Cantilever-Based Sensor for the Detection of Different Chromophore Isomers

Rosangelly Flores-Perez,[†] Amit K. Gupta,[‡,§,&] Rashid Bashir,[‡,§,▽] and Albena Ivanisevic*[,†,▽]

Department of Chemistry, Birck Nanotechnology Center, School of Electrical and Computer Engineering, and Weldon School of Biomedical Engineering, Purdue University, West Lafayette, Indiana 47907

We report the use of microcantilevers (MCs) for the detection of three retinoid isomers: 9-*cis*-retinal, 13-*cis*-retinal and all-*trans*-retinal. Detection of synthetic and natural retinoids in topical cosmetic products is important, and their presence can be used to predict reactions with the skin surface. In this study the MC surfaces were functionalized in order to promote the formation of covalent bonds with the chromophores. The lowest mass shift we detected with the functionalized MCs was 1.2 ppt, which is in the range needed by the cosmetics industry. Our results indicate that properly designed and functionalized microcantilevers can be used to construct economical, fast, and sensitive sensors for quality control in cosmetics.

The development of new label-free, fast, and easy chemical detection methods has received a lot of attention in the past two decades. In particular, the need for this format of sensing for biomolecules has become apparent in areas ranging from homeland security to clinically relevant diagnostics. In recent years, the use of resonant mechanical sensors such as microcantilevers (MCs)[1] has shown promise to fulfill the requirements for a number of fields where sensing is done in vitro.[2,3] Various research groups have shown the versatility, sensitivity, and superior detection limits of MC sensors. Some recent examples include the biomolecular detection and recognition of peptides,[2,3] DNA,[1,4−6] bacteria or bacterial cells,[7−9] poison agents, and heavy metals.[10−13] Many of the reported MC sensing platforms use simple principles of detection. The capture of molecules on the surface of the cantilever can result in a torque that bends the beam and can thus generate motion. The recorded changes are due to the adsorption of molecules to the cantilever surface, and therefore one can use the same MC structures to detect a number of different chemical or biological entities as long as the cantilever surface is properly functionalized to capture the target entities.

The use of MCs as economical and user-friendly sensors for compounds with relevance in cosmetic products has not been explored to date. In such products, the detection of synthetic and natural retinoids is of great importance.[14] Retinoids are classified as derivatives of vitamin A. Among these derivatives retinal is the most efficient molecule used in topical cosmetics. Retinal is capable of reacting nonenzymatically with many biomolecules on the skin surface. Studies have also shown that retinal is readily metabolized. In the past, traditional analytical detection methods such as high-performance liquid chromatography (HPLC)[15] have been used to detect retinal. In addition, electrodeposited metal electrodes[16] have also been used to distinguish among different derivatives. These techniques are highly sensitive but can be labor intensive and time-consuming.

In this paper we report a fast, sensitive, and efficient strategy for the detection of 9-*cis*-retinal, 13-*cis*-retinal, and all-*trans*-retinal (Figure 1A) using a resonant-based MC. Our previous studies[17,18] have focused on strategies to capture chromophore molecules on surfaces by forming a covalent bond using Schiff base chemistry (Figure 1B). We translate this knowledge to the modification of MCs and utilize them to detect the presence of chromophores in

* To whom correspondence should be addressed. Tel.: 765-496-3676. Fax: 765-494-1193. E-mail: albena@purdue.edu.

† Department of Chemistry.

‡ Birck Nanotechnology Center.

§ School of Electrical and Computer Engineering.

▽ Weldon School of Biomedical Engineering.

⊥ Present address: BioMEMS Resource Center, Massachusetts General Hospital, Harvard Medical School, and Shriners Burns Hospital, Boston, Massachusetts.

(1) Fritz, J.; Baller, M. K.; Lang, H. P.; Rothuizen, H.; Vettiger, P.; Meyer, E.; Guntherodt, H. J.; Gerber, C.; Gimzewski, J. K. *Science* **2000**, *288*, 316−318.

(2) Mukhopadhyay, R.; Sumbayev, V. V.; Lorentzen, M.; Kjems, J.; Andreasen, P. A.; Besenbacher, F. *Nano Lett.* **2005**, *5*, 2385−2388.

(3) Valiaev, A.; Abu-Lail, N. I.; Lim, D. W.; Chilkoti, A.; Zauscher, S. *Langmuir* **2007**, *23*, 339−344.

(4) Alvarez, M.; Carrascosa, L. G.; Moreno, M.; Calle, A.; Zaballos, A.; Lechuga, L. M.; Martinez-A, C.; Tamayo, J. *Langmuir* **2004**, *20*, 9663−9668.

