Electronic Supplementary Information for:

Multiplexed detection of microRNAs by a competitive DNA microarray-based resonance light scattering assay

Xia Liu[a], Rongrong Tian[a,b], Jiaxue Gao[a,b], Dianjun Liu[a], Zhenxin Wang [a,*]

[a]*State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun 130022, P. R. China,*

[b]*University of Chinese Academy of Sciences, Beijing 100049, P. R. China.*

[*]Corresponding author. Tel./fax: +86 431 85262243. E-mail: wangzx@ciac.ac.cn (ZW)

Additional Experimental Section

Additional Table S1~S5 and Fig. S1~S9

Additional References

1. Additional Experimental Section

1.1. Preparation of GNPs and ssDNAs$_n$@GNPs

The 13 nm GNPs were synthesized by the traditional ‘‘Turkevich-Frens’’ method. [1, 2] ssDNAs$_n$@GNPs were prepared according to a previously developed protocol. [3] Generally, 500 μL 6 nM GNP solution was incubated with 24 μL 40 μM disulfideprotected ssDNA mixture of label ssDNAs and a blocking ssDNA (named La to Lg, Li and L_0, see Table S1 for details) overnight. The mole ratio of each label ssDNA in the total ssDNAs is 1:11. Subsequently, 500 μL 0.2 M NaCl and 10 mM phosphate buffer was added to the mixture. After further incubation for 4 h, the solution was evaporated to 100 μL by vacuum centrifugation. Excess oligonucleotides were removed by repeated centrifugation (9000 rpm, 3 times). Finally, the ssDNAs$_n$@GNPs were redispersed into hybridization solution (1×TAE buffer with 0.1 M NaCl, 0.1% (w/v) SDS) with a final concentration of 5 nM and stored at 4 °C. The multiple ssDNAs conjugated gold nanoparticles were denoted as ssDNAs$_n$@GNPs, where n indicates label DNA species which is a to g, or i, corresponding to miRNA let-7a to 7g, and 7i, respectively.

1.2. Fabrication of 3D slides

The 3D slides were generated and activated as our previously reported method.[4] The aldehydesilane-coated glass slides were immersed into 30 mL solution containing 0.025% of PAMAM G4, G5, G6 dendrimers or different concentrations of PAMAM G6 dendrimers in methanol at room temperature under gentle agitation for 12 h. After washed using 30 mL methanol for 3 min (3 times) and dried by centrifugation (480 g

for 1 min), the slides were soaked in 30 mL $NaBH_4$ (10 mM) for 30 min. Subsequently, the PAMAM dendrimer-modified surface was activated by 30 mL GA solution (5% in PBS buffer (pH 7.4, 50 mM PB, 0.15 M NaCl)) for 4 h, followed by washing with 30 mL PBS buffer (3 times), 30 mL water (3 times), dried by centrifugation (480 g for 1 min) and stored at 4 °C. The activated PAMAM dendrimer-modified slides were named as 3D slides.

1.3. Fabrication of the 3D DNA microarray

The probe ssDNAs (named Pa to Pg and Pi, sequence shown in Table S1) were dissolved in printing solution (3×SSC, 1.5 M betaine, 0.005% (w/v) SDS) with desired concentrations and printed on the 3D slides by a SmartArrayer 136 system (Capitalbio Ltd., Beijing, China) under contact printing mode. After an overnight incubation under 65% humidity at 37 °C, the slides were rinsed with 30 mL washing solution (1×SSC, 0.01% (w/v) SDS) and water for 3 min (3 times) and then incubated in the blocking solution (pH 7.4, 50 mM PB, 0.15 M NaCl supplemented with 5 mg/mL PEG-NH_2) at 30 °C for 1 h to inactivate remaining free aldehyde groups. Finally, the slides were washing using DEPC water for 3 min (3 times) and stored at 4 °C.

1.4. The hybridization reaction on the 3D DNA microarray

Firstly, the $ssDNAs_n$@GNPs were mixed with an equal volume of target ssDNAs (named Tb and Ti, corresponding to the miRNA let-7b and 7i) or miRNA let-7a to 7g, and 7i with different concentrations in hybridization solution (1×TAE buffer, 0.1 M NaCl, 0.1% (w/v) SDS) or hybridization solution supplemented with 10% (v/v) serum.

