



# Application of displacement principle for detecting heavy metal ions and EDTA using microcantilevers

X. Chen<sup>a</sup>, W. Chen<sup>b,\*</sup>, A. Mulchandani<sup>b,\*</sup>, U. Mohideen<sup>a,\*</sup>

<sup>a</sup> Department of Physics and Astronomy, University of California, Riverside, CA 92521, USA

<sup>b</sup> Department of Chemical and Environmental Engineering, University of California, Riverside, CA 92521, USA

## ARTICLE INFO

### Article history:

Received 7 June 2011

Received in revised form

30 September 2011

Accepted 10 October 2011

Available online 29 October 2011

### Keywords:

Microcantilever

Sensor

Displacement reaction

Reversible

Heavy metal ions

EDTA

## ABSTRACT

A microcantilever sensor based on a reversible displacement reaction was developed. The demonstration was performed with the Ni–NTA–EDTA sample system. Ni ions, a typical heavy metal, were injected and adsorbed onto a NTA-functionalized AFM cantilever in a fluid cell. This adsorption resulted in tensile stress which was detected by monitoring the corresponding deflection of the microcantilever. In the next step, EDTA was injected into the cell to release the adsorbed Ni ions from the surface of the cantilever. Since EDTA molecules have a stronger affinity for Ni ions compared to NTA, they compete with and eventually replace the NTA molecules and take over the binding positions on Ni. The displacement of Ni ions from the cantilever by EDTA, followed by water flow removes the tensile stress and restores the cantilever deflection to its original value. In this way, both heavy metal ions and EDTA can be specifically recognized. From the cantilever deflection, the concentration of Ni ions and EDTA can be quantified. The detection limits are presently at the micromolar level. Another weaker Ni ion binder, imidazole, was unable to dissociate Ni ions from NTA and produce similar effects. The demonstrated principle can be used for microcantilever sensors which are capable of regeneration for multiple uses. The method presented can be generalized to detect other reactants.

© 2011 Elsevier B.V. All rights reserved.

## 1. Introduction

Label-free physical, chemical and biological sensors have experienced a rapid development in recent decades. Microcantilever based sensors [1–11], surface plasmon resonance (SPR) sensors [12–15], quartz crystal microbalances (QCM) [16–19], piezoelectric or surface acoustic wave (SAW) devices [20–22] and nanowire/nanotube-based transistors [23–26] are the most commonly used label-free detection systems. In contrast to the many varieties of sensor systems, the principles of detection used remain limited to the adsorption of the targeted analyte, usually based on specific molecular recognition pairs such as antibody–antigen and receptor–ligand/antagonist. A detection signal is generated when an analyte, chemical A, is bound to its interaction pair, chemical B, which is immobilized on the surface of the detection device. In contrast, in the displacement method of detection, chemical A can also be detected as it is displaced from the surface by a third chemical C, which has a stronger affinity to A compared to B. In principle, the detection limit of displacement should be equivalent to that of the adsorption method while the speci-

ficity of the detection is increased because the detected analyte A should have affinity to both B and C with the binding affinity  $AB < AC$ . Alternatively, if A and B are both known, then C can be specifically detected in the above dissociation process based on the affinity relation given by  $AB < AC$ . Previously, displacement of the analyte was only used in the regeneration of detection devices and seldom used for the detection itself. Even though competitive assays have a close relation to the displacement strategy, it still belongs to the category of adsorption based detection [27]. This paper will demonstrate, using a microcantilever based sensor, the feasibility of the displacement concept for detecting heavy metal ions, such as  $Ni^{2+}$  and ethylenediaminetetraacetic acid (EDTA).

Interactions between divalent transition metal ions (such as Ni, Cu and Co) and chelators (such as nitrilotriacetic acid (NTA), EDTA and His-tag) have been intensively studied and well documented [28–30]. The relationship between these chemicals is best illustrated by their most well known application, Ni-NTA chromatography. In this methodology, His-tagged recombinant proteins are purified by utilizing the interactions of Ni with NTA, His-tag and EDTA (or imidazole). Ni is pre-loaded into a chromatogram column and bound to a NTA-functionalized matrix packed inside. The targeted His-tagged protein mixed along with other impurities is then run through the column. The His-tagged protein is captured by the Ni matrix while the impurities are eluted.

\* Corresponding authors. Tel.: +1 951 827 5390; fax: +1 951 827 4529.

E-mail addresses: [wilfred@engr.ucr.edu](mailto:wilfred@engr.ucr.edu) (W. Chen), [adani@engr.ucr.edu](mailto:adani@engr.ucr.edu) (A. Mulchandani), [umar.mohideen@ucr.edu](mailto:umar.mohideen@ucr.edu) (U. Mohideen).