(5) Mukhopadhyay, R.; Lorentzen, M.; Kjems, J.; Besenbacher, F. *Langmuir* **2005**, *21*, 8400−8408.

(6) Hagan, M. F.; Majumdar, A.; Chakraborty, A. K. *J. Phys. Chem. B* **2002**, *106*, 10163−10173.

(7) Gupta, A.; Akin, D.; Bashir, R. *Appl. Phys. Lett.* **2004**, *84*, 1976−1978.

(8) Johnson, L.; Gupta, A. T. K.; Ghafoor, A.; Akin, D.; Bashir, R. *Sens. Actuators, B−Chem.* **2006**, *115*, 189−197.

(9) Gupta, A.; Akin, D.; Bashir, R. *J. Vac. Sci. Technol. B* **2004**, *22*, 2785−2791.

(10) Ji, H. F.; Thundat, T.; Dabestani, R.; Brown, G. M.; Britt, P. F.; Bonnesen, P. V. *Anal. Chem.* **2001**, *73*, 1572−1576.

(11) Dutta, P.; Chapman, P. J.; Datskos, P. G.; Sepaniak, M. J. *Anal. Chem.* **2005**, *77*, 6601−6608.

(12) Xu, X.; Thundat, T. G.; Brown, G. M.; Ji, H.-F. *Anal. Chem.* **2002**, *74*, 3611−3615.

(13) Boiadjiev, V. I.; Brown, G. M.; Pinnaduwage, L. A.; Goretzki, G.; Bonnesen, P. V.; Thundat, T. *Langmuir* **2005**, *21*, 1139−1142.

(14) Sorg, O.; Antille, C.; Kaya, G.; Saurat, J. H. *Dermatol. Ther.* **2006**, *19*, 289−296.

(15) Speek, A. J.; Vanagtmaal, E. J.; Saowakontha, S.; Schreurs, W. H. P.; Vanhaeringen, N. J. *Curr. Eye Res.* **1986**, *5*, 841−845.

(16) Wang, L. H. *Anal. Chim. Acta.* **2000**, *415*, 193−200.

(17) Kinsella, J. M.; Ivanisevic, A. *Appl. Surf. Sci.* **2005**, *243*, 7−10.

(18) Flores-Perez, R.; Ivanisevic, A. *Appl. Surf. Sci.* **2007**, *253*, 4176−4181.

