

Fig. S4. Normalized remaining DNA hybrid signals for sequences P2 and P3 after denaturation with (a) UPW, (b) urea solution, and (c) TE buffer at 90 °C for 60 min. One-way ANOVA was conducted to compare the effectiveness of each denaturant across different DNA sequences (*p*-value for UPW = 0.4021; urea solution = 0.5716; TE buffer = 0.6894, all not significant [ns]). Error bars indicate the SD from four replicate sensors (n = 4).

Table S2. Normalized average remaining and hybridization signals after denaturation with UPW, urea solution, and TE buffer at 90 °C for indicated incubation times.

Denaturants	Incubation time (min)	Remaining signals after denaturation (%)	Hybridization signals after regeneration (%)
UPW	10	78.4	-*
	30	46.4	-*
	60	42.2	-*
Urea solution	10	2.1	37.1
	30	0.7	19.6
	60	0.8	21.2
TE buffer	10	1.4	82.6
	30	0.2	51.6
	60	0.6	40.9

*The presence of excess unhybridized target DNAs interfered with the hybridization process.

Table S3. Melting temperatures of DNA hybrids with varying DMSO concentrations.

DMSO / T_M (°C)	20 %	30 %	40 %	60 %	80 %
P1-1	61.5	36.5	24.0	11.5	-13.5	-38.5
P1-2	63.5	38.5	26.0	13.5	-11.5	-36.5
P1-3	64.5	39.5	27.0	14.5	-10.5	-35.5
P1-4	63.2	38.2	25.7	13.2	-11.8	-36.8
P2-1	66.0	41.0	28.5	16.0	-9.0	-34.0
P2-2	62.9	37.9	25.4	12.9	-12.1	-37.1
P2-3	61.8	36.8	24.3	11.8	-13.2	-38.2
P2-4	64.1	39.1	26.6	14.1	-10.9	-35.9
P3-1	63.0	38.0	25.5	13.0	-12.0	-37.0
P3-2	63.6	38.6	26.1	13.6	-11.4	-36.4
P3-3	61.3	36.3	23.8	11.3	-13.7	-38.7
P3-4	61.7	36.7	24.2	11.7	-13.3	-38.3
P4-1	61.7	36.7	24.2	11.7	-13.3	-38.3
P4-2	62.9	37.9	25.4	12.9	-12.1	-37.1