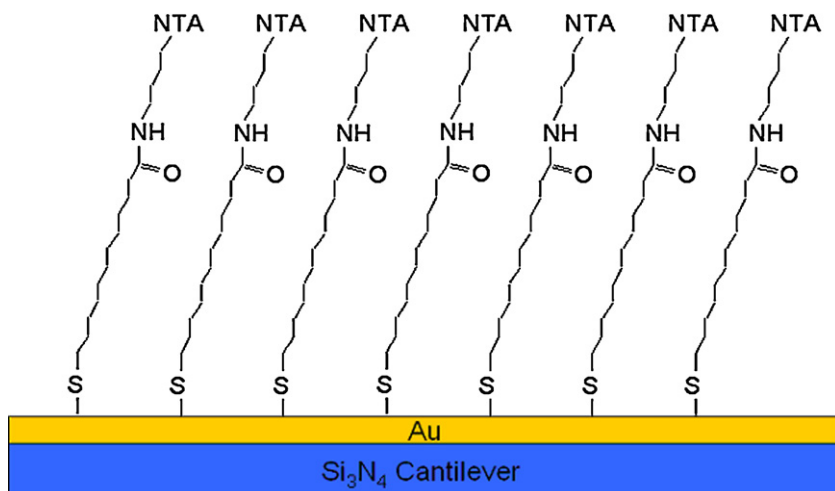


Fig. 1. Schematic representation of the molecular structure of the SAM of NTA on a gold-coated silicon nitride AFM cantilever.

As a final step, proteins are released by rinsing with EDTA or imidazole. EDTA competes with NTA and cleaves Ni from NTA while imidazole competes with His-tag and removes the protein from Ni. This technology has been solidly established as a standard procedure for the purification of His-tagged proteins and provides a classic model system to detect heavy metal ions and EDTA.

Heavy metal ions are highly toxic for bioorganisms, sometimes even at trace concentrations. This makes the detection of heavy metal ions extremely critical in environmental control and food processing. Likewise, the detection of EDTA is also important due to its wide use in industry, food and agriculture [31].

In this paper, using the sample Ni–NTA–EDTA system, the detection principle of reversible displacement is demonstrated using an AFM cantilever for both heavy metals and EDTA. The microcantilever based sensor was used for its improved dynamic response, greatly reduced size, high precision, and increased reliability compared with conventional sensors [32]. In this demonstration system, Ni ions, a typical heavy metal ion, are delivered in situ in the water flow and immobilized onto an AFM cantilever via interaction with the self-assembled monolayer (SAM) of NTA on the cantilever. Due to the stress resulting from the adsorption of Ni ions, the cantilever bends and a deflection change is detected. This is followed by the injection of EDTA, which is used to remove Ni ions from the cantilever by competing with NTA. This change causes another detectable bending of the cantilever which is read-out. Concentrations of Ni ions and EDTA were varied independently up to the detection limits for each analyte. Since adsorbed Ni can be completely cleaned from the cantilever, this detection system can be regenerated and reused, significantly saving time, labor and cost of materials. Although microcantilever-based sensors have been reported for detection of heavy metal ions, the displacement method has not been applied previously [33,34]. Detection of peptides with replacement of water molecules around the topmost NTA–Ni layer was reported [35]. However, since peptides were detected via their adsorption to Ni ions, the methodology is different from the displacement method detailed in this paper. To the authors' knowledge, this is the first demonstration of the displacement principle on a microcantilever based EDTA sensor.

## 2. Material and methods

$\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ , ethylenediaminetetraacetic acid disodium salt dihydrate ( $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ ), imidazole, NaCl and N-[ $\text{N}_\alpha, \text{N}_\alpha$ -Bis(carboxymethyl)-L-lysine]-12-mercaptododecanamide (NTA-Thiol) were purchased from Sigma–Aldrich and used as received.

EDTA solutions were prepared from  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ , as pure EDTA has very low solubility in water [36]. For simplicity, EDTA is used as shortcut for the  $\text{Na}_2\text{EDTA}$  solution unless explicitly stated. Ultrapure deionized water ( $18.2 \text{ M}\Omega \text{ cm}$ ) was obtained with a Milli-Q water system (Millipore). The V-shaped silicon nitride cantilever (Veeco Instruments, Santa Barbara, CA) used in the experiments was  $310 \mu\text{m}$  long,  $20 \mu\text{m}$  wide and  $0.55 \mu\text{m}$  thick with a spring constant of  $0.01 \text{ N/m}$ . The top of the cantilever was coated with  $60 \text{ nm}$  of gold over a  $15 \text{ nm}$  of chromium adhesion layer.

Multiple steps were taken to clean the cantilever before it was functionalized with NTA SAM. The cantilever was first immersed in acetone overnight and then washed with ethanol and dried in a stream of nitrogen. After 60 min of UV irradiation, the cantilever was immersed in Piranha solution ( $3:1 \text{ H}_2\text{SO}_4 (98\%)/\text{H}_2\text{O}_2 (30\%)$ ) for 5 min. (Caution: Piranha solution reacts violently with many organics and should be handled with great care.) After Piranha treatment, the cantilever was rinsed with copious water followed by ethanol. Next, the cantilever was functionalized with a SAM of NTA by incubation in a NTA-thiol saturation ethanolic solution ( $<1 \text{ mM}$ ) for 7 days. Fig. 1 shows the surface structure of the NTA SAM on the AFM cantilever.

