Supporting Information

Analytical Formulation of Cantilever Mechanics. The resonant frequency of a cantilever beam is a function of its spring constant and its effective mass. In this work, the cantilever beams were rectangular, and the idealized theoretical spring constant can be obtained by using values of cantilever parameters such as Young's modulus of the cantilever material and the dimensions of the cantilever beams (1). When the protein layer attached on the cantilever beam is in the same thickness range as the cantilever beam, an increase in the overall spring constant value is expected, even if the Young's modulus of the attached layer is much less than that of the cantilever beam material. The spring constant of the cantilever beam with the attached layer is given as

$$k_{C+A} = \frac{E_{\text{comp}}(t_C + 2t_A)^3 W_C}{4L_C^3}, \qquad [1]$$

where E_{comp} is the Young's modulus of the composite layer and can be extracted by using the method of transformed section of a composite material (2). The effective mass of the composite cantilever beam is given as

$$m_C + m_A = 0.24 W_C L_C (t_C + 2t_A)\rho_{\text{comp}} = 0.24 W_C L_C t_C \rho_C + 0.48 W_C L_C t_A \rho_A, \qquad [2]$$

where ρ_{comp}, ρ_C, and ρ_A are the densities of the composite protein/cantilever layer, silicon material, and protein layer, respectively. The minimum or critical protein layer thickness that will cause the resonant frequency to increase ($\omega_r^A > \omega_r$) can be given as

$$t_A \geq \frac{t_C}{2}\left(\sqrt{\frac{\rho_{\text{comp}} E_C}{\rho_C E_{\text{comp}}}} - 1\right). \qquad [3]$$

The minimum mass change that can be detected is related to the minimum detectable resonant frequency shift. The minimum detectable frequency shift is limited by thermomechanical noise (3) and is given as

$$\Delta f_{\min} = \frac{1}{\langle \hat{A} \rangle} \sqrt{\frac{f_0 k_B T B}{2\pi k Q}}, \qquad [4]$$

where k_B is Boltzmann's constant, T is temperature in Kelvin, B is the bandwidth measurement of the frequency spectra, Q is the quality factor, and $\langle \hat{A} \rangle$ is the square root of the mean-square amplitude of the vibration (3, 4).

Fluorescent Microscopy. In scheme 1, the two main protein molecules used were biotinylated BSA and biotinylated Ab to vaccinia virus (source rabbit). The secondary Ab to BSA used was FITC-conjugated chicken anti-BSA (Immunology Consultants Laboratory, Inc., Newburg, OR). The secondary Ab to the rabbit vaccinia virus biotinylated Ab used was goat TRITC-conjugated anti-rabbit IgG (Biodesign International, Kennebunkport, ME). The fluorescent imaging was done on an Axiotech vario Materials Microscope (Carl Zeiss Light Microscopy, Thornwood, NY). The fluorescent images were taken by using a pco.1600 camera (pco.imaging, Kelheim, Germany). This camera produces only gray-scale images unless attached to a color wheel, which was not used in this experiment. The software ImageJ (National Institutes of Health freeware; http://rsb.info.nih.gov/ij) was used to calculate the fluorescence intensity from the images. The fluorescent images were taken separately by using the FITC (green) and the TRITC (red) filters. All of the images were taken at the same lamp intensity and for the same exposure time (50 ms). When doing the analysis, however, the images from the separate filters were digitally combined. The background surrounding the image of the object (in this case, cantilevers) was used as the baseline.

1. Tortonese, M. (1997) *IEEE Eng. Med. Biol.* **16,** 28-33.

2. Beer, F. P. & Johnston, E. R., Jr. (1981) *Mechanics of Material* (McGraw–Hill, New York), pp. 168-172.

3. Martin, Y., Williams, C. C. & Wickramasinghe, H. K. (1987) *J. Appl. Phys.* **61,** 4723-4729.

4. Albrecht, T. R., Grütter, P., Horne, D. & Rugar, D. (1991) *J. Appl. Phys.* **69,** 668-673.