10.1021/ac0703000 CCC: $37.00
Published on Web 05/18/2007

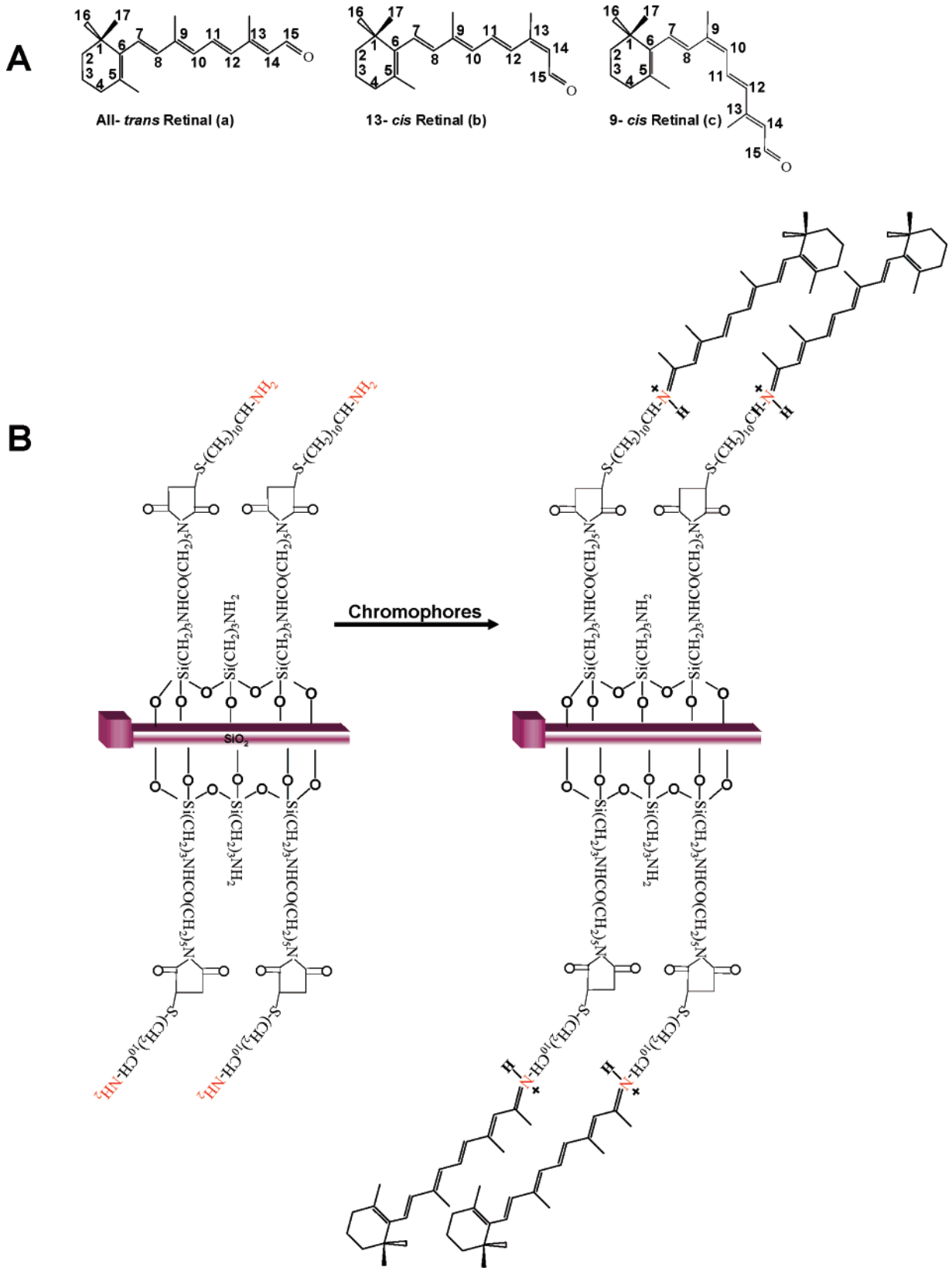

Figure 1. (A) Structures of the three retinal molecules used in this study; (B) scheme of chromophore immobilization on NH_2-terminated MC surfaces.

solution. Concentration dependence and competition experiments were done in order to understand the capabilities of the MC sensors for the detection of retinal molecules. We note that the concentrations tested in this study are not biologically relevant. However they are relevant to analysis in cosmetics. Previous reports in the literature have focused on investigating retinal