Then, 80 μL the mixtures were immediately applied to 3D DNA microarrays, respectively. After incubation at 48 °C for 3 h, the slides were subjected to a series of rinsing steps: 30 mL hybridization solution at 48 °C for 5 min (3 times); (2) 30 mL washing solution at room temperature for 5 min (3 times); (4) 30 mL DEPC water for 3 min (3 times), then dried by centrifugation (480 g for 1 min).

1.5. Silver enhancement and detection

After hybridization reaction, 1 mL silver enhancer mixture (1:1 mixed solutions of solution A ($AgNO_3$) and B (hydroquinone)) were applied to each subarray for 8 min, washed with water for 3 min (3 times), and dried by centrifugation (480 g for 1 min). Then, the slides were ready for detection by ArrayIt SpotWare Colorimetric Microarray Scanner (Telechem. International Inc. USA). According to the manufacturer's preset parameters, all images were collected with broad spectrum white light source.

2. Additional Tables and Figures

Table S1. Sequence of DNA oligomers in the assay.

Name	Sequence (5' to 3')	Functionality
Pa	AAAAAACTATACAACCTACTACCTCATCCCT$_5$(CH$_2$)$_6$-NH$_2$	Probe ssDNA for miRNA let-7a
Pb	AACCACACAACCTACTACCTCAT$_5$(CH$_2$)$_6$-NH$_2$	Probe ssDNA for miRNA let-7b
Pc	TCTAAACCATACAACCTACTACCTCAACCCT$_5$(CH$_2$)$_6$-NH$_2$	Probe ssDNA for miRNA let-7c
Pd	AACTATGCAACCTACTACCTCTT$_5$(CH$_2$)$_6$-NH$_2$	Probe ssDNA for miRNA let-7d
Pe	AACTATACAACCTCCTACCTCAT$_5$(CH$_2$)$_6$-NH$_2$	Probe ssDNA for miRNA let-7e
Pf	AACTATACAATCTACTACCTCAT$_5$(CH$_2$)$_6$-NH$_2$	Probe ssDNA for miRNA let-7f
Pg	AACTGTACAAACTACTACCTCAT$_5$(CH$_2$)$_6$-NH$_2$	Probe ssDNA for miRNA let-7g
Pi	AACAGCACAAACTACTACCTT$_5$(CH$_2$)$_6$-NH$_2$	Probe ssDNA for miRNA let-7i
Tb	TGAGGTAGTAGGTTGTGTGGTT	Target ssDNA corresponding to miRNA let-7b
Ti	TGAGGTAGTAGTTTGTGCTGTT	Target ssDNA corresponding to miRNA let-7i
La	AGGTTGTATAGTTT$_{10}$(CH$_2$)$_6$-S-S-(CH$_2$)$_6$OH	Label ssDNA for miRNA let-7a
Lb	AGGTTGTGTGGTTT$_{10}$(CH$_2$)$_6$-S-S-(CH$_2$)$_6$OH	Label ssDNA for miRNA let-7b
Lc	AGGTTGTATGGTTT$_{10}$(CH$_2$)$_6$-S-S-(CH$_2$)$_6$OH	Label ssDNA for miRNA let-7c
Ld	AGGTTGCATAGTTT$_{10}$(CH$_2$)$_6$-S-S-(CH$_2$)$_6$OH	Label ssDNA for miRNA let-7d
Le	AGGTTGTATAGTTT$_{10}$(CH$_2$)$_6$-S-S-(CH$_2$)$_6$OH	Label ssDNA for miRNA let-7e
Lf	GTAGTAGATTGTATAGTTT$_{10}$(CH$_2$)$_6$-S-S-(CH$_2$)$_6$OH	Label ssDNA for miRNA let-7f
Lg	AGTTTGTACAGTTT$_{10}$(CH$_2$)$_6$-S-S-(CH$_2$)$_6$OH	Label ssDNA for miRNA let-7g
Li	AGTTTGTGCTGTTT$_{10}$(CH$_2$)$_6$-S-S-(CH$_2$)$_6$OH	Label ssDNA for miRNA let-7i
L_0	TTTTTTTTTTTTTT$_{10}$(CH$_2$)$_6$-S-S-(CH$_2$)$_6$OH	blocking ssDNA for protecting gold nanoparticles

Table S2. Sequence of miRNA in the assay.

