Single Molecular Detection of Transcription Factors Bound to DNA-Probe by AFM

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We have proposed a novel method for the detection of DNA-binding proteins from image analysis using atomic force microscopy (AFM). The method is based on the design of a double-stranded DNA probe, which is containing the binding sequence site of a DNA-binding protein target and site-specific labeling of DNA probe. In this method, 5'-biotinylated double-stranded DNA probe is synthesized by PCR and labeled site specifically through high affinity with streptavidin. The DNA probes were immobilized on a mica surface by the adsorption of streptavidin that attached to the 5'-end of DNA and applied for selection of the target protein in solution, and then AFM was used to image DNA prove-protein complexes. The measurement of the distance between 5'-labeled streptavidin and protein bound on DNA probes from an AFM image allowed us to identify the DNA-binding protein target. Here we describe the detection of estrogen receptor-α (ER-α) as the proof-example.

A 518 bp-long (or about 176 nm) DNA probe was made by PCR from a plasmid template (Fig.1). At the 5' end of the probe, biotin is covalently attached. A consensus oligonucleotide sequence that is binding site of estrogen receptor-α (ER-α) was inserted in the probe prior to PCR. Streptavidin was then bound to the biotin probe. Streptavidin functions as a pin-holder to the mica substrate and as 5' label. The probe is the physically immobilized onto a treated mica substrate. A solution containing ER-α protein was dropped onto the DNA immobilized on mica surface. After incubation and other treatments, the mica surface was observed by AFM.

ER-α could be observed on the DNA prove containing binding site of ER-α by AFM (Fig. 2). Specific binding of particular DNA-binding protein is observed by measuring the distance between the sites where binding was occur, to the streptavidin label. This distance can then be compared to the expected distance value obtained during probe design.

We have established the method for detecting DNA binding protein with 5'-streptavidin labeled DNA probe, using AFM as the sensitive analytical tool. The next step is simultaneous detection of several kinds of proteins.

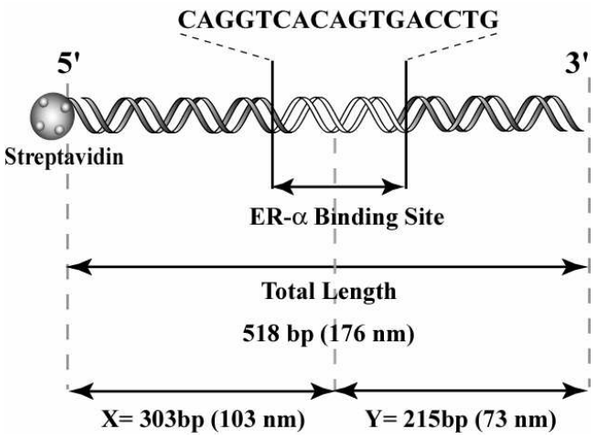

Fig. 1 Design of DNA probe for detecting ER-α

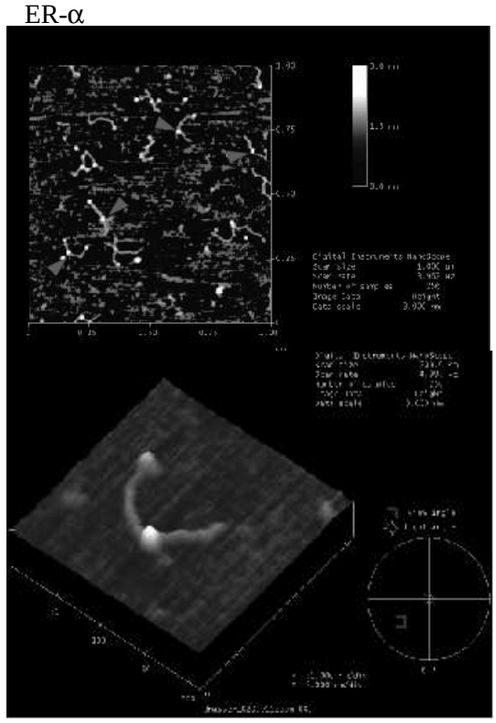

Fig. 2 The AFM image of the complex between ER-α and DNA-probe