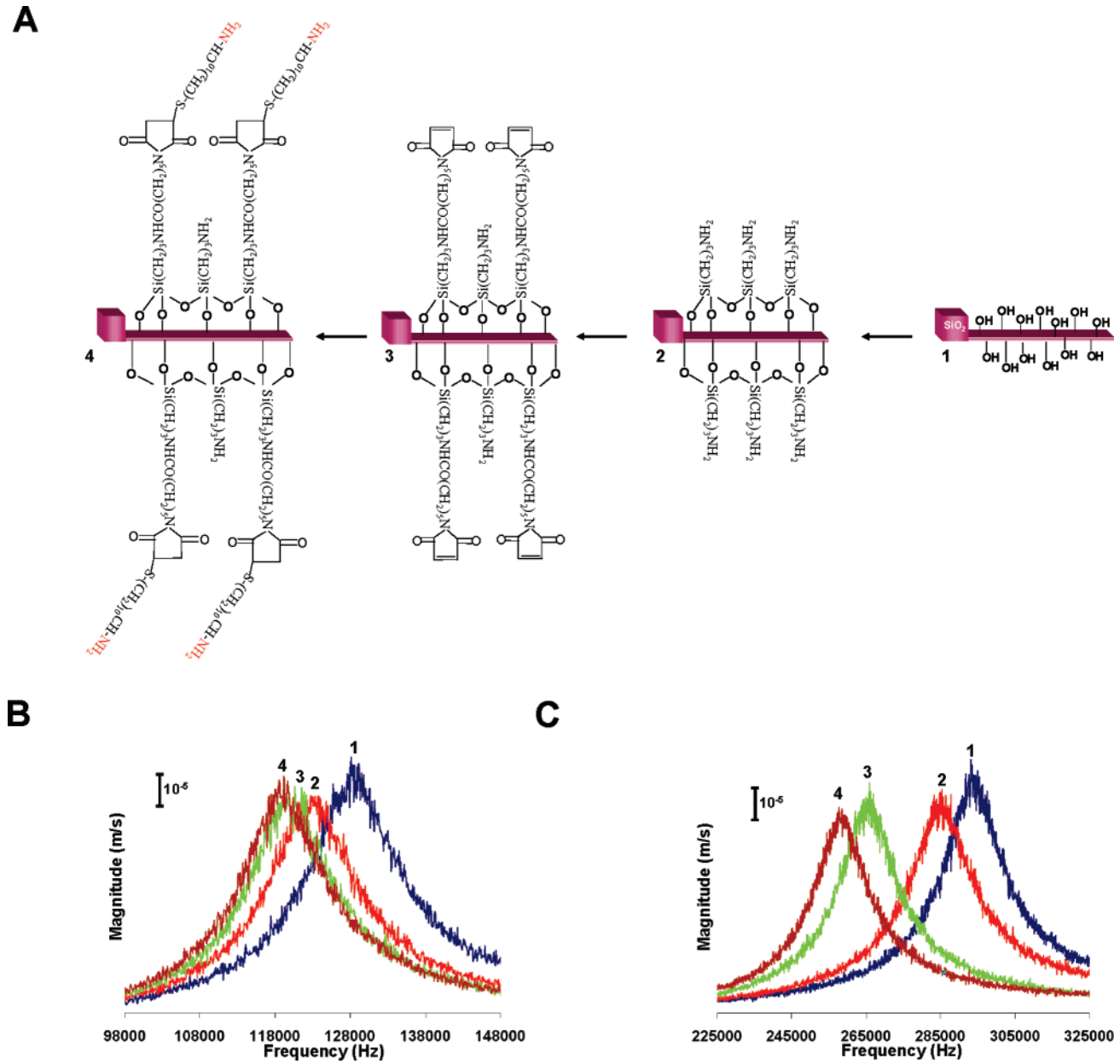

Figure 2. (A) Surface functionalization scheme prior to the chromophore immobilization; plots of the resonant frequencies of clean (1), APTES-terminated (2), SMPB-terminated (3), and MUAM-terminated (4) MCs with lengths of 40 μm (B) and 20 μm (C).

solutions with concentrations of about 10^{-3} M and have used liquid chromatography to validate the data.[19] This study uses the same concentration range.

EXPERIMENTAL SECTION

Reagents. 3-Aminopropyltriethoxysilane (APTES, 99%,), ethanol, dimethyl sulfoxide (DMSO), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2, 30%), phosphate-buffered saline (PBS, pH ~7.4), 9-*cis*-retinal (98%), 13-*cis*-retinal (85%), and all-*trans*-retinal (98%) were obtained from Sigma-Aldrich. *N*-succinimidyl 6-maleimidocaproate (SMPB, 98%) and 11-mercaptoundecylamine, (MUAM, 90%) were purchased from Fluka and Dojindo Laboratories, respectively.

Cantilevers Fabrication. Photolithography and chemical wet etching processes were used to fabricate the silicon microcantilevers using a previously reported protocol.[20] Each cantilever chip had a size of 1 cm^2 and two channels with an array of 80 microcantilevers with different lengths and widths. We tested MCs with two different lengths and identical widths and thicknesses, Table 1.

Cantilever Modification. Modification of the microfabricated cantilevers was done as previously described, and the role of each modification step was previously characterized using surface sensitive techniques.[18] The only modification to our previously published procedure was that no sonication or drying with a stream of N_2 was done in between cleaning steps. Changes in temperature and humidity during the modification steps caused reproducibility problems particularly in the silanization step and resulted in insufficient coverage of the surface. The amine-terminated MCs were incubated in different chromophore solu-

(19) Ceugniet, C.; Lepetit, L.; DeViguerie, N. L.; Jammes, H.; Peyrot, N.; Riviere, M. *J. Chromatogr. A* **1998**, *810*, 237−240.

(20) Gupta, A.; Denton, J. P.; McNally, H.; Bashir, R. *J. Microelectromech. Syst.* **2003**, *12*, 185−192.