Name	Sequence (5’ to 3’)
miRNA let-7a	UGAGGUAGUAGGUUGUAUAGUU
miRNA let-7b	UGAGGUAGUAGGUUGUGUGGUU
miRNA let-7c	UGAGGUAGUAGGUUGUAUGGUU
miRNA let-7d	AGAGGUAGUAGGUUGCAUAGUU
miRNA let-7e	UGAGGUAGGAGGUUGUAUAGUU
miRNA let-7f	UGAGGUAGUAGAUUGUAUAGUU
miRNA let-7g	UGAGGUAGUAGUUUGUACAGUU
miRNA let-7i	UGAGGUAGUAGUUUGUGCUGUU

Table S3. Comparison of different detection methods for miRNAs.

Name	Detection limit (pM)	Linear range (pM)	Detection method	Reference
miRNA let-7a	0.2	0.2-100	a competitive DNA microarray-based RLS assay	This work
	1	1-5000	a hybridization chain reaction/graphene oxide-based assay	S5
	0.38	1-10000	a circular exponential amplification assay	S6
	0.18	0.1-10000	rolling circle amplification	S7
miRNA let-7b	0.8	0.8-500	a competitive DNA microarray-based RLS assay	This work
	3.2	10-10000	A cascade signal amplification strategy	S8
miRNA let-7c	0.2	0.2-100	a competitive DNA microarray-based RLS assay	This work
miRNA let-7d	0.2	0.2-100	a competitive DNA microarray-based RLS assay	This work
	10	10-400	rolling circle replication	S9
miRNA let-7e	0.2	0.2-100	a competitive DNA microarray-based RLS assay	This work
miRNA let-7f	0.8	0.8-500	a competitive DNA microarray-based RLS assay	This work
	28000	28000-200000	a surface-enhanced Raman scattering-based assay	S10
miRNA let-7g	0.8	4-500	a competitive DNA microarray-based RLS assay	This work
miRNA let-7i	0.8	0.8-200	a competitive DNA microarray-based RLS assay	This work
	1	1-1000	a size-coded ligation-mediated polymerase chain reaction method	S11

Table S4. DLS analysis data for the hydrodynamic diameter of GNPs.

Name	Diameter (d.nm)
GNPs	23.4±1.00
$ssDNAs_n$@GNPs in hybridization solution	61.65±6.83
$ssDNAs_n$@GNPs in hybridization solution with 10% serum	214±13.95

Table S5. The recovery determined using the competitive DNA microarray-based RLS assay via spiking miRNAs into human serum samples.

sample	Spiking value (pM)	Assayed value (pM)	Recovery (%)
miRNA let-7a	5.0	5.1	102.0
	50.0	43.2	86.4
miRNA let-7b	5.0	6.2	124.0
	50.0	62.9	125.8
miRNA let-7c	5.0	5.1	102.3
	50.0	58.4	116.8
miRNA let-7d	5.0	5.8	116.0
	50.0	43.2	86.4
miRNA let-7e	10.0	11.6	116.0
	100.0	118.0	118.0
miRNA let-7f	10.0	12.3	123.0
	100.0	110.8	110.8
miRNA let-7g	10.0	9.6	96.0
	100.0	85.1	85.1
miRNA let-7i	10.0	8.5	85.0
	100.0	91.8	91.8

