Methods 105 (2016) 1–2

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Guest Editor's Introduction

Single molecule probing by fluorescence and force detection

Introduction

The field of single molecule biophysics has rapidly grown in diverse directions. Enabled by improved microscopes and cameras, the temporal and spatial resolution of molecular detection has reached sub-nanometer and microsecond levels. An exciting new trend involves developing combination of fluorescence and force microscopy. Such tools allow for maneuvering and capturing extremely fine movement and rotation of molecules simultaneously. On the other hand, DNA curtain method offers a unique opportunity to study processes on dimensions more relevant to genomic length scale. Another area of growth is the suite of new technology put forth for probing cellular content in its native context. Single molecule pull-down assays developed in several laboratories have established a reliable way of isolating single proteins and protein complexes directly from cell lysate without going through purification procedure [1–3]. Super resolution microscopy technique [4–7] has matured over the past decade and has become a main cell imaging tool in many laboratories. Another exciting area in single molecule research is the DNA sequencing through nanopores, which has led to manufacturing of sequencing chips that enable high throughput single molecule sequencing in a small volume. This review chapter presents recent progresses in all the topics mentioned above (see References below).

References

1. Fareh, M., Loeff, L., Szczepaniak, M., Haagsma, A.C., Yeom, K. H., Joo, C. "Single-molecule pull down for investigating protein-nucleic acid interactions" Method-D-15-00289 R1, 2016
2. Hodeib, S., Raj, S., Manosas, M., Zhang, W., Bagchi, D., Ducos, B., Allemand, J.F., Bensimon, D., Croquette, V., "Single molecule studies of helicases with magnetic tweezers", Method-D-16-00048 R1, 2016
3. Qi, Z., Greene, E., "Visualizing recombination intermediates with single-stranded DNA curtains", Method-D-16-0007 R1, 2016
4. Kamsma, Dl, Creyghton, R., Sitters, G., Wuite, G., Peterman, E. J., "Tuning the music: Acoustic Force Spectroscopy (AFS) 2.0", Method-D-16-00023 R1, 2016
5. Jeong, J., Le, T., Kim, H., "Single-molecule fluorescence studies of DNA looping", Method-D-15-00282 R1, 2016
6. Laszlo, A. H., Derrington, I. M., Gundlach, J., "MspA nanopore as a single-molecule tool: from sequencing to SPRNT", Method-D-15-00281 R1, 2016
7. Seol, Y., Strub, M.P., Neuman, K., "Single molecule measurements of DNA helicase activity with magnetic tweezers and t-test based step-finding analysis", Method-D-16-00040 R1, 2016
8. Cattoni, D., Mutin, T., Chamousset, D., Nollmann, M., "Nanometer resolved single-molecule colocalization of nuclear factors by two-color super resolution microscopy imaging", Method-D-16-0003 R2, 2016
9. Long, X., Parks, J.W., Stone, M., "Integrated magnetic tweezers and single-molecule FRET for investigating the mechanical properties of nucleic acid", Method-D-16-00014 R1, 2016
10. Koeber, M., Dekker, N.H., "High-throughput, high-force probing of DNA-protein interactions with magnetic tweezers", Method-D-15-00248 R1, 2016
11. Koh, H., Wang, X., Myong, S., "Visualizing repetitive diffusion activity of double-strand RNA binding proteins by single molecule fluorescence", Method-D-16-00032 R1, 2016
12. Duboc, C., Graves, E., Strick, T., "Simple calibration of total internal reflection field depth using the supercoiling response of DNA", Method-D-16-00021 R1, 2016

Summary

Two articles presented study of RNA binding proteins by combining single molecule fluorescence and pull-down technique. Fareh et al. demonstrates how to determine function and stoichiometry of proteins (Drosha, Dicer and TUT4) which are involved in RNA silencing pathway. Unlike many other single molecule pull-down applications focusing on stoichiometry determination, they investigated the protein functions using the platform. In particular, they introduce in vivo biotinylation of protein complexes and immobilization on a surface via biotin-streptavidin conjugation, which has not been reported in previous single-molecule pull-down method. Koh et al. provides a comprehensive guide on measuring diffusion activity of double strand (ds) RNA binding proteins which are pulled down from mammalian cell lysate. The ATP-independent sliding of dsRNA binding proteins are visualized by one-color assay termed protein induced fluorescence enhancement (PIFE) developed in the lab, two- and three-color FRET detection method. The same sliding activity is observed in vitro and by pull-down protein, strongly suggesting such mobility as cellular activity.

Hodeib et al. presents a detailed mechanical study of helicases on DNA and RNA using a high resolution magnetic tweezers. In

http://dx.doi.org/10.1016/j.ymeth.2016.06.026

particular, they focus on two configurations referred to as unpeeling and hairpin assays which enable study of helicase induced unwinding from a nick or a fork construct. Such platform led them to discover some unexpected behavior of strand switching and random migration of DNA junctions. Seol et al. also provides a guideline on the usage of magnetic tweezers for DNA helicase activity. They describe an algorithm based on Student's *t*-test by which single molecule trajectories can be analyzed for not only discrete changes but also for continuous transitions. Such automation facilitates data acquisition in varying conditions of salt, ATP and temperature.

