Sensing single molecule under micromanipulation to quantify nucleic acids

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Sensing single molecule under micromanipulation to quantify nucleic acids

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Abstract

Rapid molecular diagnosis using nucleic acid biomarkers is important for timely identification of acute pathogenic infections. We introduce an active kinetic approach called sensing single molecule under micromanipulation (SSM^3) to quantify nucleic acids, which circumvents hydrodynamic limits of reaction rate, sensitivity and specificity. We demonstrate the 15-minute assay to detect synthetic miRNAs with subfemtomolar limit of detection and high-confidence discrimination of single-base-pair mismatch.

Nucleic acid biomarkers from various origins have been widely used in disease diagnosis[1], but timely quantification of nucleic acids with sufficient sensitivity has been challenging. The standard assays in clinical settings are based on polymerase chain reaction (PCR), which usually requires time-consuming extraction and amplification steps.[2] For example, the practical nucleic acid tests of coronavirus disease (COVID-19) take hours[3], which could delay the prevention of spread. Several amplification-free approaches have been developed by digitally counting single nucleic acid molecules in either conventional endpoint assays[4, 5] or more advanced kinetic assays.[6-9] Especially, state-of-the-art single-molecule recognition through equilibrium Poisson sampling (SiMREPS) have achieved ultrahigh sensitivity and specificity by exploiting the repeated binding of a short fluorescently labeled DNA probe to an analyte immobilized on sensor surfaces.[6, 10, 11] However, these typically require incubation of analyte molecules onto the sensor surface for at least tens of minutes before detection, due to the limitation by mass transportation and thermodynamics of hybridization.[6, 7, 12, 13] **(Supplementary Note 1)**

Here we present bioconjugated nanoparticles assisted single molecule kinetic assay to circumvent the above limits in nucleic acid detection. In the sensing single molecule under micromanipulation (SSM[3]) approach (**Fig. 1a**), surface plasmon resonance microscopy (SPRM)[14] dynamically images DNA probe conjugated nanoparticles as the reporter of binding and dissociation events (**Fig. 1b**). To beat the diffusion limit, analyte molecules are captured by longer detection probes (12- nucleotides, nt) conjugated to the nanoparticles and actively brought to the sensor surface by applying an external driving force (**Supplementary Fig.1**). The use of longer probes ensures a higher binding rate to capture analyte, but it also inevitably slows down the dissociation rate. Inspired by the single molecule force spectroscopy[15, 16], in SSM[3], we tuned the

dissociation rate by applying a load on the molecular bound through micromanipulation of the nanoparticles to promote the repeated binding (**Supplementary Fig.1 and Note 2**). In our customized SPRM system, each binding event is discriminated to be specific molecular binding or nonspecific background binding by analyzing the bound lifetime related to the distinctive kinetic signature (**Supplementary Fig.2**).