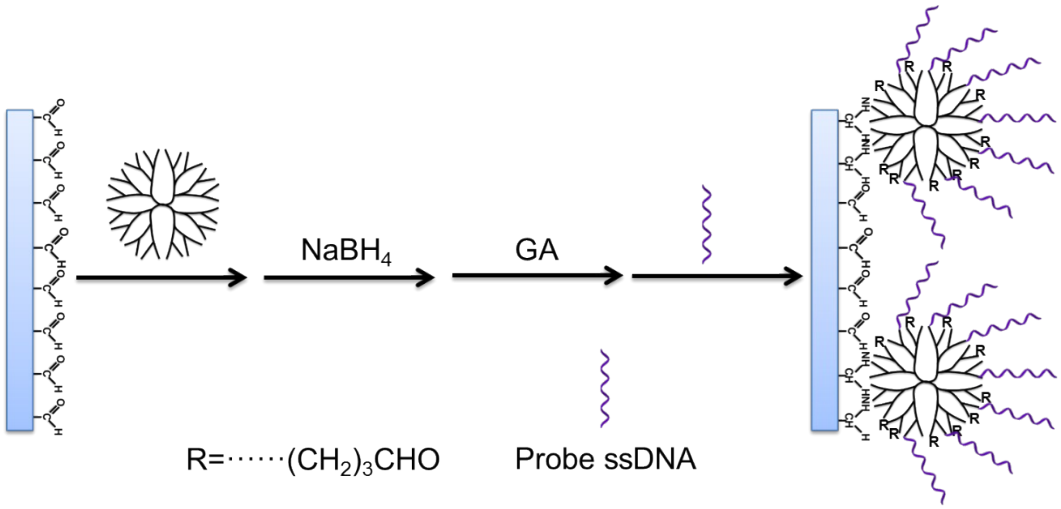

Fig. S1. Fabrication process of 3D DNA microarray.

The 3D DNA microarray was fabricated by our previously reported strategy.[4] Briefly, the PAMAM dendrimer, the monodisperse, hyper-branched and globular polymer, was immobilized on the aldehydesilane-coated glass slide by formation of Schiff base linkage and reduction of $NaBH_4$. Then, the PAMAM dendrimer-modified slide (3D slide) was activated by glutaraldehyde (GA). Subsequently, probe ssDNAs were directly immobilized on the glutaraldehyde-activated 3D slide through the reactions of the aldehyde groups on the external end of dendrimers and amino groups of probe ssDNAs.

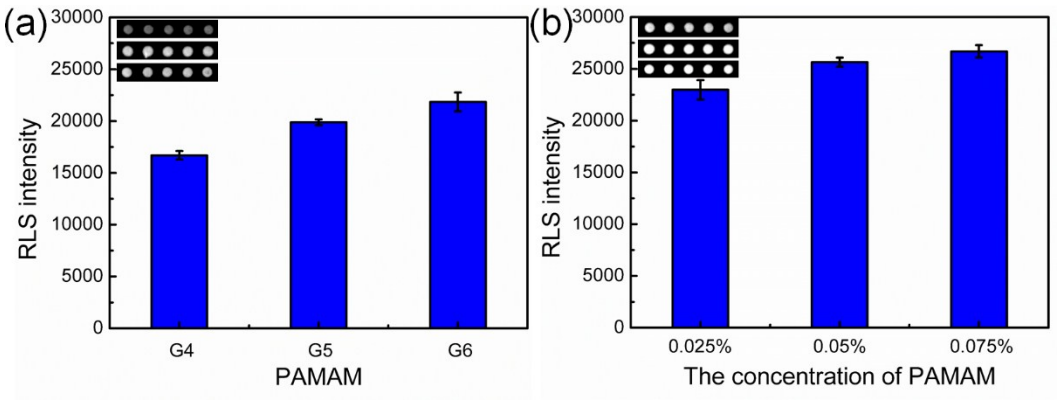

Fig. S2. RLS images (inset) and corresponding RLS intensities of 3D DNA microarrays fabricated on different kinds of dendrimer (0.025 % (w/w) PAMAM G4, G5, G6) (a) and different concentrations of PAMAM G6 dendrimer (b) modified slides. 300 pM ssDNAs$_i$@GNPs were hybridized with immobilized Pi on 3D DNA microarray at 42 °C. The concentration of Pi in printing solution is 0.5 μM.