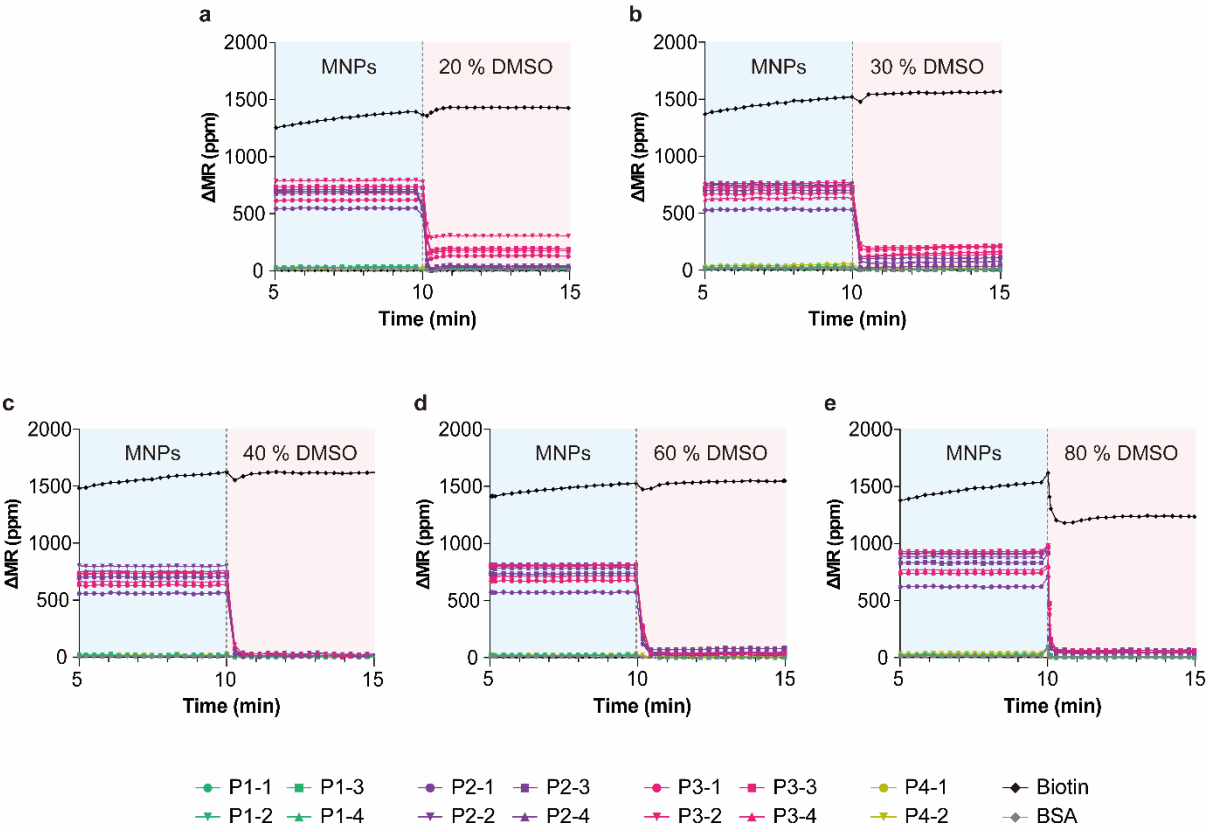

Fig. S5. Real-time denaturation signals when (a) 20 %, (b) 30 %, (c) 40 %, (d) 60 %, and (e) 80 % DMSO were added to each chip.

Table S4. Normalized average remaining and hybridization signals after denaturation with different concentrations of DMSO.

Concentration (%)	Remaining signals after denaturation (%)	Hybridization signals after regeneration (%)
20	17.5	49.8
30	16.8	52.7
40	2.0	64.8
60	4.9	72.5
80	5.8	67.9

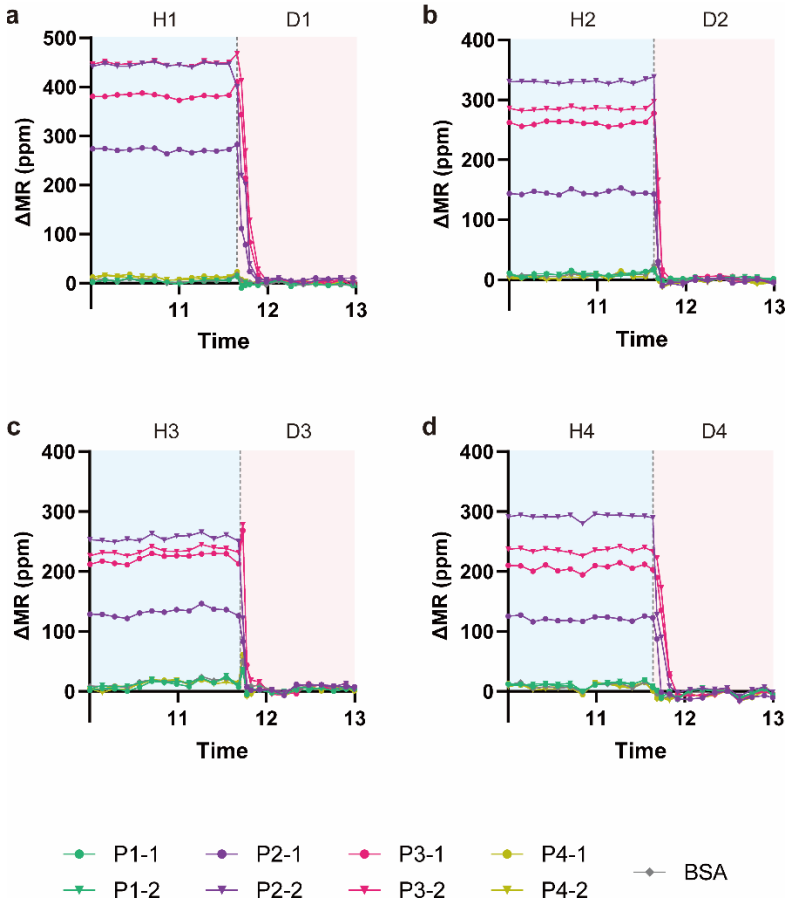

Fig. S6. Real-time denaturation signals during the (a) first, (b) second, (c) third, and (d) fourth regeneration cycles of identical chip using 40 % DMSO, corresponding to Fig. 5. H and D denote signals measured after hybridization and denaturation in each cycle, respectively. Blue shading indicates MNP conjugation to the hybrids (MNPs were added at 1 min; not shown), while red shading represents the application of 40 % DMSO.

