Supporting Information

Development of a Nanomechanical Biosensor for Analysis of Endocrine Disrupting Chemicals

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Experimental

Aminoethanethiolhydrochloride (AET), glutaraldehyde (GA), the salts employed for the preparation of buffer solutions, and all other reagents were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO) or Fisher at highest available purity and used as received. The proteins human immunoglobulin G (hIgG, reagent grade), protein A, and anti-human IgG were also obtained from Sigma-Aldrich.

Smooth gold (40nm) and dealloyed (50nm, 100nm, and 200nm) MCs were prepared (See Cantilever Modification in the main text) and functionalized with anti-human IgG antibody. Random functionalization (without protein A) of both the anti-human IgG antibody and human IgG was achieved by dipping the AET and GA functionalized MCs (see the Cantilever Modification under Experimental section in the main text) into 50mg/L solutions of protein in 10 mM phosphate buffered saline (PBS, pH 7) for 5 hours. Oriented functionalization (with protein A) was performed as follows. Initially, the AET and GA functionalized cantilevers were dipped into 100mg/L solutions of protein A in PBS for 1 hour, washed with PBS for several times and then the protein A functionalized cantilevers were dipped into 50mg/L solutions of anti-human IgG/human IgG in PBS for 5 hours. Both proteins were separately immobilized on the functionalized surfaces of different cantilevers from different microcantilever arrays.

Results and Discussion

Response characteristics of nanostructured MCs

Figure SI-1 shows comparison of bending responses of antibody functionalized nanostructured MC to similarly functionalized smooth gold MC using anti human IgG antibody. When the nanostructured MC, functionalized with anti human IgG antibody was exposed to 50mg/L of human IgG, specific interaction of antibody-antigen exhibited 480mV (~380nm) cantilever tip deflection that corresponds to a compressive surface stress change. Upon flushing the cell with background buffer, the response was reversed. Conversely, similarly functionalized MC with a smooth gold surface shows a largely irreversible compressive response on exposure to the same concentration of human IgG; which was also observed by other researchers for specific interactions of different antibody-antigen pairs (See references *16, 17*, and *28* in the main text). When comparing a smooth to a nanostructured MC surface, 50nm dealloyed gold coating increases MC response by roughly a factor of two. In some prior applications not involving bioaffinity functionalized MC, the nanostructuring resulting in as much as 2-3 orders of magnitude enhancements in chemi-mechanical responses (References *10* and *21* in the main text show direct comparisons of dealloyed versus smooth gold surfaces) for both self-assembled monolayers and thin films of responsive phases. Some of this enhancement in response has been attributed to increased surface area for the nanostructured surfaces.

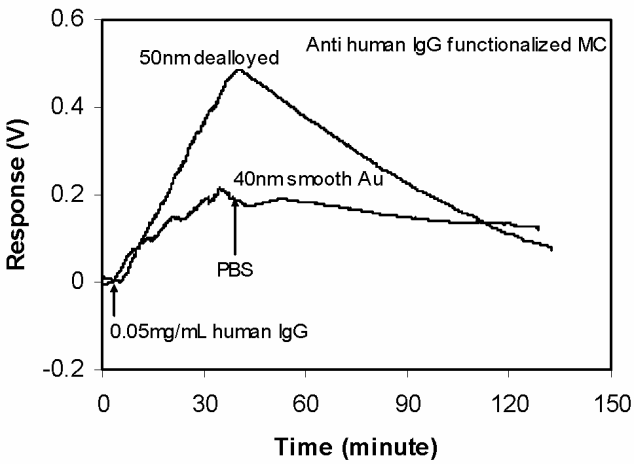

Figure SI-1. Comparison of bending signals of anti human IgG antibody functionalized nanostructured (dealloyed) MC to similarly functionalized smooth gold MC.