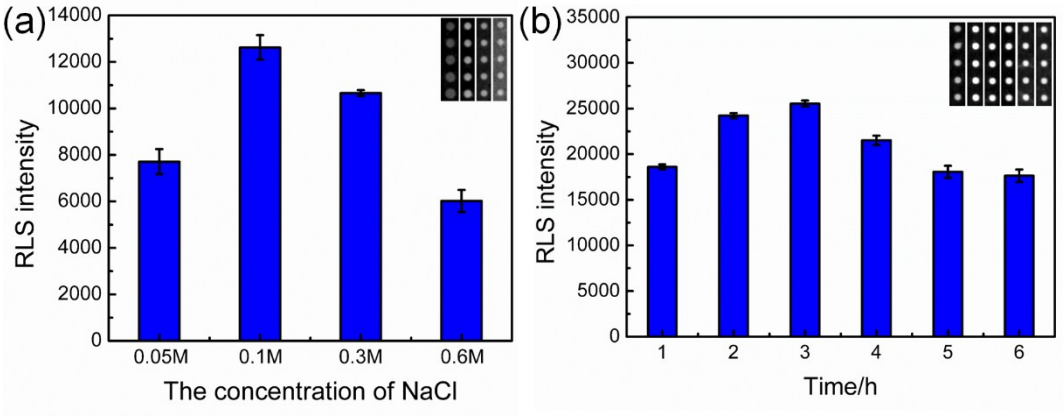

Fig. S3. Effects of ionic strengthen (a) and hybridization time (b) on the hybridization of 5 nM ssDNAs$_i$@GNPs with immobilized Pi on 3D DNA microarray. (a) ssDNAs$_i$@GNPs in hybridization solution containing various concentrations of NaCl were hybridized with immobilized Pi on 3D DNA microarray at 42 °C for one hour, while (b) ssDNAs$_i$@GNPs in hybridization solution containing 0.1 M NaCl were hybridized with immobilized Pi on 3D DNA microarray at 42 °C for different hybridization times. The concentration of Pi in printing solution is 0.2 μM.

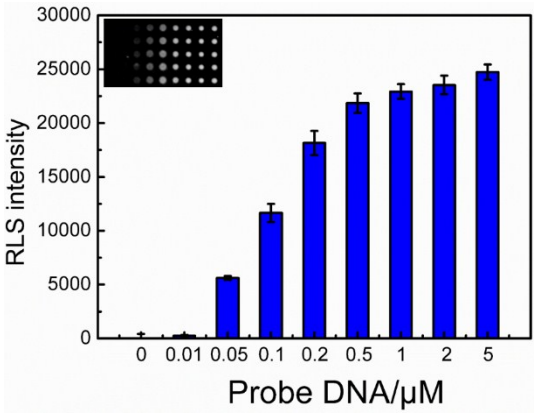

Fig. S4. RLS images (inset) and corresponding RLS intensities of the 3D DNA microarray with different concentrations of Pi in printing solutions: 0 μM, 0.01 μM, 0.05 μM, 0.1 μM, 0.2 μM, 0.5 μM, 1 μM, 2 μM, and 5 μM. The concentration of ssDNAs$_i$@GNPs is 300 pM. The hybridization temperature is 42 °C.

