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A biofunctional polymeric coating for microcantilever molecular recognition

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ABSTRACT

An innovative route to activate silicon microcantilevers (MCs) for label free molecular recognition is presented. The method consists in coating the underivatized MCs with a functional ter-polymer based on N,N-dimethylacrylamide (DMA) bearing N-acryloyloxysuccinimide (NAS) and 3-(trimethoxysilyl)propyl-methacrylate (MAPS), two functional monomers that confer to the polymer the ability to react with nucleophilic species on biomolecules and with glass silanols, respectively. The polymer was deposited onto MCs by dip coating. Polymer coated MCs were tested in both static and dynamic modes of actuation, featuring detection of DNA hybridization as well as protein/protein interaction. In the dynamic experiments, focused on protein detection, the MCs showed an average mass responsivity of 0.4 Hz/pg for the first resonant mode and of 2.5 Hz/pg for the second resonant mode. The results of the static experiments, dedicated to DNA hybridization detection, allowed for direct estimation of the DNA duplex formation energetics, which resulted fully consistent with the nominal expected values. These results, together with easiness and cheapness, high versatility, and excellent stability of the recognition signal, make the presented route a reliable alternative to standard SAM functionalization (for microcantilevers (MCs) and for micro–electro–mechanical systems (MEMS) in general).

1. Introduction

Microcantilevers (MCs) are versatile and sensitive devices for label free molecular recognition. They display unique nanomechanical signal transduction, allowing direct detection of molecules and investigations of molecular interactions. Furthermore high sensitivity, label free, real time detection, small size, fast response and the natural implementability for multiplex and/or differential analysis make MCs an outstanding promise for biological, chemical and medical research [1–4].

MC beams are typically 0.2–3 μm thick, 20–100 μm wide and 100–800 μm long, and are connected to one end to an appropriate support, appearing like the diving board of a swimming pool. In MC biosensing, the beam is functionalized with a probe (receptor) that can selectively bind the target biochemical species (ligand). Specific adsorption of the target species induces a nanomechanical response of the MC, that pro-

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vides the transduction/sensing mechanism. The read-out of the response is commonly achieved by an optical lever or a piezoresistive film integrated with the MC. The following responses can be monitored: (1) bending of the MC induced by the surface stress that originates by the binding of the target species with the probes immobilized on one face of the MC (static mode); (2) changes in the eigenfrequencies of the MC upon the ligand mass load (dynamic mode). The first transduction mechanism is energy-based [5], while the latter is mass-based [1].

Properly functionalized MCs have recently demonstrated remarkable performances as biosensors. In this field, several experiments have been successfully performed, sensing DNA hybridization (including detection of single nucleotide polymorphisms) and detecting proteins and antibodies, single virus particles and bacteria [1–4]. The state-of-the-art, after the proof of concept experiments, is now moving towards competitive sensitivity and reliability in comparison with more mature label-based and label free biosensing methods [1–4,6]. MCs may reach sensitivities from picograms to attograms, which are comparable with those of other mature techniques, such as Surface Plasmon Resonance (SPR) spectroscopy and acoustic sensors, including Quartz Crystal Microbalances (QCM) [1]. Apart from on–off detection, (static mode actuated) MCs can play a key role in critical molecular recognition experiments and other fundamental biophysics investigations. Actually, thanks to direct mechanical transduction of the energy involved in the binding reactions, they can be employed in cases of low molecular weight (LMW) species or in presence of conformational changes or multimeric interactions. In these cases, QCM and SPR are inherently limited by their transduction principles [1,7]. For example, MCs unique capabilities in experimental investigation of protein conformational changes [8–11] and cooperative work of DNA molecular motors [12] have been already recently proven.

Probes immobilization is of crucial importance for the efficiency and sensitivity of any surface supported biosensor. Immobilization of the probes on the transducer without a significant change in their physicochemical nature is the first critical step in developing surface supported bioassays. Accessibility, stability and efficiency of surface functional groups, applicability to a wide range of relevant compounds and robustness of the protocols are further decisive issues. In the case of static MC biosensors, an additional request is that the procedure ensures significant target binding-induced surface stress. According to the literature, this issue has been faced through direct adsorption of the probes onto the MC surface or through specific functionalization methods. The use of thiolated molecules on gold coated MCs is the most common functionalization method for DNA, protein and antibodies. Bacteria, viruses [13,14] and in some cases proteins [15], have been immobilized by activation techniques based on organosilanization. An alternative approach to gold coating and organosilanization chemistry is to coat the underivatized MCs with appropriate functional polymers. This approach has found application in MC chemical sensing [16–18] and has been adopted in biosensing by Gunter et al., who used a poly-ethylene-oxide (PEO) coating to immobilize vaccinia polyclonal antibodies [19].