All experiments were carried out in a fluid cell installed in a Veeco Multimode optical head (Veeco Instruments, Santa Barbara, CA). This system is shown in Fig. 2. The cantilever is mounted on the glass fluid cell containing an inlet and outlet. The bottom is closed with a coverslip or a silicon wafer using a Teflon O-ring. The volume of the sealed chamber is about  $60 \mu\text{l}$ , ensuring a fast

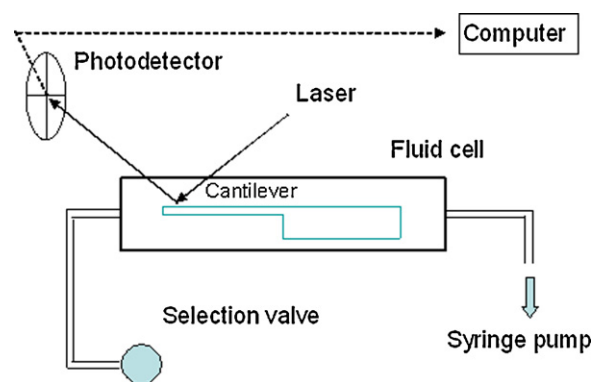
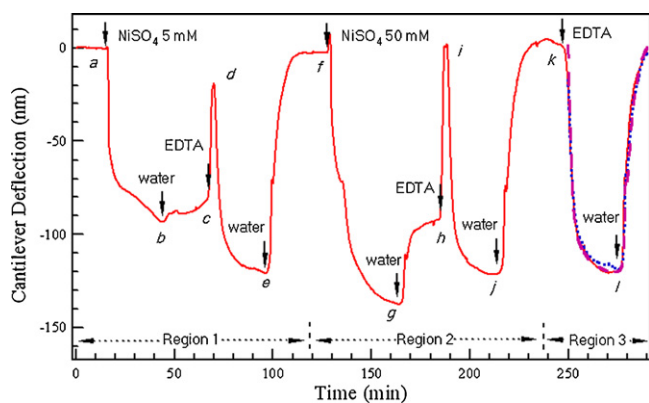


Fig. 2. Schematic diagram of the experimental setup.



**Fig. 3.** Deflection response of a NTA-functionalized cantilever to injections of  $\text{NiSO}_4$  solution, a 50 mM EDTA solution and water introduced at the time marked with arrows. In region 3, the dotted line and the dashed line are the segments *def* and *ijk* respectively, which are overlaid on the last EDTA curve. The excellent match demonstrates the reversibility and reproducibility of the sensor.

replacement of the solution during flow. The whole system is placed on an isolation table to reduce vibration. A laser beam is incident onto the top side of the free cantilever (beam positioned at its apex), and reflected onto a four-quadrant photodetector. A positive change (rise) in the deflection means the cantilever bends up, while a negative change (drop) corresponds to the cantilever bending down, which is away from the sensing surface. The upward bending indicates tensile surface stress due to attractive interactions and the downward bending represents compressive surface stress caused by repulsive interactions, respectively. The deflection signal was acquired by an AFM controller and transferred to a computer. Acquisition software was programmed in Labview. The flow through the fluid cell was controlled at a constant rate of 2 ml/h by a syringe pump, which was connected to the outlet of the fluid cell, operating in a withdrawal mode. This flow method leads to less noise. To reduce the effect of temperature fluctuations during measurement, the fluid cell was housed in a temperature-controlled box and stabilized at  $26 \pm 0.05^\circ\text{C}$ . The switch between different solutions was done by using a 6-way selection valve. In each experiment, the flow began with water and was followed by 1 ml of 50 mM EDTA. This step ensured the removal of any possible trace heavy metal ions absorbed onto the cantilever surface in the previous steps. Eventually, the cantilever was stabilized in the water flow before switching to the analyte.

### 3. Results and discussion

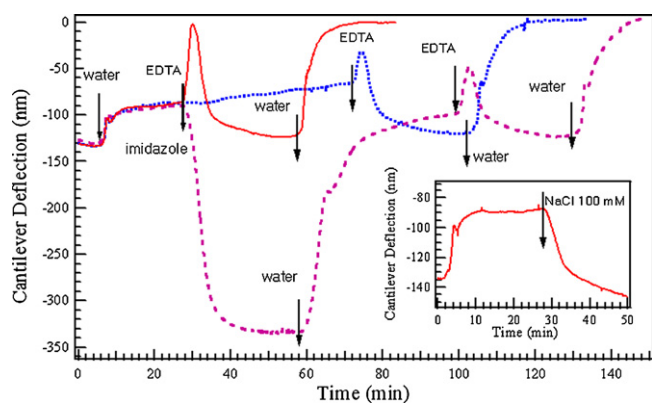
The cantilever deflection associated with the Ni adsorption onto the NTA SAM is thought to result from the change in charge density of the surface. A Ni ion in solution bears 2 positive charges, which are tightly bound to the cantilever surface after capture by NTA. This change can be reversed with the injection of EDTA which removes Ni ions trapped on the cantilever and thus restores the original charge distribution on the cantilever surface. Since the effect of the charge and the related electrostatic interaction can be screened by salts in solution, pure water was chosen as the medium and all analytes were dissolved in water without adding additional electrolytes. The baselines in all experiments were obtained in pure water in order to minimize the screening effect of charges, and in turn to optimize the signal.

Fig. 3 shows a typical cantilever deflection measurement performed as a function of time. The displayed data include three separate regions, with each being a complete measurement. All basic essential information can be extracted from Fig. 3 by comparing the three sections.