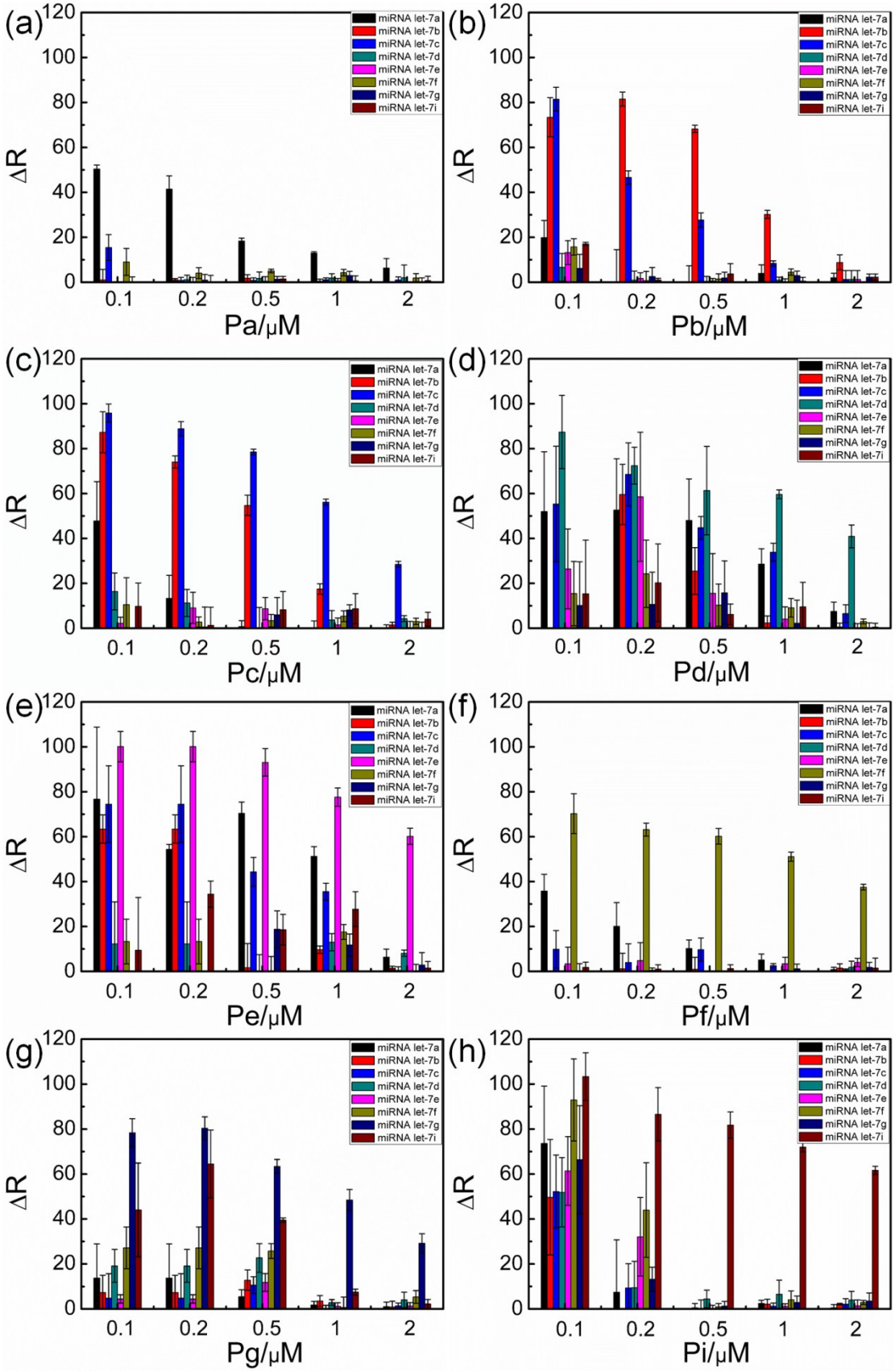

Fig. S5. The effect of probe ssDNA concentration on the assay selectivity. The 8

probe ssDNA with various concentrations (0.1 μM, 0.2 μM, 0.5 μM, 1 μM, and 2 μM) in printing solutions were printed in a subarray which was hybridized with 200 pM pure miRNA (miRNA let-7a to 7g, and 7i) solutions with 30 pM $ssDNAs_n$@GNPs at 48 °C, respectively.