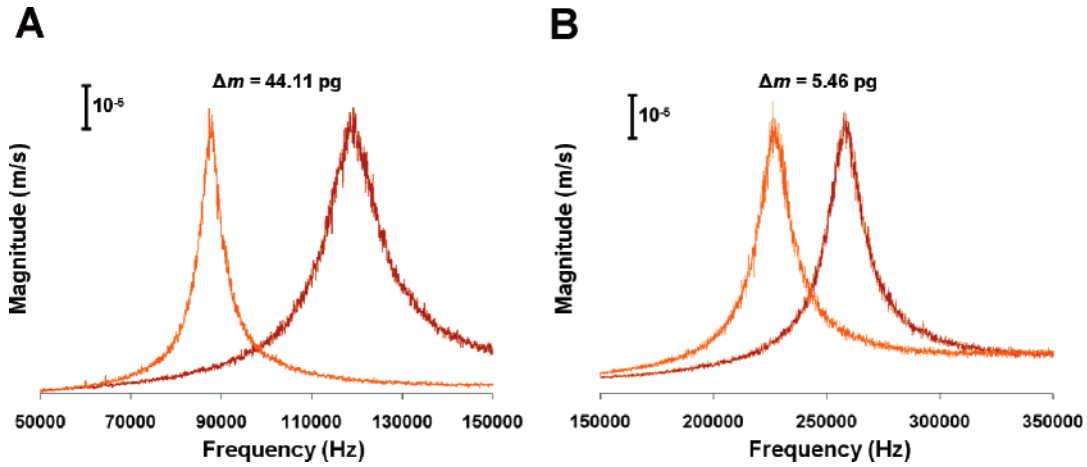

Figure 3. Resonant frequency of MUAM-terminated MCs (brown spectrum) and frequency changes after incubation in all-*trans*-retinal solution (orange spectrum). Part A shows data collected with 40 μm long MC, and part B represents data measured with 20 μm long MC.

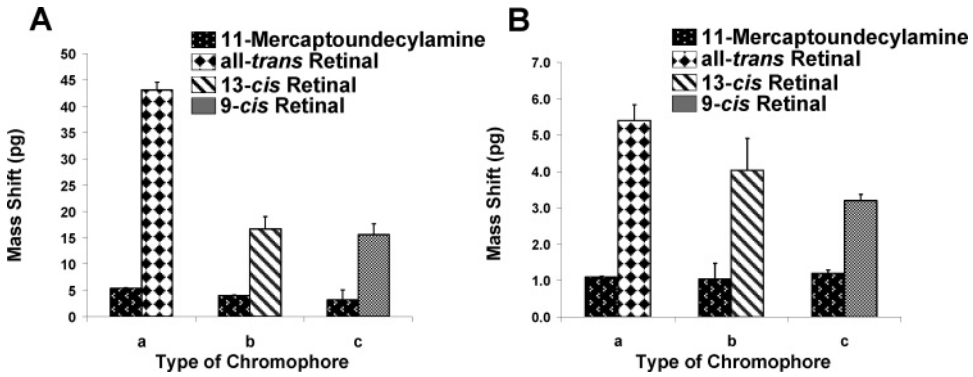

Figure 4. Mass shifts for all-*trans*-retinal (a), 13-*cis*-retinal (b), and 9-*cis*-retinal (c) measured with 40 μm long (A) and 20 μm long (B) MCs.

Table 1. Unloaded Silicon Cantilever Beams Planar Dimensions and Calculated and Theoretical Parameters

cantilever no.	length, width, and thickness	theoretical resonant frequency (kHz)	theoretical spring constant (N/m)	theoretical quality factor (Q) in air	minimum detectable mass (pg)	minimum detectable frequency (kHz)	measured spring constant (k, N/m)	measured quality factor (Q)
1	40 μm × 9 μm × 200 nm	147	0.037	16.3	0.78	1.43	0.025 ± 0.009	12 ± 2
2	20 μm × 9 μm × 200 nm	595	0.30	38.7	0.16	2.44	0.078 ± 0.01	26 ± 3

tions (9-*cis*-retinal, 13-*cis*-retinal, and all-*trans*-retinal) and subsequently washed multiple times with ethanol solvent and dried at room temperature. Competition experiments were done in the same fashion except that the solution contained an equimolar mixture of retinals (1:1, v:v). The combinations that were tested were mixtures of 9-*cis*-retinal:13-*cis*-retinal; all-*trans*-retinal:13-*cis*-retinal; and all-*trans*-retinal:9-*cis*-retinal.

Instrumentation and Data Evaluation. A Polytec PI (MSV-300) instrument equipped with a fiber interferometer, microscope scanner unit, vibrometer, and scanner controllers was used to measure the individual resonance frequency of MCs after each modification step. All data were processed with the PSV 8.1 software. Each FFT-spectrum was set up with the following parameters: bandwidth of 1000 kHz, frequency range between 0−1000 kHz, displacement of 2 MHz and a velocity of 25 mm/s. The cantilevers were driven using thermal noise. The obtained spectral data was then evaluated by using the least-square method fitted to the amplitude responses of a simple harmonic oscillator (SHO). This was done using a MATLAB code.[7−9,20] In Table 1, we summarize the planar dimensions and calculated values of the unloaded microcantilevers.

