

BIOLOGICAL APPLICATION OF NANO-SCALE IMAGING AND SINGLE-MOLECULE MANIPULATION TECHNIQUES

Kunio Takeyasu, Masatoshi Yokokawa, Hirohide Takahashi, Yasuhiro Hirano, R.L. Ohniwa, Kohji Hizume and Shige H. Yoshimura

Graduate School of Biostudies, Kyoto University, Kyoto, Japan

*Corresponding author: takeyasu@lif.kyoto-u.ac.jp (Tel: +81 75 753 7905)

We have been using atomic force microscopy (AFM) for studying the structural organization and dynamics of various biological macromolecules and assemblies [1-5]. Here we shall summarize our most recent results using AFM.

The topics include: (1) Similarities and differences between the eukaryotic and prokaryotic genome organizations in cells. (2) Importance of the topology controls of DNA in architecturing the higher-order structures. (3) Application of fast-scanning AFM to the analyses of enzyme reaction. (4) Development of a novel method for a site-specific attachment of any glutathione S-transferase (GST)-fused proteins to the cantilever in a desired direction, which allows the applications to the measurement of interaction between chromatin and inner nuclear membrane proteins such as the lamin B receptor (LBR). (5) Successful application of the PicoTrec™ mode that can simultaneously obtain a topographic image together with a recognition signal by using protein- (antibody-) coupled cantilever (recognition imaging). Using the PicoTrec™ mode combined with our GSH- and antibody-cantilevers, we could detect specific interactions between LBR and chromatin, and between DNA and nuclear matrix proteins such as SP120.

[References]

1. J. Kim, S.H. Yoshimura, K. Hizume, R.L. Ohniwa, A. Ishihama & K. Takeyasu (2004) A fundamental structural unit of the *Escherichia coli* nucleoid revealed by atomic force microscopy. *Nucleic Acid Res.*, 32: 1982-1992.
2. K. Hizume, S.H. Yoshimura & K. Takeyasu (2005) Linker histone H1 *per se* can induce three-dimensional folding of chromatin fiber. *Biochemistry*, 44: 12978-12989.
3. S.H. Yoshimura, H. Takahashi, S. Ohtsuka & K. Takeyasu (2006) Development of glutathione-coupled cantilever for the single-molecule force measurement by scanning force microscopy. *FEBS Lett.*, 580: 3961-3965.
4. M. Yokokawa, C. Wada, T. Ando, N. Sakai, A. Yagi & K. Takeyasu (2006) Single-molecule reaction analysis reveals the ATP-ADP-dependent conformational changes of chaperonin GroEL. *EMBO J.*, 25: 4567-4576.
5. R.L. Ohniwa, K. Morikawa, J. Kim, T. Ohta, A. Ishihama, C. Wada & K. Takeyasu (2006) Dynamic state of DNA topology is essential for genome condensation in bacteria. *EMBO J.*, 25: 1-13.