Supplementary Information

Ultrasensitive detection of lung cancer-associated miRNAs by multiple primers-mediated rolling circle amplification coupled with a graphene oxide fluorescence-based (MPRCA-GO) sensor

Krissana Khoothaim[a,b,c], Kiatnida Treerattrakoon[d], Tawin Iempridee[d], Patraporn Luksirikul[e,f], Tararaj Dharakul[*b] and Deanpen Japrung[*d]

[a.]Graduate Program in Immunology, Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

[b.]Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

[c.]Division of Microbiology and Parasitology, School of Medical Science, University of Phayao, Phayao, Thailand

[d.]National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), Thailand Science Park, Pathumthani, Thailand

[e.]Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand

[f.]Center for Advanced Studies in Nanotechnology for Chemical, Food and Agricultural Industries, KU Institute for Advanced Studies, Kasetsart University, Bangkok, Thailand

*Corresponding author: Deanpen Japrung
Email address: deanpen@nanotec.or.th

*Corresponding author: Tararaj Dharakul
Email address: tararaj.dha@mahidol.ac.th

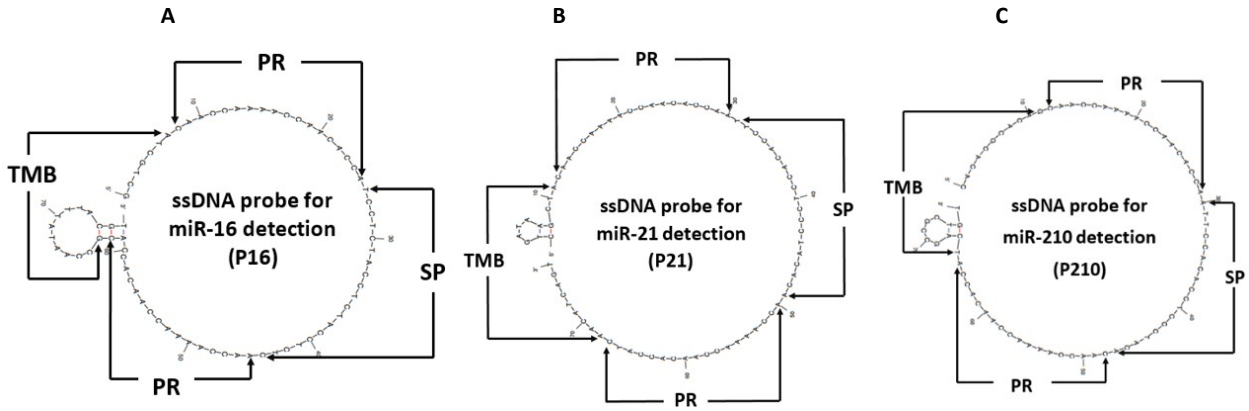

Fig. S1 Secondary structure of ssDNA probe for miR-16 (A), miR-21 (B) and miR-210 (C) detection predicted by using Mfold software analysis. TMB, PR and SP represent target miRNA binding site, primer binding region and signaling probe identical sequence, respectively.

A B

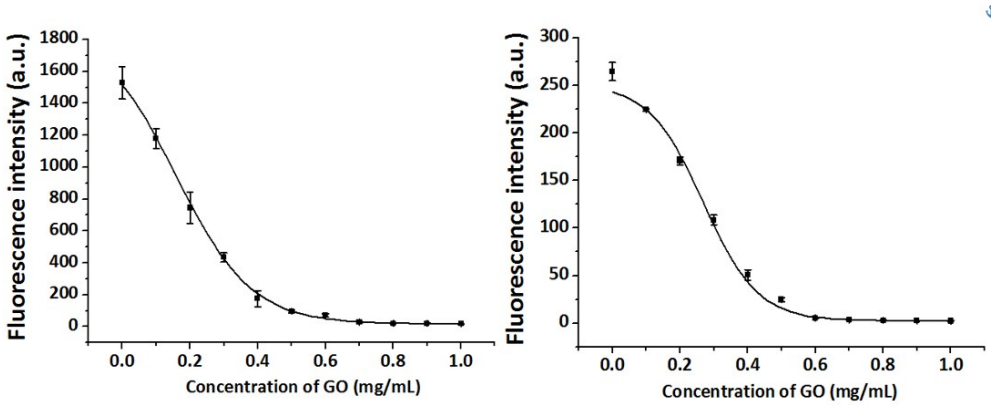

Fig.S2 Effect of GO concentration on the fluorescence intensity of fluorescence labelled ssDNA tag. (A) and (B) are the fluorescence intensity of Cy5-FP and ROX-FP, respectively, in the presence of GO concentration between 0 and 1 mg/mL. The fluorescence spectra of Cy5 and ROX were individually excited and emitted at 630/670 nm and 570/610 nm, respectively.

Table S1. Comparison of sensitivity by different isothermal miRNA detection methods

Limit of detection	Linear rang	Detection methods	References
0.4 pM	1 fM - 50 pM	RCA combined with GO-based fluorometric assay	Hong et al. (2016)[1]
0.38 pM	1 pM - 10 nM	Hairpin probe-mediated circular exponential isothermal amplification (EXPAR)	Wang and Zhang (2012)[2]
290 fM	0.5 pM - 12.5 nM	Electrochemical biosensor coupled with hairpin probe-mediated RCA	Li et al. (2018)[3]
10 fM	0.025 - 1 pM	Branched-RCA (BRCA)	Cheng et al. (2009)[4]
10 fM	0.2 fM - 1 nM	Hairpin probe-mediated RCA	Li et al. (2013)[5]
1.67 fM	5 fM - 50 pM	Electrochemiluminescent biosensor coupled with hybridization chain reaction and hemin	Zhang et al. (2014)[6]
1 fM	1 fM - 100 nM	Ramification amplification (RAM)	Yao et al. (2009)[7]
0.87 fM	1 fM - 10 pM	MPRCA coupled with GO-sensing biosensor	This study
0.68 fM	1 fM - 1 pM	RCA-mediated DNA machine	Zhuang et al. (2014)[8]
0.02 fM	0.02-75 fM	BRCA combined with bioluminescent pyrophosphate detection	Mashimo et al. (2011)[9]

References

1. C. Hong, A. Baek, S. S. Hah, W. Jung and D. E. Kim, Anal Chem, 2016, 88, 2999-3003.
2. G.-l. Wang and C.-y. Zhang, *Analytical Chemistry*, 2012, **84**, 7037-7042.
3. Q. Li, F. Zeng, N. Lyu and J. Liang, *Analyst*, 2018, **143**, 2304-2309.
4. Y. Cheng, X. Zhang, Z. Li, X. Jiao, Y. Wang and Y. Zhang, *Angewandte Chemie* (International ed. in English), 2009, **48**, 3268-3272.
5. Y. Li, L. Liang and C.-y. Zhang, *Analytical Chemistry*, 2013, **85**, 11174-11179.
6. P. Zhang, X. Wu, Y. Chai and R. Yuan, *Analyst*, 2014, **139**, 2748-2753.
7. B. Yao, J. Li, H. Huang, C. Sun, Z. Wang, Y. Fan, Q. Chang, S. Li and J. Xi, *Rna,* 2009, **15**, 1787-1794.
8. J. Zhuang, W. Lai, G. Chen and D. Tang, *Chemical communications* (Cambridge, England), 2014, **50**, 2935-2938.
9. Y. Mashimo, M. Mie, S. Suzuki and E. Kobatake, *Analytical and Bioanalytical Chemistry*, 2011, **401**, 221-227.