RESULTS AND DISCUSSION

Our study was aimed at validating that properly functionalized MCs can be used to detect different chromophore isomers. The method relies on detecting changes in resonance frequency due to the attachment of molecules to the MC surface. One can record a reduction in resonant frequencies upon the addition of mass on the cantilever surface. Cantilevers with different lengths have similar behavior in sensor systems. In general, the sensitivity of the MCs operated as resonant mass sensors increases as their

size decreases.[8,21] One can quantify the amount of material captured on the MC surfaces using the following equation[7,9,20]

$$\Delta m = k/(4\pi^2 n)(1/f_1^2 - 1/f_0^2) \quad (1)$$

where k is the transverse spring constant of the microcantilever beam, f_0 is the initial or previous resonant frequency, f_1 is the resonant frequency after a certain modification, $n = 0.24$ when its assumed that the added mass is distributed uniformly on the cantilever surfaces, and that the spring does not change with the added mass.[8,9] We carried out three surface coupling reactions in order to terminate the MCs surfaces on amine groups, as shown in Figure 2A. We began our sensor development by validating that the functionalization of a given MC resulted in the addition of mass to its surface and thus lowering its resonance frequency, as shown in Figure 2B. We tested the procedure using many different batches of cantilevers, and the amount of mass added to the cantilever was dependent upon the amount of time, solvent, and concentrations used to achieve each surface modification. In subsequent experiments with chromophore molecules, we only used cantilevers with the appropriate surface size (that is length and width) that showed a substantial amount of frequency shift after the last functionalization step. We note that the capture of chromophores on the cantilever is irreversible. Repeated washing and soaking in buffer solutions did not show a change in the resonance frequency.

All chromophore testing was verified using cantilevers that were 20 and 40 μm long. Sample data with all-*trans*-retinal is presented in Figure 3. This experiment was done using a 5 mM solution of all-*trans*-retinal. Using eq 1. the mass changes were calculated. On the 40 and 20 μm long MCs after chromophore immobilization they were 44.11 pg and 5.46 pg, respectively. All measurements were done in air. More chromophore molecules were captured on the longer cantilevers resulting in a larger frequency shift, which is expected due to the greater surface area available for retinal binding. We verified that the change in frequency shift is irreversible by using variable reaction times and copious solvent washes. This is consistent with our surface design, as shown in Figure 1B, which is used to anchor the retinal on the surface via a covalent bond. The shifts we observed with millimolar concentrations were rather large and indicated that properly functionalized MCs can be used to easily interpret the presence of all-*trans*-retinal in solution. Subsequently we tested the response to the different retinal isomers using various batches of cantilevers functionalized on different days. A typical set of data is presented in Figure 4. All cantilevers used to do the experiments with a particular chromophore were from the same batch, but showed slightly different shifts as a result of the last functionalization step—the attachment of 11-mercaptoundecylamine. The data in Figure 4 compares the mass shifts after a solution of each retinal isomer was used to soak the chosen cantilever. The concentrations of all isomer solutions were the same. We repeated this experiment with many sets of cantilevers that were successfully functionalized. We observed that the magnitude of the frequency shift for a specific chromophore was very dependent upon the success of the coupling reaction with 11-mercaptoundecylamine.