The reversibility of response for the nanostructured MC, despite very high sensitivity (see main text Figure 4 for example), is surprising and counter to the expected large affinity constants. The morphology and chemical nature of these two types of MC surfaces are not the same and perhaps chemical attachment of the bioreceptor in the dealloyed case shows a decrease in affinity constants that is compensated by the inherent larger responses of the large surface area nanostructured MC. It is also worth noting that we do not allow the establishment of equilibrium upon exposure to analyte (sampling only occurs for several minutes before we return to buffer flow). Long term exposure to the sample may not show the same reversibility. In any event, the fortuitous nature of high sensitivity with reversibility is a unique and

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valuable attribute of our bioaffinity nanomechanical sensing approach.

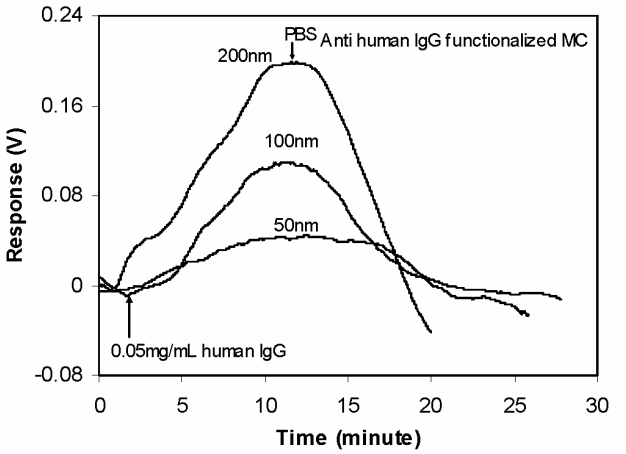

Figure SI-2. Response of anti human IgG antibody functionalized nanostructured (dealloyed) MC with different thicknesses of the dealloyed surfaces on exposure to 0.05mg/mL of human IgG in 10mM pH7 PBS.

Concurrent with the studies described in the main text, we performed some optimization studies involving the dealloying process. Figure SI-2 demonstrates the responses of anti human IgG antibody functionalized dealloyed MCs with different thicknesses of the dealloyed surfaces. On exposure to 0.05mg/mL of hIgG in 10 mM pH7 PBS for 10 minutes, antibody functionalized MCs showed increased response with increasing thickness of the dealloyed MCs. The response of the antibody functionalized 100nm and 200nm dealloyed MCs are 2.5 and 5 fold greater, respectively, than the response of the similarly functionalized 50nm dealloyed MCs. The work with EDC receptors was performed with the thinner 50nm dealloyed layer, thus even greater sensitivity than is demonstrated in the main text for EDC detection may be possible.

Random and Oriented functionalization of antibody and antigen

Both oriented and randomly functionalized MCs with anti human IgG or human IgG showed reversible compressive stress responses (expansion of the active surface) on exposure to different concentrations of human IgG (0.001mg/mL) or anti human IgG (0.05mg/mL) in PBS. It is interesting to observe that the random functionalization shows a three times larger response than the oriented case when the cantilever is functionalized with anti human IgG antibody. Conversely, when MC is functionalized with human IgG, oriented functionalization shows greater response than the random one when exposed to the antibody (See Table SI-1). These two different experiments demonstrate that proper orientation of bioreceptor proteins on the MC surface do not always yield improvements in response, presumably because of the stress induction response requirement (i.e. conformational changes upon analyte binding are important but depending on the nature of the linkage to the MC surface this conformational change may not translate into a large apparent surface stress). Note that both this specific monoclonal antibody and the protein A (the protein A may act in some cases as a spacer that reduces the induction of stress) are specific for the Fc portion of the IgG protein. Because of these complications, affinity constants determined by the nanomechanical approach would only be apparent ones and of only minor significance.

Table S-1: Comparison of the response of antibody and antigen functionalized MC on exposure to antigen/antibody with random (without Protein A) and oriented (with Protein A) functionalization.

	Antibody functionalized MC	Antigen functionalized MC
	0.001mg/mL human IgG (5 mins response) (V)	0.05mg/mL anti human IgG (5mins response) (V)
With Protein A	0.043	0.518
Without Protein A	0.126	0.321

125