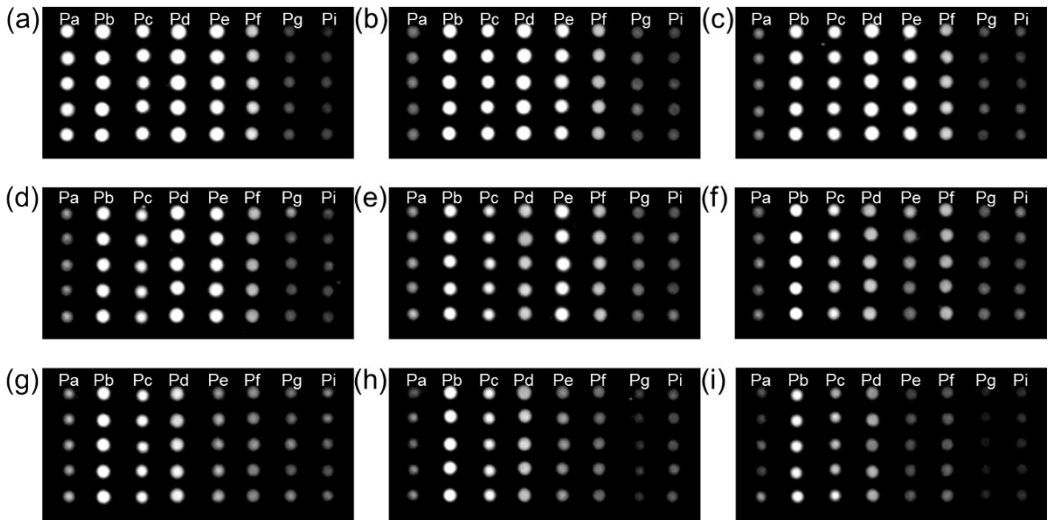

Fig. S6. Selectivity of the competitive DNA microarray-based RLS assay. The subarrays were incubated with different miRNA mixtures: (a) without any miRNA, (b) only miRNA let-7a, (c) miRNA let-7a and 7b, (d) miRNA let-7a to 7c, (e) miRNA let-7a to 7d, (f) miRNA let-7a to 7e, (g) miRNA let-7a to 7f, (h) miRNA let-7a to 7g, and (i) miRNA let-7a to 7g, and 7i, respectively. The subarray contains 8 probe ssDNAs (Pa to Pg, and Pi). The concentrations of Pa to Pg, and Pi in printing solutions are 0.2 μM, 1 μM, 2 μM, 2 μM, 2 μM, 1 μM, 1 μM, and 0.5 μM, respectively. The concentrations of miRNA let-7a to 7g, and 7i are 200 pM. The concentration of $ssDNAs_n$@GNPs is 30 pM. The hybridization temperature is 48 °C.

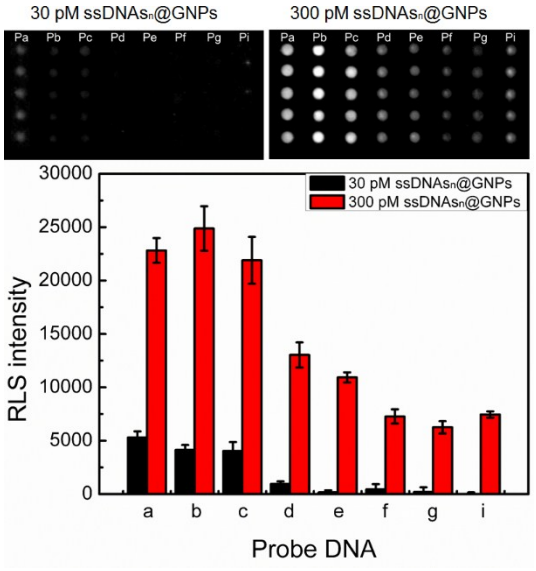

Fig. S7. RLS images (top) and corresponding RLS intensities of a subarray containing eight probe ssDNAs (Pa to Pg, and Pi) after hybridized with 30 and 300 pM $ssDNAs_n$@GNPs in hybridization solution supplemented with 10% serum. The concentrations of Pa to Pg, and Pi are 0.2 μM, 1 μM, 2 μM, 2 μM, 2 μM, 1 μM, 1 μM, and 0.5 μM, respectively. The hybridization temperature is 48 °C.