By integrating magnetic tweezers and single molecule FRET, Long et al. provides a step by step procedure for assembly of the hybrid instrumentation, preparation of DNA construct which requires digestion, ligation and attachment to two chemically distinct beads and manipulating nanoscale structural transitions of DNA over a wide range of forces. This system allows study of many biologically important secondary structures that form in nucleic acid such as G-quadruplex, trinucleotide repeats, DNA-RNA hybrid and stalled replication forks. Koeber et al. presents a high-throughput, high-force magnetic tweezers for the study of DNA-protein interactions. In particular, they describe a DNA assembly method that emerges from refinement and improvement of the preexisting protocol for designing and producing a wide variety of DNA constructs. Using such DNA substrates, they demonstrate a high-throughput assay on a protein (Tus) binding to DNA hairpin and an enzymatic unwinding activity of a helicase (LTag) on dsDNA.

DNA curtain has enabled studies of genomic processes that occur in kilo bases of DNA. Qi et al. presents thorough stepwise protocol for a recently developed single stranded (ss) DNA curtain. Unlike the dsDNA curtain used in most previous studies, the ssDNA curtain provides a platform to capture molecular event that occurs when the duplex DNA is unwound. Such was showcased by their study of the Rad51 presynaptic complexes in homologous recombination. Remarkably, the study revealed DNA sampling, capture and microhomology domain search process performed by Rad51 presynaptic complexes. Laszlo et al. describes the single molecule picometer resolution nanopore tweezers (SPRNT) which enables detecting the motion of nucleic acid through molecular motors with 40 pm and sub-millisecond resolution. The signal output reports on an exact location of the protein with respect to DNA sequence. This striking sensitivity provides the capability to perform single molecule DNA sequencing. Many exciting future applications await.

Despite the rigidity, dsDNA displays a propensity to form a loop. Jeong et al. utilizes single molecule FRET to study DNA looping and performs an extensive theoretical calculations to reexamine the J factor between experimental and theoretical settings. In addition, they demonstrate an interesting case of DNA looping assisted protein diffusion mechanism, termed intersegmental transfer using the one color PIFE assay aforementioned. Also taking advantage of dsDNA looping, Duboc et al. presents a combination of magnetic trap and fluorescence microscope to image proteins acting on DNA. Interestingly, they used this tool to calibrate the evanescent field used for total internal reflection (TIR) microscope. They demonstrate how the TIR field depth can be mapped as a function of the angle of incident beam.

The Acoustic Force Spectroscopy (ASF) is a newly improved high throughput single molecule method designed for examining structural and mechano-chemical properties of biomolecules. Kamsma et al. describes detailed steps involved in modeling, optimization and experimental validations of the ASF. The new generation ASF provides an innovative solution by implementing a transparent piezo, high numerical aperture objectives and optimizing force profile with a MATLAB modeling. Last but not least, Cattoni et al. presents a protocol for two color single molecule localization microscopy (SMLM) which essentially combines PALM and STORM. The main advantage of SMLM is that two or multiple components labeled with distinct colors can be imaged simultaneously. Detailed protocol on sample preparation, cell fixation, antibody labeling, data acquisition and software data processing is covered in the article.

As demonstrated clearly in this issue, the field of single molecule biophysics has far exceeded the stage of proof of concept measurement and moved deeply into probing molecules in physiologically relevant regime. It is truly exciting to witness the harmonious balance between the development of new tools and the application to new biology. Looking into the future, it will be critically important to maintain this balance while engaging into more biological and potentially biomedical problems.

References

[1] A. Jain, R. Liu, B. Ramani, E. Arauz, Y. Ishitsuka, K. Ragunathan, J. Park, J. Chen, Y. K. Xiang, T. Ha, Nature 473 (2011) 484–488.
[2] K.H. Yeom, I. Heo, J. Lee, S. Hohng, V.N. Kim, C. Joo, EMBO Rep. 12 (2011) 690–696.
[3] H.W. Lee, T. Kyung, J. Yoo, T. Kim, C. Chung, J.Y. Ryu, H. Lee, K. Park, S. Lee, W.D. Jones, D.S. Lim, C. Hyeon, W.D. Heo, T.Y. Yoon, Nat. Commun. 4 (2013) 1505.
[4] M.J. Rust, M. Bates, X. Zhuang, Nat. Methods 3 (2006) 793–795.
[5] M. Kaksonen, D.G. Drubin, Dev. Cell 11 (2006) 438–439.
[6] K.I. Willig, S.O. Rizzoli, V. Westphal, R. Jahn, S.W. Hell, Nature 440 (2006) 935–939.
[7] G. Shtengel, J.A. Galbraith, C.G. Galbraith, J. Lippincott-Schwartz, J.M. Gillette, S. Manley, R. Sougrat, C.M. Waterman, P. Kanchanawong, M.W. Davidson, R.D. Fetter, H.F. Hess, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 3125–3130.

Sua Myong
Biophysics department, Johns Hopkins University, United States
E-mail address: smyong1@jhu.edu

Available online 30 June 2016