The first region shows the cantilever deflection caused by the flow of 5 mM  $\text{NiSO}_4$  solution and the reversal by the subsequent flow of EDTA solution. The functionalized cantilever was first equilibrated in water for several hours until a stabilized flat baseline was obtained in the temperature controlled chamber. The background deflection due to temperature drift is negligible (maximum drift of a few nanometers per hour). This stability enables long time data collection. At the time point *a*, indicated by the first arrow, a 5 mM  $\text{NiSO}_4$  solution was flowed into the reaction chamber and continued for 30 min. As the  $\text{NiSO}_4$  flow entered the reaction chamber the cantilever bends down leading to a total deflection drop of 93 nm to point *b*. The direction of the deflection indicates a tensile stress on the top surface of the cantilever. This stress is thought to result from the adsorption of Ni ions onto the NTA layer. It should be noted that other effects, such as pH variation and non-specific adsorption [37], can also lead to the bending of the cantilever. Thus, the origin of the cantilever deflection needs to be carefully analyzed and the direct contribution from the adsorption of Ni ions should be separated from the background mentioned above. To remove ambiguities from pH and non specific adsorption, pure water was next flowed into the reaction chamber at position *b* as shown by the arrow in Fig. 3. The water flow causes the deflection to reduce as shown in Fig. 3. In the first 10 min after the start of the water flow, there was a rapid rise in deflection, which most likely corresponds to the change in pH of the reaction chamber and the removal of the physically adsorbed  $\text{Ni}^{2+}$  or  $\text{SO}_4^{2-}$ . This is followed by a further slow and almost linear increase in the deflection from *b* to *c*. This observation suggests the slow dissociation of Ni ions from NTA due to the flow of water. About 25 min later, a 50 mM EDTA solution was introduced into the fluid cell by using a selection valve. This led to a two-step change in the deflection. First, a sharp rise from *c* to *d* was seen immediately after the start of flow of EDTA which then turned to a quick drop in a few minutes, leading to the formation of a narrow peak. In the following half hour, the signal decreased to a minimum (position *e*) before the flow was again switched back to water. This behavior is not what is expected from the complete removal of Ni ions from NTA. EDTA should remove Ni ions from the surface, thereby decreasing the tensile stress and restoring the cantilever to zero deflection. The situation, however, is not as simple as outlined above. Along with the removal of the immobilized Ni ions, a change in the pH value and physical adsorption of EDTA can also occur. This would lead to competing influences on the cantilever as observed. To check the net effect of EDTA, water flow was again restored at point *e* as shown in Fig. 3. The deflection rises due to the removal of the influence of pH and EDTA adsorption. After 30 min of water flow the cantilever deflection was restored to its original zero value as at the start of the experiment prior to the introduction of the Ni ions. This means all the chemicals,  $\text{NiSO}_4$  or EDTA, which were introduced into the fluid cell, were removed and the state of the cantilever was regenerated and made ready for subsequent use.

In the second region of Fig. 3, another cycle of flow experiments as above was repeated with a 50 mM  $\text{NiSO}_4$  solution and the same concentration of EDTA. The same features were reproduced except that the decrease in cantilever deflection caused by  $\text{NiSO}_4$  was deeper and the subsequent peak caused by EDTA flow was higher. These changes correspond well to the larger concentration of  $\text{NiSO}_4$  used. At the end of this cycle, the deflection level again returned to the original zero as at the beginning of the experiment. From these two regions the magnitude of the deflection change with Ni and EDTA can be correlated to the concentration of the Ni ions used.

To further understand the formation of the peak in the deflection generated by EDTA, a control experiment was done in the third region of Fig. 3. After the water flow cycle in region 2, EDTA flow was started at point *k*, without the flow of  $\text{NiSO}_4$  beforehand. As water was replaced with EDTA, the cantilever deflection signal dropped



**Fig. 4.** Deflection response of a NTA-functionalized cantilever to injections of EDTA (solid line), imidazole (dashed line), and water (dotted line). The cantilevers were loaded with Ni ions by flowing a 50 mM NiSO<sub>4</sub> solution prior to the start of the experiments. The inset shows the deflection change due to the flow of a 100 mM NaCl solution.

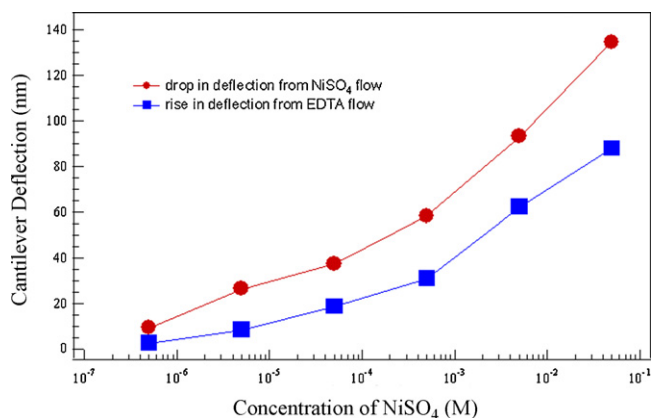
immediately. Unlike in the previous two regions there is no initial increase in the deflection as here no Ni ions are present. Next EDTA flow was shut off and the water flow to the fluid cell turned on at point *l*. The cantilever deflection increased till it was restored to its original zero value. We can now understand the role of EDTA in the cantilever deflections in the experiments in regions 1 and 2 by comparing the corresponding sections to the control experiment in region 3. For this, in Fig. 3 those sections showing a decrease during the EDTA flow and the subsequent rise with the water flow (sections *def* and *ijk*) are cut and overlaid on region 3. The overlaid segments from region 1 (section *def*) and region 2 (section *ijk*) are shown with dotted and dashed lines in Fig. 3. No normalization of the segments was done for the comparison. A perfect match between all 3 experiments is observed. This points to the individual role of EDTA in the cantilever deflection. Thus the initial rise in the cantilever deflection after introduction of EDTA (sections *cd* and *hi*) can be attributed to the removal of Ni ions from NTA. The peak formation in the cantilever deflection in regions 1 and 2 is the result of the competition between the rise from the displacement of Ni and the drop due to the individual role of EDTA. The excellent overlap between the solid, dashed and dotted lines also points to the fact that all prior effects of Ni ion absorption are lost after the flow of EDTA. There is no memory of the interaction of Ni allowing easy regeneration for multiple reuses of the sensor. This also provides a proof of the chemical stability of the functionalized cantilever. Based on the negligible change in sensor function between cycles, the cantilever should be usable for a few tens of cycles.