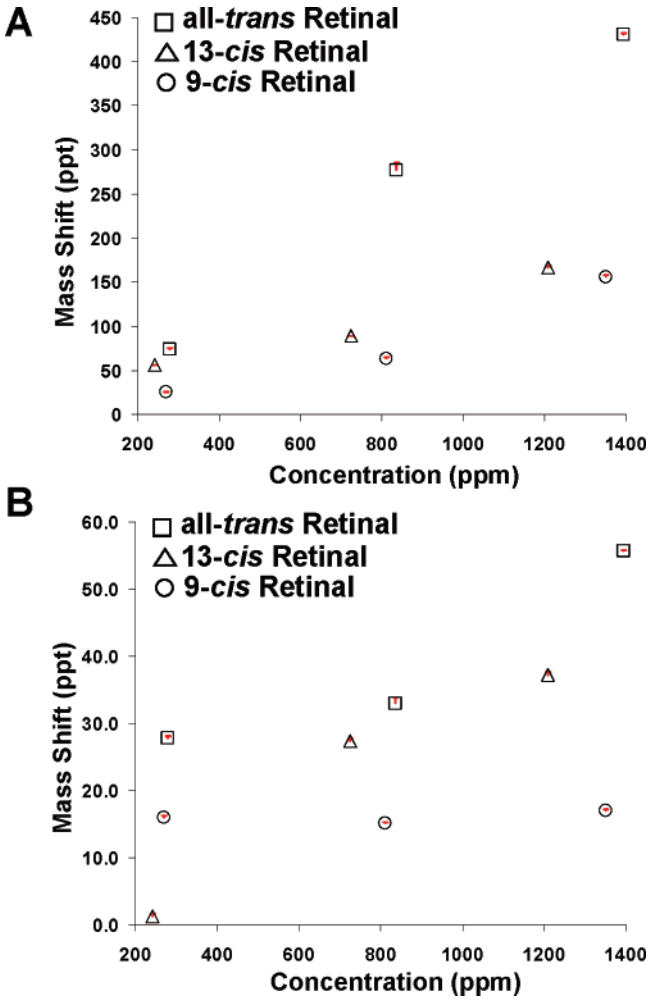

Figure 5. Responses as a function of concentration with with 40 μm long (A) and 20 μm long (B) MCs.

The concentration dependence of the frequency shifts was also tested using solutions with retinal masses that are relevant to the level detection needed in cosmetics. Generally the data showed an increase in the mass shift as the concentration of each chromophore solution we used increased. However, the MCs responses did not show a linear response, and we did not reach a saturation level with the highest concentration we tested (1400 ppm). The nonlinearity is expected due to the variation in the available surface binding sites on the MCs. Since we have an extremely large surface area one needs to use very concentrated solutions in order to reach the saturation level. Detecting such high concentrations is of no practical value. From our experiments we determined that the lowest mass shift we can detect with our functionalized MCs was 1.2 ppt, Figure 5. Our detection limit is in the same range of what has been reported by other detection methods.[16,19] Our experiments show that functionalized MCs are sensitive enough to detect concentrations of retinal that might be in topical cosmetics and therefore present an alternative faster and easier method for the detection of these compounds.

We carried out direct competition experiments with the different isomers of retinal in order to reveal if our simple surface functionalization on the MCs can be used to discriminate among

(21) Gupta, A. K.; Nair, P.; Akin, D.; Ladisch, M. R.; Broyles, S.; Alam, M. A.; Bashir, R. *Proc. Natl. Acad. Sci.* **2006**, *103*, 13362−13367.