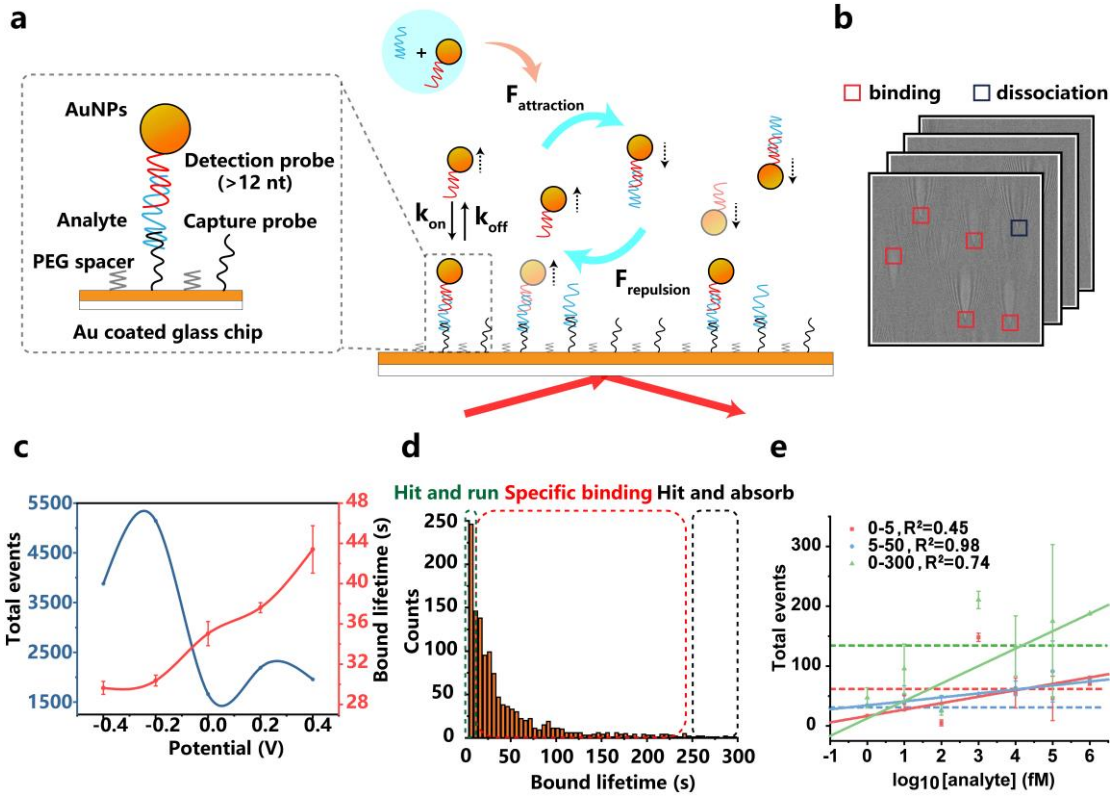

Fig.1 Detection of nucleic acids with SSM[3]. (a) Schematic illustration of SSM[3]. (b) Image acquisition and processing. (c) The tuning of bound lifetime and number of binding and dissociation events with different potential applied. (d) Setting time windows to identify specific bindings of *hsa-miR-29a*. (e) Sensing performance of *hsa-miR-29a* when selecting different time windows for counting binding events including 0-5 s, 5-50 s and 0-300 s in (d). Dashed lines are the control without analyte.

As an example, we explored the micromanipulation of DNA probes conjugated on 50-nm Au nanoparticles (AuNPs) by applying an electrical field between the Au surface and a reference

electrode in solution, owing to the compatibility of SPRM with electrochemical systems.[17, 18] The number of total binding and dissociation events (N) and the bound lifetime (t_b) for a probe with 12-nt complementary bases (**Supplementary Table 1)** were analyzed when different voltages were applied (**Fig. 1c**). Consistent with our expectation, t_b decreased with a pulse force at negative voltages, indicating the tuning of dissociation rate. But N increased by over 3 folds at –0.2 V (**Supplementary Note 3**). With the micromanipulation, the specific bindings to microRNA (miRNA) *hsa-miR-29a* (*miR-29a*) could be identified by applying an appropriate window on the t_b histogram to reject nonspecific bindings (**Fig.1d**). When choosing the 5-50 s window, the sensitivity and linearity in concentration detection were greatly improved (**Fig. 1e**).

While longer detection time ensures higher sensitivity and specificity (**Supplementary Fig. 3**), we demonstrated a 15-minute assay to prove the effectiveness and generality using three miRNAs including *hsa-miR-21-5p* (*miR-21*), *hsa-miR-155-5p* (*miR-155*) and *hsa-miR-362-5p* (*miR-362*) (**Fig.2a**), which is acceptable waiting time in an out-patient visit. Despite varied binding kinetics, t_b were all separated from unspecific bindings by setting a threshold at 5 s (**Supplementary Fig. 4**). Digital counting of only specific binding events provides ultrahigh sensitivity with subfemtomolar limit of detection (LoD) (**Fig. 2b**). The LoD for *miR-21*, *miR-155* and *miR-362* were 0.26, 0.17 and 0.15 fM respectively, with linear detection ranges over 7 orders of magnitude. In the receiver operating characteristic (ROC) plot, the area under curve (AUC) values were 0.997 for *miR-21*, 0.988 for *miR-155* and 0.967 for *miR-362*, indicating high specificity against nonspecific bindings (**Fig. 2c**). A more rigorous threshold setting could further improve the specificity, but at the cost of less countable events.