To further confirm the specificity of EDTA on the displacement of Ni, an additional set of control experiments were conducted and the results are displayed in Fig. 4. The same protocol as used in experiments shown in Fig. 3 was followed. These experiments were started at the point corresponding to *g* after a 30 min flow of 50 mM NiSO<sub>4</sub> in Fig. 3, so as to have the same initial state of the cantilever, i.e. loaded with Ni ions. All the changes in flow are indicated with arrows. The flow through the reaction chamber was then switched to water for 25 min to flush out ions in the solution as well as those loosely bound to the cantilever surface. Three different experiments were done following the water flush. In the first experiment, shown by the solid line in Fig. 4, the flow was switched to 50 mM EDTA for 30 min and followed by water, similar to the experiments described in region 2 of Fig. 3. We reproduced the previous results in Fig. 3. In the second experiment, shown by the dotted line in Fig. 4, no flow of EDTA was started and the water was continuously run for an additional 45 min. The cantilever deflection shows a slow rise related to gradual dissociation of Ni ions by the water. As discussed

above, the Ni dissociation from NTA in water is much slower than that in EDTA. Thus even after 45 min the cantilever deflection does not go back to its original zero value as observed when EDTA is used. The lag in recovery reveals the importance of EDTA in the release of Ni. To confirm that Ni was still left on the surface, 50 mM EDTA was introduced after 45 min flow of water. The expected rise in the cantilever deflection resulted. As some of the Ni ions were removed by the extended water flow, the peak due to EDTA is smaller than that shown by the solid line. The third experiment shown by the dashed line in Fig. 4 was similar to the first one shown by the solid line, except that imidazole was flowed instead of EDTA. The cantilever deflection decreased further with the start of the imidazole flow. The deflection rise *hi* seen in Figs. 3 and 4 when EDTA flow was started was absent. This is due to the difference in the Ni dissociation constants. Unlike the Ni–EDTA complex, which has a dissociation constant of 10<sup>-19</sup> M, the Ni–imidazole complex is much weaker with a dissociation constant of 10<sup>-3</sup> M. In comparison, the Ni–NTA complex has a dissociation constant of 10<sup>-11</sup> M, and thus imidazole does not displace Ni from NTA as rapidly as does EDTA [28,38]. It should be noted that imidazole caused an even larger change in the signal magnitude compared to that by EDTA. This might seem strange under the conventional scenario where all cantilever bending signals are caused by adsorption and thus a stronger interaction usually results in a larger signal in the same bending direction. However, it is not surprising when one realizes that there are different underlying mechanisms for the two phenomena. In the case of EDTA, the bending shows a tensile stress generated by the release of Ni ions. In contrast, the injection of imidazole leads to a drop in signal indicating compressive stress, probably resulting from physical adsorption of imidazole onto the surface of the cantilever. When the water flow was restored after 30 min of imidazole, the deflection rose. Thus water flow removes the imidazole adsorbed on the Ni surface of the cantilever. After 40 min of water flow, the deflection was much lower compared to the solid line of experiment 1, corresponding to a cantilever state coated with primarily Ni ions. It is also interesting to note that the deflection level is even lower than that at the start of the imidazole flow. This points to additional tensile stress from the presence of some residual imidazole on the cantilever. As the last step, EDTA was run to regenerate the cantilever. The characteristic peak due to the Ni–EDTA interaction again appeared. The subsequent flow of water restored the deflection was to its initial zero level indicating the complete removal of Ni ions from the cantilever.

As Na<sub>2</sub>EDTA was used instead of pure EDTA, there is concern that salts might play a role in the deflection detections of the Ni–EDTA interaction. To resolve this, the experiments were repeated by replacing EDTA flow with the flow of a 100 mM NaCl solution after the standard procedure of Ni loading and water wash (point *c* in Fig. 3). The results of the deflection with NaCl flow are displayed in the inset of Fig. 4. As seen, the characteristic peak due to Ni–EDTA interaction is absent. This further confirms the attribution of the peak to the role of EDTA.