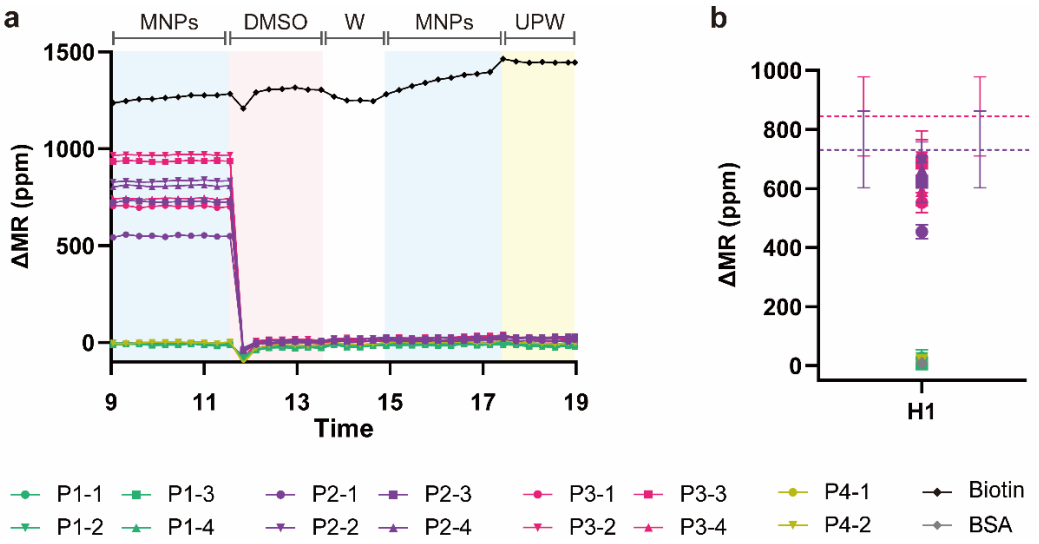

Fig. S7. Assessment of denaturation efficiency and residual target DNA. (a) Saturated signals were obtained after adding MNPs (indicated by first MNPs) and 40 % DMSO was added to denature the target-MNP complexes from the surface-bound probes (DMSO). The chip was briefly washed with washing buffer (W) and MNPs were introduced to the chip (second MNPs) to detect the remaining target DNAs. The chip was rinsed with UPW and re-used to measure the same target DNAs. (b) The same chip was re-incubated with the same target groups P2 and P3 to evaluate the removal of target DNAs and the preservation of the probes. Dashed lines represent the average hybridization signals prior to denaturation (purple: P2 and red: P3). Error bars indicate the SD from four identical sensors (n = 4).

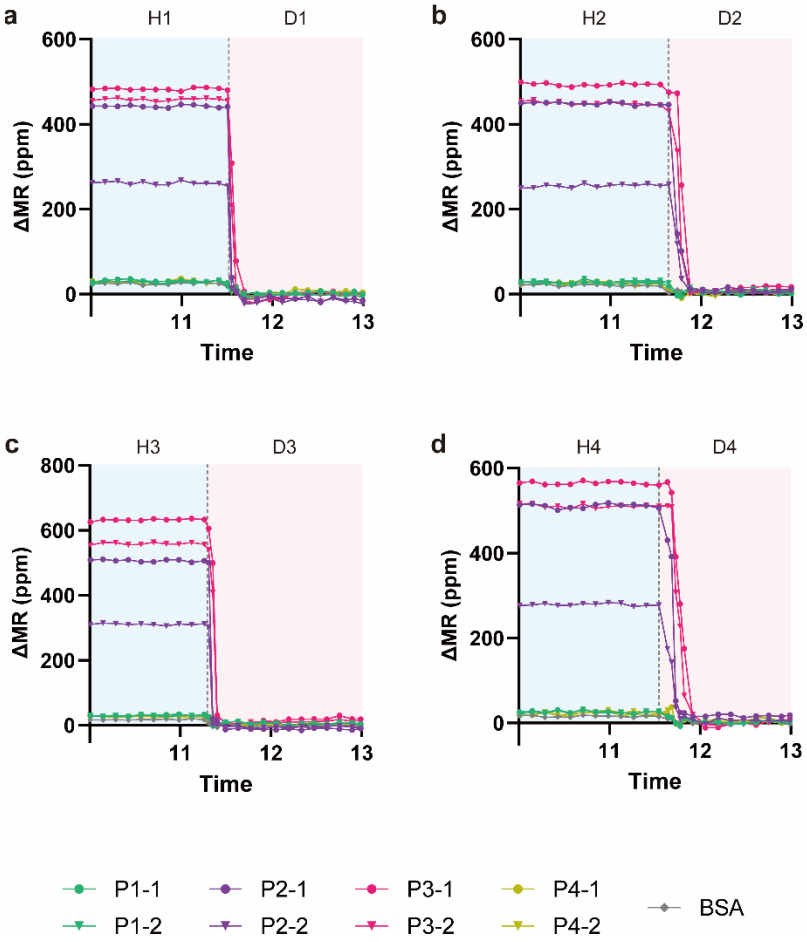

Fig. S8. Real-time denaturation signals during the (a) first, (b) second, (c) third, and (d) fourth regeneration cycles of identical chip using 40 % DMSO, corresponding to Fig. 6. H and D denote signals measured after hybridization and denaturation in each cycle, respectively. Blue shading indicates MNP conjugation to the hybrids (MNPs were added at 1 min; not shown), while red shading represents the application of 40 % DMSO.