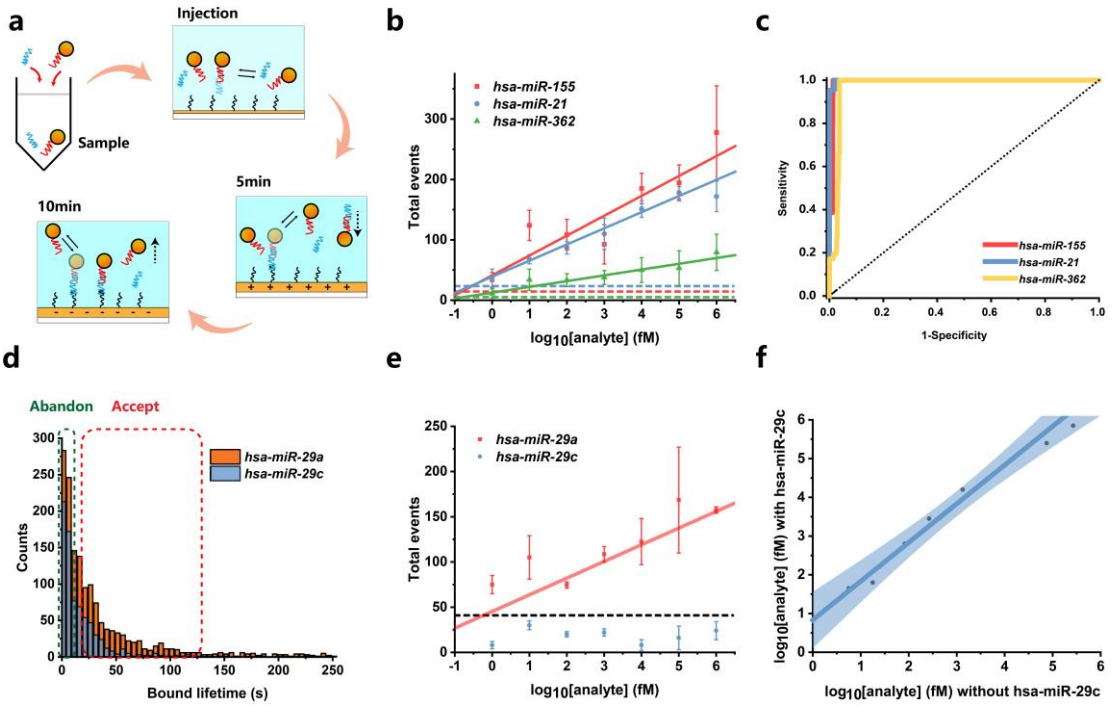

Fig.2 The 15-minute assay for miRNA detection and single nucleotide mismatch discrimination. (a) Workflow of the 15-minute assay. (b) Calibration curves and corresponding (c) ROC curves of three disease-related miRNAs. Dashed lines represent the reference without analyte (mean + 3 × std). (d) Bound time filter for high-confidence distinguishing *hsa-miR-29a* from *hsa-miR-29c*. (e) Calibration curves of *hsa-miR-29a* and *hsa-miR-29c.* The dashed line indicates the number of nonspecific binding events. (f) The detection of *hsa-miR-29a* with or without the presence of 100-fold higher concentration of *hsa-miR-29c*. Blue region indicates the 95% confidence level. All experiments were performed in triplicates.