Next we studied the concentration dependence of Ni ions and EDTA to determine the detection limit of each analyte. To investigate the deflection dependence on the Ni ion concentration, solutions of NiSO<sub>4</sub> with concentrations from 0.5 μM to 50 mM were flowed through the reaction chamber for 30 min. After each NiSO<sub>4</sub> flow, the cantilever was regenerated by flowing 50 mM EDTA for 30 min followed by water flow for 40 min. The results are shown by the solid circles in Fig. 5. The drop in the deflection (such as the change from *a* to *b* in Fig. 3) is plotted as a function of the NiSO<sub>4</sub> concentration. The change in the deflection is observed to increase with the NiSO<sub>4</sub> concentration. Alternatively, the Ni ion concentration can be detected from the height of the peak due to the flow of EDTA (change in deflection from *c* to *d* in Fig. 3). This change in deflection is shown by the solid squares as a function of NiSO<sub>4</sub>

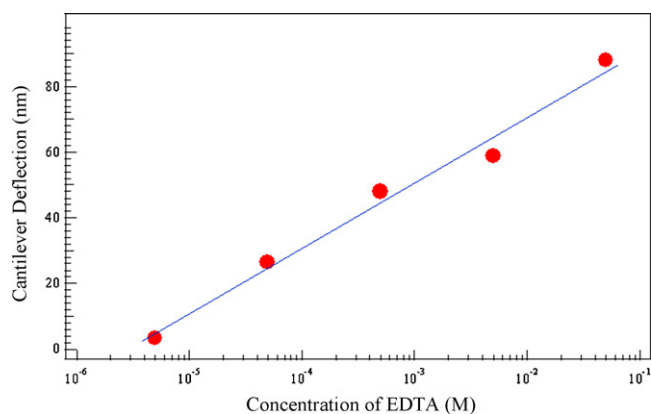


**Fig. 5.** Bending response for a NTA-functionalized cantilever as a function of the change in concentration of Ni<sup>2+</sup>. The circles represent the change due to the injection of Ni (the drop before water wash) and the squares represent the change due to the injection of EDTA (the height of the rise).

concentration. Again the change in deflection increases with the NiSO<sub>4</sub> concentration.

The Ni ion detection limit is 0.5 μM for both methods. The detection of Ni ions using the displacement principle is essentially equivalent to the result given by the direct traditional adsorption method. Although the displacement detection has slightly reduced signals than that from adsorption, it has a much faster response to the chemical injection and is thus more convenient and feasible for sensor applications. Moreover, it should be pointed out that the demonstrated displacement method of monitoring the dissociation peak due to the interaction of EDTA is more specific since it provides information on the relative affinities of Ni, NTA and EDTA. This advantage is not restricted to this choice of chemicals and can be applicable to displacement reactions in general. This is a more general improvement inherent to the new detection strategy.

The concentration dependence of EDTA on the cantilever deflection was studied similarly. Here, the NTA coated cantilevers were functionalized with a flow of 50 mM NiSO<sub>4</sub> through the reaction chamber for 30 min. The concentration of EDTA was changed from 5 μM to 50 mM. The rises in the deflection from the flow of EDTA (e.g. *c* to *d* in Fig. 3) are measured as a function of the EDTA concentration. The results are shown as solid circles in Fig. 6. The deflection increases with EDTA concentration. The detection limit for EDTA is 5 μM in this approach.



**Fig. 6.** Cantilever deflection change for a Ni–NTA-functionalized cantilever as a function of the change in concentration of EDTA. The straight line is the best linear fit to the data.

## 4. Conclusions

A microcantilever sensor employing the principle of displacement has been demonstrated using the model system Ni–NTA–EDTA. To detect heavy metal ions, such as Ni<sup>2+</sup>, a cantilever functionalized with NTA SAM was used. The flow of the Ni ions led to a downward deflection of the cantilever. The subsequent flow of EDTA led to a characteristic rise in the deflection. The flow of water after EDTA restored the initial state of the cantilever. The specificity of the cantilever response to the Ni–EDTA interaction was verified using imidazole and salts. The concentration dependence of the cantilever deflection due to the flow of Ni ions and EDTA was measured. A detection limit of 0.5 μM for Ni and 5 μM for EDTA respectively was determined. Compared to the conventional adsorption approach used in detection, the displacement principle provides an alternative method, equivalent in most respects, but with more specificity to molecule recognition. The principle allows regeneration of the substrate for multiple repeated use. The method can be further generalized to detect other analytes.

Another advantage of this displacement method is that it provides a convenient way to investigate relative affinities of different molecules/ions. It is very common for a target molecule/ion to bind with a variety of other molecules with different affinities. As the target molecule/ion is immobilized onto the surface of cantilever, the affinity between this target molecule/ion and its partners can be checked in sequence with the same cantilever in one single experiment.

Although this paper focuses on the methodology at the current stage, it also provides a convenient, inexpensive and rapid method to fulfill the detection requirement with reasonable sensitivity [39]. While the sample preparation and the equilibration of the equipment require a long time, one can use pre-functionalized and equilibrated cantilevers for rapid detection. On the other hand, the fast response and the capacity for multiple use of the detection device (cantilever) in a one-time setup may be the most attractive features for situations such as handling a batch of solution samples in a real test. At the current stage, we only focus on the methodology. We hope to optimize the setup time in future work. For example, the equilibration time and the amount of reagents can both be reduced using improved design of the AFM chamber. The incubation time of the sample could also be shortened and optimized with a careful investigation of the temperature/time effect in the cantilever functionalization. The sensitivity should also be substantially improved, potentially to the order of nM, which is very common for microcantilever based sensors. To achieve such a sensitivity, the conditions of the cantilever, such as the coating of the gold surface and the surface functionalization need to be systematically examined and optimized in future work [40].

## Acknowledgements

The authors acknowledge the financial support from the National Science Foundation (Grant 0617240) and the UC Labs Fees Grant 116505 (XC and UM).

