STABILITY OF THIOL-IMMOBILIZED DNA ON MICROCANTILEVER SENSORS

Karen A. Stevenson, Adosh Mehta,
Karolyn M. Hansen, and Thomas G. Thundat
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

Sensors designed on the AFM microcantilever platform, either singly or in array format, show great potential. Recent studies have demonstrated the utility of microcantilevers as biosensors for the detection of antibody:antigen interactions[1] and DNA hybridization and mismatch detection[2-4]. Biomolecule interaction is detected as bending of the bimetallic cantilevers with the specific sensing layer immobilized on only one side of the cantilever. Cantilevers are typically gold-coated silicon (or silicon nitride) and biomolecule immobilization strategy is typically thiol-based due to the high affinity of thiols for gold.

Enzymatic manipulation of immobilized biomolecules, either for detection of interaction or for sensing layer patterning, often requires the use of thiol-containing reducing agents (e.g. DTT (dithiothreitol), β-mercaptoethanol) in reaction buffers. Thiol exchange at surfaces is known to occur when additional thiol compounds are available in overlying solutions; magnitude and rate of exchange have been attributed to size and concentration of thiolated compounds, surface coating defects, and surface morphology[5-9].

In this study, gold-coated rectangular silicon cantilevers (Silicon-MDT, Moscow) were functionalized with double-stranded thiolated DNA and exposed to reaction buffer without and then with the small reducing agent DTT (dithiothreitol). Significant cantilever deflection occurred upon exposure to buffer containing DTT (figure 1) indicating either reaction of the DTT on the gold side (at interstitial locations) or exchange of immobilized DNA for DTT. To verify thiol exchange at the surface, microcantilevers were functionalized with thiolated fluoroscein-labeled dsDNA and exposed to DTT-containing reaction buffer. Cantilevers exposed to reaction buffer without DTT retain fluorescence (figure 2A) while those exposed to buffer containing DTT retain little or no fluorescence (figure 2B). These results indicate that stability of thiol-immobilized biomolecules at gold surfaces is reduced in the presence of free thiol compounds present in overlying solutions. While surface functionalization strategies employing thiolated biomolecules on gold surfaces are efficient and of great utility, consideration should be given to any subsequent manipulation of the surfaces to avoid the possibility of sensing layer exchange and loss.

ACKNOWLEDGEMENTS

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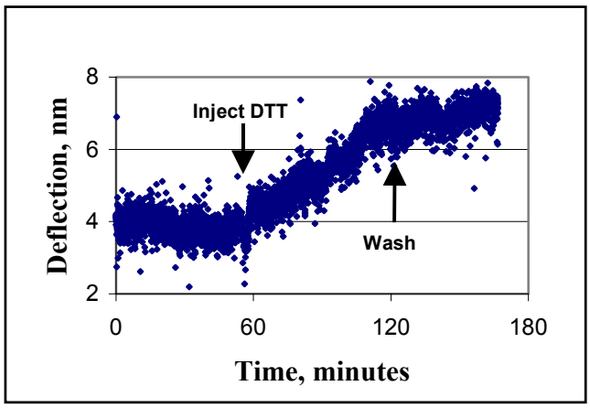

Figure 1. Deflection of thiol-DNA-functionalized Au/Si microcantilever exposed to buffer containing DTT (dithiothreitol).

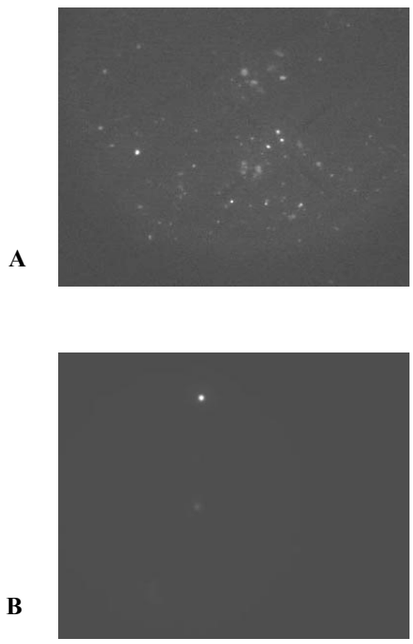

Figure 2. Fluorescence images of thiol-DNA-functionalized Au/Si microcantilevers exposed to: A. buffer without DTT; B. buffer with DTT. (Scan size 44 x 38 microns.)