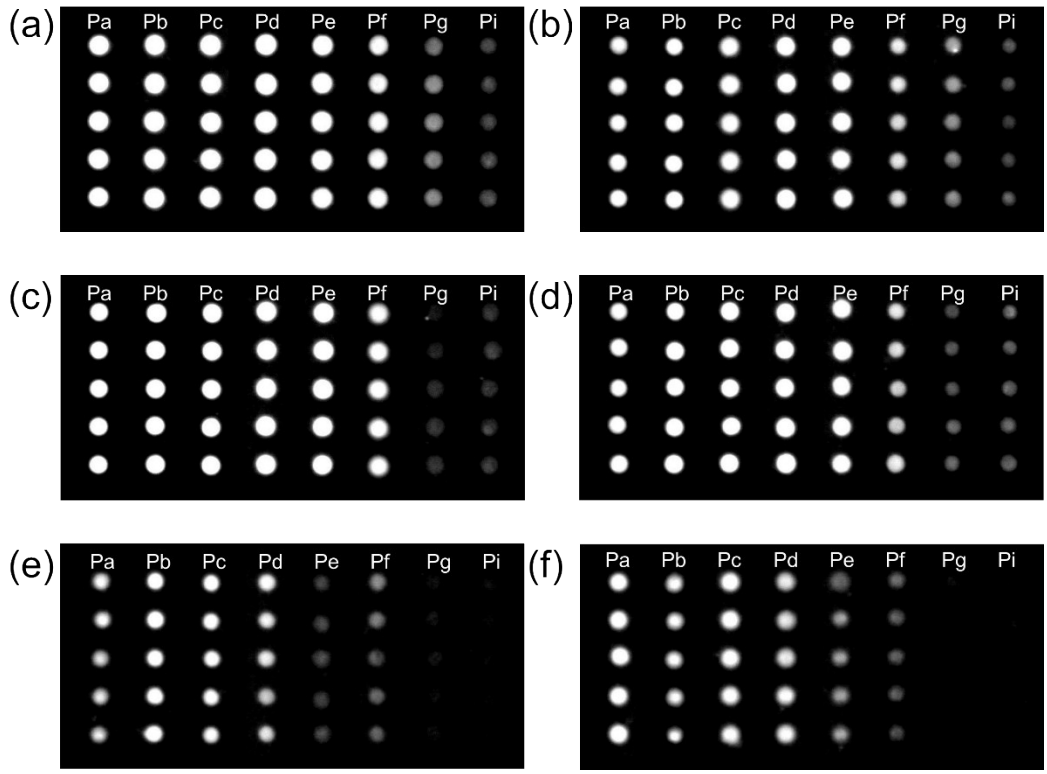

Fig. S8. Cellular miRNA expression levels of different cell lines. The subarrays were incubated with hybridization solution without RNA extracts (a) and with 920 ng total RNA extracts of living A549 (b), SW480 (c), SW620 (d), L-929 (e) and HeLa (f) cells with 30 pM ssDNAs$_n$@GNPs. The concentrations of Pa to Pg, and Pi in printing solutions are 0.2 μM, 1 μM, 2 μM, 2 μM, 2 μM, 1 μM, 1 μM, and 0.5 μM, respectively. The hybridization temperature is 48 °C.

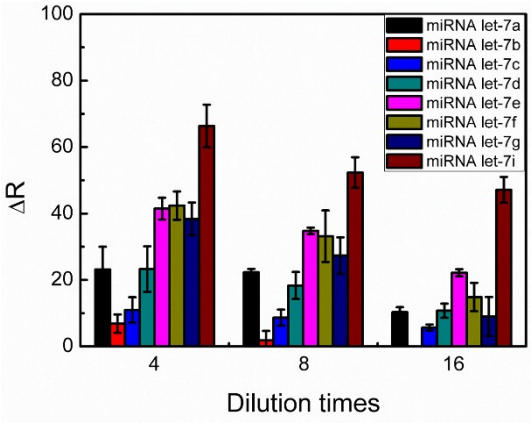

Fig. S9. Data analysis of subarrays after incubated in hybridization solutions with diluted RNA extracts from living L-929 cells. The subarrays were incubated with diluted RNA extracts of living L-929 cells supplemented with 30 pM ssDNAs$_n$@GNPs. The concentrations of Pa to Pg, and Pi in printing solutions are 0.2 μM, 1 μM, 2 μM, 2 μM, 2 μM, 1 μM, 1 μM, and 0.5 μM, respectively. The total RNA concentration is 46 μg/mL. The hybridization temperature is 48 °C.

3. Additional References

S1 G. Frens, *Nat. Phys. Sci.*, 1973, **241**, 20–22.

S2 J. Turkevich, P. C. Stevenson and J. Hillier, *J. Discuss. Faraday Soc.*, 1951, **11**, 55–75.

S3 A. G. Kanaras, Z. X. Wang, A. D. Bates, R. Cosstick and M. Brust, *Angew. Chem., Int. Ed.*, 2003, **115**, 201–204.

S4 X. Li, J. Gao, D. Liu and Z. Wang, *Anal. Methods*, 2010, **2**, 1008–1012.

S5 L. Yang, C. Liu, W. Ren and Z. Li, *ACS Appl. Mater. Interfaces*, 2012, **4**, 6450–6453.

S6 G.-l. Wang and C.-y. Zhang, *Anal. Chem.*, 2012, **84**, 7037–7042.

S7 L.-r. Zhang, G. Zhu and C.-y. Zhang, *Anal. Chem.*, 2014, **86**, 6703–6709.

S8 R. Wang, L. Wang, H. Zhao and W. Jiang, *Biosens. Bioelectron.*, 2016, **86**, 834–839.

S9 F. Xu, H. Shi, X. He, K. Wang, D. He, Q. Guo, Z. Qing, L. a. Yan, X. Ye and D. Li, *Anal. Chem.*, 2014, **86**, 6976–6982.

S10 J. D. Driskell and R. A. Tripp, *Chem. Commun.*, 2010, **46**, 3298–3300.

S11 E. Arefian, J. Kiani, M. Soleimani, S. A. M. Shariati, S. H. Aghaee-Bakhtiari, A. Atashi, Y. Gheisari, N. Ahmadbeigi, A. M. Banaei-Moghaddam and M. Naderi, *Nucleic Acids Res.*, 2011, **39**, e80.