## References

- [1] N.V. Lavrik, M.J. Sepaniak, P.G. Datskos, Cantilever transducers as a platform for chemical and biological sensors, *Review of Scientific Instruments* 75 (2004) 2229–2253.
- [2] A. Majumdar, Bioassays based on molecular nanomechanics, *Disease Markers* 18 (2002) 167–174.
- [3] J.K. Gimzewski, C. Gerber, E. Meyer, R.R. Schlittler, Observation of a chemical reaction using a micromechanical sensor, *Chemical Physics Letters* 217 (1994) 589–594.
- [4] L.G. Carrascosa, M. Moreno, M. Álvarez, L.M. Lechuga, Nanomechanical biosensors: a new sensing tool, *Trends in Analytical Chemistry* 25 (2006) 196–206.

- [5] K.S. Hwang, S.-M. Lee, S.K. Kim, J.H. Lee, T.S. Kim, Micro- and nanocantilever devices and systems for biomolecule detection, *Annual Review of Analytical Chemistry* 2 (2009) 77–98.
- [6] J. Fritz, Cantilever biosensors, *Analyst* 133 (2008) 855–863.
- [7] H.-F. Ji, H. Gao, K.R. Buchapudi, X. Yang, X. Xu, M.K. Schulte, Microcantilever biosensors based on conformational change of proteins, *Analyst* 133 (2008) 434–443.
- [8] G. Shekhawat, S.-H. Tark, V.P. Dravid, MOSFET-embedded microcantilevers for measuring deflection in biomolecular sensors, *Science* 311 (2006) 1592–1595.
- [9] H. Lang, C. Gerber, Microcantilever Sensors, in: P. Samori (Ed.), *STM and AFM Studies on (Bio)molecular Systems: Unravelling the Nanoworld*, Springer, Berlin/Heidelberg, 2008, pp. 1–27.
- [10] W. Liu, V. Montana, E.R. Chapman, U. Mohideen, V. Pappas, Botulinum toxin type B micromechanosensor, *Proceedings of the National Academy of Sciences of the United States of America* 100 (2003) 13621–13625.
- [11] G.A. Campbell, R. Mutharasan, Detection and quantification of proteins using self-excited PZT-glass millimeter-sized cantilever, *Biosensors and Bioelectronics* 21 (2005) 597–607.
- [12] J. Homola, S.S. Yee, G. Gauglitz, Surface plasmon resonance sensors: review, *Sensors and Actuators B: Chemical* 54 (1999) 3–15.
- [13] I. Abdulhalim, M. Zourob, A. Lakhtakia, Surface plasmon resonance for biosensing: a mini-review, *Electromagnetics* 28 (2008) 214–242.
- [14] J. Mitchell, Small molecule immunosensing using surface plasmon resonance, *Sensors* 10 (2010) 7323–7346.
- [15] A. Abbas, M.J. Linman, Q. Cheng, New trends in instrumental design for surface plasmon resonance-based biosensors, *Biosensors and Bioelectronics* 26 (2011) 1815–1824.
- [16] J. Handley, Product review quartz crystal microbalances, *Analytical Chemistry* 73 (2001) 225A–229A.
- [17] K. Kanazawa, N.-J. Cho, Quartz crystal microbalance as a sensor to characterize macromolecular assembly dynamics, *Journal of Sensors* 2009 (2009) 17.
- [18] G.N.M. Ferreira, A.-C. da-Silva, B. Tomé, Acoustic wave biosensors: physical models and biological applications of quartz crystal microbalance, *Trends in Biotechnology* 27 (2009) 689–697.
- [19] K.A. Marx, Quartz crystal microbalance: a useful tool for studying thin polymer films and complex biomolecular systems at the solution–surface interface, *Biomacromolecules* 4 (2003) 1099–1120.
- [20] G. Zhang, Nanostructure-enhanced surface acoustic waves biosensor and its computational modeling, *Journal of Sensors* 2009 (2009).
- [21] K. Länge, B. Rapp, M. Rapp, Surface acoustic wave biosensors: a review, *Analytical and Bioanalytical Chemistry* 391 (2008) 1509–1519.
- [22] E. Benes, M. Gröschl, W. Burger, M. Schmid, Sensors based on piezoelectric resonators, *Sensors and Actuators A: Physical* 48 (1995) 1–21.
- [23] N. Sinha, J. Ma, J.T.W. Yeow, Carbon nanotube-based sensors, *Journal of Nanoscience and Nanotechnology* 6 (2006) 573–590.
- [24] C. Li, E.T. Thostenson, T.-W. Chou, Sensors and actuators based on carbon nanotubes and their composites: a review, *Composites Science and Technology* 68 (2008) 1227–1249.
- [25] B. Mahar, C. Laslau, R. Yip, Y. Sun, Development of carbon nanotube-based sensors: a review, *IEEE Sensors Journal* 7 (2007) 266–284.
- [26] T. Zhang, S. Mubeen, N.V. Myung, M.A. Deshusses, Recent progress in carbon nanotube-based gas sensors, *Nanotechnology* 19 (2008) 332001.
- [27] D. Shankaran, T. Kawaguchi, S. Kim, K. Matsumoto, K. Toko, N. Miura, Evaluation of the molecular recognition of monoclonal and polyclonal antibodies for sensitive detection of 2,4,6-trinitrotoluene (TNT) by indirect competitive surface plasmon resonance immunoassay, *Analytical and Bioanalytical Chemistry* 386 (2006) 1313–1320.
- [28] S. Knecht, D. Ricklin, A.N. Eberle, B. Ernst, Oligohis-tags: mechanisms of binding to Ni<sup>2+</sup>-NTA surfaces, *Journal of Molecular Recognition* 22 (2008) 270–279.
- [29] L. Nieba, S.E. Nieba-Axmann, A. Persson, M. Hämäläinen, F. Edebratt, A. Hansson, J. Lidholm, K. Magnusson, Å.F. Karlsson, A. Plückthun, BIACORE analysis of histidine-tagged proteins using a chelating NTA sensor chip, *Analytical Biochemistry* 252 (1997) 217–228.
- [30] J. Schmitt, H. Hess, H.G. Stunnenberg, Affinity purification of histidine-tagged proteins, *Molecular Biology Reports* 18 (1993) 223–230.
- [31] Y.T. Zarouila, M.D.C. Valencia Mirón, L.F. Capitán-Vallvey, Disposable chemiluminometric sensor for rapid determination of EDTA, *Microchimica Acta* 157 (2007) 63–71.
- [32] A. Boisen, T. Thundat, Design and fabrication of cantilever array biosensors, *Materials Today* 12 (2009) 32–38.
- [33] S. Cherian, R.K. Gupta, B.C. Mullin, T. Thundat, Detection of heavy metal ions using protein-functionalized microcantilever sensors, *Biosensors and Bioelectronics* 19 (2003) 411–416.
- [34] K. Liu, H.-F. Ji, Detection of Pb<sup>2+</sup> using a hydrogel swelling microcantilever sensor, *Analytical Sciences* 20 (2004) 9–11.
- [35] F. Gambinossi, L. Lorenzelli, P. Baglioni, G. Caminati, Silicon oxide surface functionalization by self-assembled nanolayers for microcantilever transducers, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 321 (2008) 87–93.
- [36] J. Kragten, L.G. Decnop-Weever, Solubility and protonation of EDTA, DCTA and DPTA in acidic perchlorate medium, *Talanta* 30 (1983) 623–626.
- [37] J. Fritz, M.K. Baller, H.P. Lang, T. Strunz, E. Meyer, H.J. Güntherodt, E. Delamarche, C. Gerber, J.K. Gimzewski, Stress at the solid–liquid interface of self-assembled monolayers on gold investigated with a nanomechanical sensor, *Langmuir* 16 (2000) 9694–9696.
- [38] S.A. Lauer, J.P. Nolan, Development and characterization of Ni-NTA-bearing microspheres, *Cytometry* 48 (2002) 136–145.
- [39] C.-M. Wu, L.-Y. Lin, Immobilization of metal ions by surface plasmon resonance, *Biosensors and Bioelectronics* 20 (2004) 864–871.
- [40] H.-F. Ji, B.D. Armon, Approaches to increasing surface stress for improving signal-to-noise ratio of microcantilever sensors, *Analytical Chemistry* 82 (2010) 1634–1642.