The 15-minute SSM[3] assay could also discriminate single-base mismatch. Two diseases-related miRNAs, the *miR-29a* and *hsa-miR-29c* (*miR-29c*), were synthesized with a single nucleotide mismatch. The t_b were found to be 21.47 ± 0.45 s and 10.42 ± 0.96 s (mean ± std) respectively with an exponential fitting (**Fig. 2d**). We thus empirically set a threshold at 14 s, and observed the distinct sensing performance for *miR-29a* and *miR-29c* with a discrimination factor over 141 (**Fig. 2e and Supplementary Note 4**). Even in the presence of *miR-29c* at a 100-fold higher concentration, the LoD of *miR-29a* was 0.63 fM with an AUC of 0.816 (**Supplementary Fig. 4**). The

detection of *miR-29a* with or without the existence of *miR-29c* further verified the effectiveness (slope = 0.92, R>0.99) (**Fig.2f**).

We have presented a method for rapid detection of specific nucleic acid biomarkers by fine tuning of molecular binding kinetics through nanoparticle micromanipulation. The SSM^3 enables the 15-minute assay with subfemtomolar detection limit and single base mismatch discrimination, which is a critical step towards practical testing in an outpatient visit.

METHODS

Methods and any associated references are submitted separately.

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AUTHOR CONTRIBUTIONS

H.Y. conceived the idea. Q.Z. Y.Y. and H.Y. designed the experiments. Q.Z., X.Z. and Y.Y. carried out the experiments and analyzed the results. Q.Z., X.Z., Y.Y. J.W., C.Z. and H.Y. interpreted the results and wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing interests.

REFERENCES

1. Schwarzenbach, H., Hoon, D.S. & Pantel, K. *Nat. Rev. Cancer* **11**, 426-437 (2011).
2. White, A.K. et al. *Proc. Natl. Acad. Sci. USA* **108**, 13999-14004 (2011).
3. Aman, R., Mahas, A. & Mahfouz, M. *ACS Synth. Biol.* **9**, 1226-1233 (2020).
4. Xu, W., Yin, P. & Dai, M. *Angew. Chem. Intl. Ed.* **130**, 14271-14275 (2018).
5. Cohen, L., Hartman, M.R., Amardey-Wellington, A. & Walt, D.R. *Nucleic Acids Res.* **45**, e137-e137 (2017).
6. Johnson-Buck, A. et al. *Nat. Biotechnol.* **33**, 730-732 (2015).
7. Sevenler, D., Trueb, J. & Ünlü, M.S. *Proc. Natl. Acad. Sci. USA* **116**, 4129-4134 (2019).
8. Visser, E.W., Yan, J., van IJzendoorn, L.J. & Prins, M.W. *Nat. Commun.* **9**, 1-10 (2018).
9. Canady, T.D. et al. *Proc. Natl. Acad. Sci. USA* **116**, 19362-19367 (2019).
10. Johnson-Buck, A., Li, J., Tewari, M. & Walter, N.G. *Methods* **153**, 3-12 (2019).
11. Hayward, S.L. et al. *J. Am. Chem. Soc.* **140**, 11755-11762 (2018).
12. Squires, T.M., Messinger, R.J. & Manalis, S.R. *Nat. Biotechnol.* **26**, 417-426 (2008).

13. Zhang, D.Y., Chen, S.X. & Yin, P. *Nat. Chem.* **4**, 208-214 (2012).
14. Yang, Y. et al. *Proc. Natl. Acad. Sci. USA* **115**, 10275-10280 (2018).
15. Merkel, R., Nassoy, P., Leung, A., Ritchie, K. & Evans, E. *Nature* **397**, 50-53 (1999).
16. Strunz, T., Oroszlan, K., Schäfer, R. & Güntherodt, H.-J. *Proc. Natl. Acad. Sci. USA* **96**, 11277-11282 (1999).
17. Shan, X. et al. *Nat. Nanotechnol.* **7**, 668 (2012).
18. Shan, X., Patel, U., Wang, S., Iglesias, R. & Tao, N. *Science* **327**, 1363-1366 (2010).

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