EuroSciCon

17th Edition of International Conference on

Emerging Trends in Materials Science and Nanotechnology

April 26-27, 2018
Rome, Italy

Joon Won Park, Nano Res Appl, Volume:4
DOI: 10.21767/2471-9838-C1-007

SINGLE DNA SEQUENCING AND VISUALIZATION OF MIR-134 IN NERVE CELLS WITH FORCE-BASED AFM

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Single-molecule sequencing methods have been developed to analyze DNA directly without the need for amplification. Here, we present a new approach to sequencing single DNA molecules using atomic force microscopy (AFM). In our approach, four surface-conjugated nucleotides were examined sequentially with a DNA polymerase-immobilized AFM tip. By observing the specific rupture events upon examination of a matching nucleotide, we could determine the template base bound in the polymerase's active site. The subsequent incorporation of the complementary base in solution enabled the next base to be read. Additionally, we observed that the DNA polymerase could incorporate the surface-conjugated dGTP when the applied force was controlled by employing the force-clamp mode. MicroRNAs (miRNAs) play key roles in controlling various cellular processes, and the expression levels of individual miRNAs can be considerably changed in pathological conditions such as cancer. Accurate and precise quantification of miRNA at the single-cell level will lead to a better understanding of miRNA function. Here, we present a direct and sensitive detection method for a specific miRNA using AFM. A hybrid binding domain (HBD)-tethering tip enabled mature miRNAs to be located individually on an adhesion force map. By scanning several sections of a micron-sized DNA spot, we were able to quantify the copy number of miR-134 from a single neuron and demonstrated that the expression was increased upon the cell activation. Additionally, we visualized individual miR-134s on fixed neurons after membrane removal and observed 2-4 miR-134s in the area of 1.0x1.0 m2 of soma. The number increased to 8-14 in stimulated neurons, and this change matches with the ensemble-averaged increase in copy number.

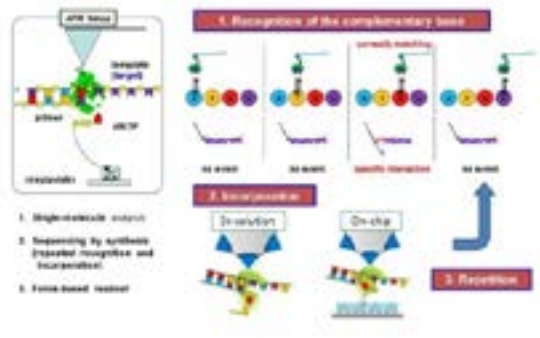

Figure 1: Three steps for the single DNA sequencing with AFM

Recent Publications

1. Hyun-Seo Koo, Ikbum Park, Yoonhee Lee, Hyun Jin Kim, Jung Hoon Jung, Joo Han Lee, Youngkyu Kim, Joung-Hun Kim and Joon Won Park (2016) Visualization and quantification of microRNA in a single cell by AFM. Journal of the American Chemical Society 138(36):11664.
2. Yoonhee Lee, Youngkyu Kim, Donggyu Lee, Dhruvajyoti Roy and Joon Won Park (2016) Quantification of fewer than ten copies of a DNA biomarker without amplification or labeling. Journal of the American Chemical Society 138(22):7075.
3. Woong Kim, Nara Kim, Joon Won Park and Zee Hwan Kim (2016) Nanostar probes for tip-enhanced spectroscopy. Nanoscale 8(2):987.
4. Dae Heon Kim, Jae-Eun Lee, Zheng-Yi Xu, Kyeong Rok Geem, Yun Kwon, Joon Won Park, Inhwan Hwang (2015) Cytosolic targeting factor AKR2A

captures chloroplast outer membrane-localized client proteins at the ribosome during translation, Nature Communications 6:6843.

5. Youngkyu Kim, Eung-Sam Kim, Yoonhee Lee, Joung-Hun Kim, Bong Chu Shim, Seong Moon Cho, Jeong Soo Lee and Joon Won Park (2014) Reading single DNA with DNA polymerase followed by atomic force microscopy. Journal of the American Chemical Society 136(39):13754

Biography

Joon Won Park has obtained his PhD at California Institute of Technology in 1988, and worked in Northwestern University as a Postdoctoral Fellow for two years, he started an independent professional career at Pohang University of Science and Technology (or POSTECH) in 1990. His initial research was focused on self-assembled monolayer and created a dendron-modified surface that provides regular nanoscaled spacing between immobilized molecules. He combined the proprietary approach with force-based atomic force microscopy. As a result, he was able to increase the reliability, reproducibility and accuracy of the analysis. He has been keen on analyzing various biomarkers including DNA, mRNA, microRNA, and protein of low abundance quantitatively without amplification as well as manipulating single biomolecules and mapping biomolecules at high resolution. He was the Head of Chemistry Department and is a Founder of NB POSTECH Inc. (a Pohang-based venture) and Nanogea Inc. (Los Angeles-based venture).

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