## Biographies

**Xiaojuan Chen** received his PhD in condensed matter physics from the University of California at Riverside in 2006. Soon after his graduation he joined Prof. Umar Mohideen's lab at UCR as a postdoctoral researcher working in the field of biophysics. Currently, he is a postdoctoral fellow in the Department of Radiation Oncology at the Medical College of Wisconsin, where he conducts his research focused on adaptive radiation therapy for cancer treatment.

**Wilfred Chen** joined the University of Delaware on January 1, 2011 as the Gore Professor of Chemical Engineering. He obtained his B.S. degree from UCLA in 1988 and his Ph.D. from Caltech in 1993, both in Chemical Engineering. After one-year post doc in Switzerland, he joined UC Riverside in 1994. He was Professor of Chemical and Environmental Engineering and the holder of the Presidential Chair until 2010. His research interests are in biomolecular engineering, biofuel production, viral infection, and nano-biotechnology. Chen has published more than 190 journal papers and delivered over 50 invited lectures. He serves on the editorial board of eight scientific publications. He is a fellow of the American Association for the Advancement of Science (AAAS) and the American Institute for Medical and Biological Engineering (AIMBE).

**Ashok Mulchandani** is a Professor in the Department of Chemical and Environmental Engineering at the University of California and the Editor-in-Chief of the *Applied Biochemistry and Biotechnology* journal. He is an elected Fellow of the American Association for Advancement of Science and the American Institute for Medical and Biological Engineering. He has received several honors and awards including Research Initiation Award from the National Science Foundation and Faculty Participation Award from the Department of Energy. He has delivered several Plenary and Keynote lectures. Prof. Mulchandani has published over 220 peer-reviewed journal publications, 13 book chapters, 12 conference proceedings articles, over 200 conference abstracts and edited four textbooks. Prof. Mulchandani's primary research interest is in the broad area of "Bio-Nanotechnology" with goals of developing novel (bio)analytical devices/assays, (bio)remediation technologies and (bio)nanomaterials.

**Umar Mohideen** obtained his PhD in Physics from Columbia University, NY, NY and worked as a postdoctoral fellow in the Physics Division at AT&T Bell Labs, Murray Hill, NJ. Since 1994 he has been a Professor in the Department of Physics & Astronomy of the University of California, Riverside, CA. His interests are in using microcantilever techniques for sensor applications and precision measurements of the Casimir force. He is the author of 75 peer reviewed journal publications with over 2000 total citations. He is also the coauthor of a 766 page monograph on the Casimir effect published by Oxford University Press, Oxford, UK. He is a Fellow of the American Physical Society (APS) and a Fellow of the American Association for the Advancement of Science (AAAS).