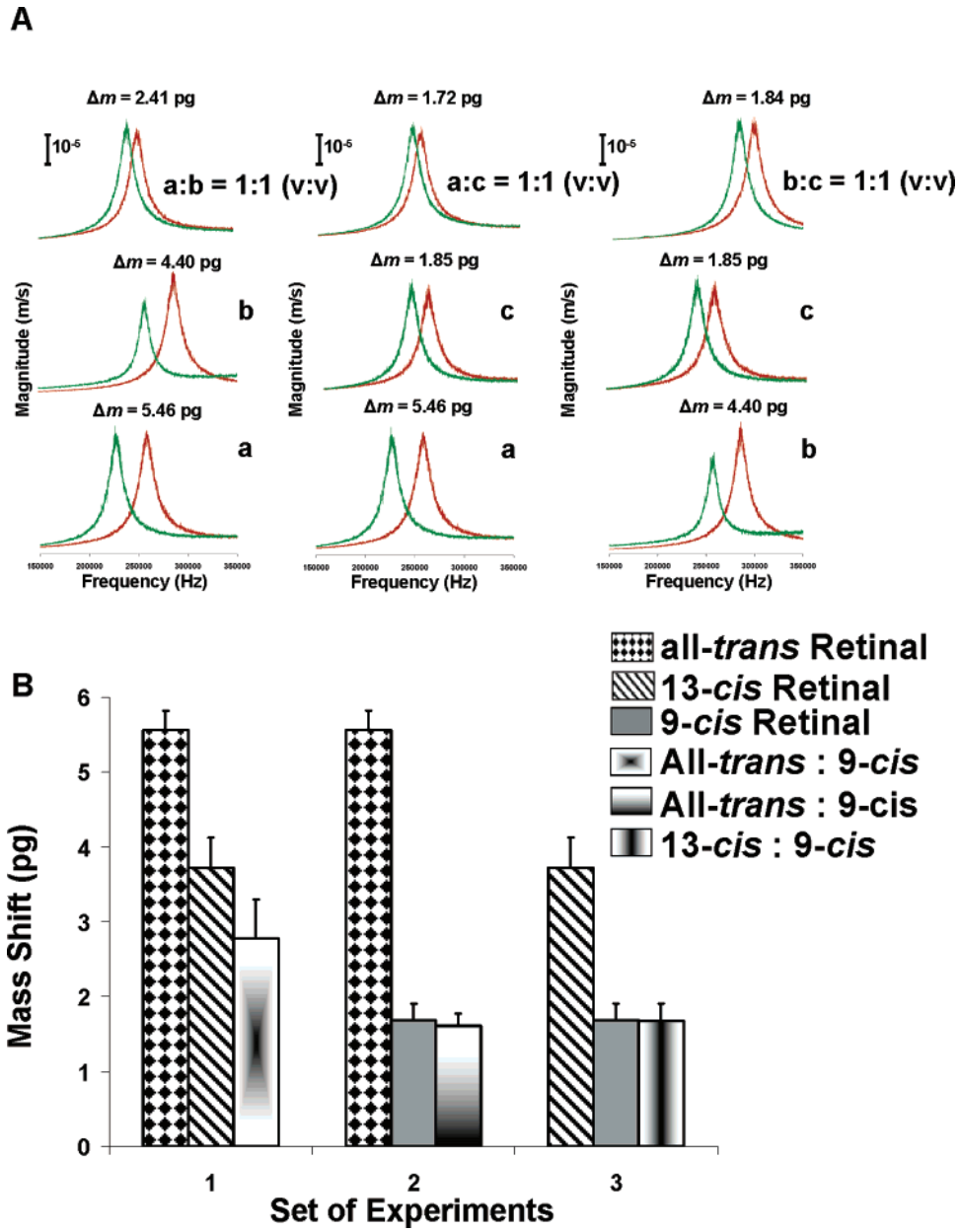

Figure 6. (A) Changes in resonance frequencies for 20 μm long MCs before (brown spectra) and after adsorption of solutions of all-*trans*-retinal (a), 13-*cis*-retinal (b), and 9-*cis*-retinal (c) and stoichiometric ratios of all-*trans*-retinal:13-*cis*-retinal (a:b), all-*trans*-retinal:9-*cis*-retinal (a:c), and 13-*cis*-retinal:9-*cis*-retinal (b:c). Data collected after the analyte adsorption is shown in the green spectra. (B) Data comparing different sets of experiments.

them. The retinal preference experiments were initiated by immersing amine-terminated MCs into a mixture of two chromophores using 1:1 (v:v) ratios. A set of sample data sets from the three possible combinations is shown in Figure 6. All data shown in Figure 6 was collected on cantilevers taken from the same batch preparation in order to minimize differences in the available amine groups on the surface. In a competition experiment with an equimolar ratio of *trans*-retinal and 13-*cis*-retinal, the first column of plots in Figure 6, the mass change was approximately midway between the change observed when each isomer was introduced separately. This result indicates that the reaction rates are the same. In a similar experiment when the behavior of *trans*-retinal and 9-*cis*-retinal was compared, the middle column of plots in Figure 6, the surface showed a strong preference for 9-*cis*-retinal. Last, a competition experiment between 13-*cis*-retinal and 9-*cis*-retinal, the third column of plots in Figure 6, revealed a preference for 9-*cis*-retinal. Taken in sum, all competition experiments demonstrate that the amine-terminated cantilever has a preference for binding the retinal isomer that is most likely to form disordered monolayers and produce lower coverage of molecules on the surface. We note that we do not know if the same coverage and binding sites are used when each isomer is adsorbed on the MC surface. However, we have verified that with both cantilever sizes and various batches of functionalized cantilevers, the MC surface shows preference for the 9-*cis*-isomer over the *trans*-isomer in competition experiments.

CONCLUSIONS

In summary we have demonstrated that functionalized MCs can be used for the detection of retinal in solution. The detection limit of this sensor is within the needs of the topical cosmetics industry. In addition, the simple surface functionalization on the MCs shows preference for binding of the 9-*cis*-retinal isomer. We note that the surface chemistry we used is not only selective for retinal isomers, and therefore in samples where other aldehydes might be present specificity will be a problem. The results of this report can be used toward the development of cheaper and user-friendly sensors for quality control in cosmetics.

ACKNOWLEDGMENT

This work was supported by the Bindley Bioscience Center at Purdue University and by NASA under award no. NCC 2-1363. A.K.G. was supported by National Institutes of Health (NIBIB) Grant R21-R33EB00778-01.

Received for review February 12, 2007. Accepted April 18, 2007.

AC0703000