In this work we present the results of molecular recognition tests performed on silicon MCs functionalized with a thin layer of a functional copolymer. The polymer, obtained by radical polymerization of three monomers, N,N-dimethylacrylamide, N-acryloyloxysuccinimide (NAS) and 3-(trimethoxysilyl)propyl-methacrylate (MAPS)], was developed for DNA and protein microarray assays on microscope glass slides [20,21]. Then the deposition methodology, based on simple dip coating, was implemented for silicon MCs [22]. In the cited papers we described the chemistry that allows the polymer to bind DNA and protein receptors and calculated the related surface coverage and efficiency of the ligand binding reaction, demonstrating that this method provides a fast, inexpensive and robust route to bind biomolecules with high density and efficiency. In this work we will prove the effectiveness of DMA–NAS–MAPS coating for MC molecular recognition in both static and dynamic mode. In particular we will present and discuss the detection of oligonucleotides hybridization in the static mode and protein interactions in the dynamic mode.

2. Experimental

2.1. Materials

The ter-polymer (DMA-co-NAS-co-MAPS) contains N,Ndimethylacrylamide (DMA), N-acryloyloxysuccinimide and 3-(trimethoxysilyl)propyl-methacrylate, respectively at 97, 2 and 1% of the total monomer mole. Hereafter the polymer will be referred as DNM. Briefly, the polymer synthesis started from the monomers' dissolution in dried tetrahydrofuran (THF). The solution was warmed to 50 °C with the addition of α,α′-azoisobutyronitrile (AIBN) under a slightly positive pressure of nitrogen for 24 h. After the polymerization, evaporation and precipitation, the polymer was dried under vacuum for 24 h at room temperature and stored at 4 °C. Detailed synthesis procedures and a full characterization of DNM analytical properties can be found in Refs. [20,21].

For the static experiments, we employed arrays of eight rectangular silicon (1 0 0) MCs (IBM, Zurich Research Laboratory, CH). The nominal dimension of each MC was 500 μm in length, 100 μm in width and 1 μm in thickness. Custom synthesized 5′-amine-modified and Cy3 labelled oligonucleotides (100 nM mL^{-1} stock solution) from MWG (Ebersberg, Germany), were dissolved in 200 mM sodium phosphate buffer. We adopted two different 23-mer amino-modified probes, named, respectively, COCU8 (5′-GCC CAC CTA TAA GGT AAA AGT GA-3′) and COCU9 (5′-TCA CTT TTA CCT TAT AGG TGG GC-3′).

For the dynamic experiments, arrays of microcantilevers with dimensions of 250–700 μm in length, 50–80 μm in width and 2 μm in thickness were fabricated using a combination of surface and bulk micromachining process, as described in detail elsewhere [23].

All the chemicals used for the molecular recognition experiments were from Sigma–Aldrich, Germany.

2.2. Procedures

The MC coating was obtained through the immersion of the MCs for 30 min in a solution of the polymer (1%, w/v in

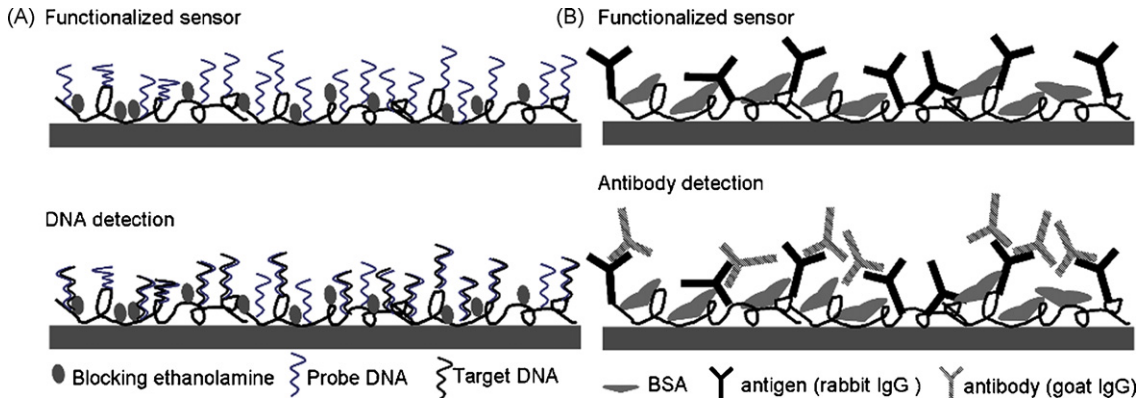

Fig. 1 – (A) Scheme of the sensor surface in the case of a static experiment. The upper drawing pictures the polymer coated surface after DNA immobilization and blocking of the free active groups with ethanolamine. The lower picture shows a scheme of the sensor after DNA hybridization. (B) Scheme of the sensor surface in the case of a dynamic experiment. The upper drawing pictures the polymer coated surface after rabbit IgG immobilization and blocking of the free active groups of the polymer with BSA. The lower picture shows a scheme of the sensor after goat IgG recognition.

a water solution of ammonium sulphate at 20% saturation level). Finally, they were accurately washed with demineralized water and dried under vacuum at 80 °C for 30 min. Details about DNM coating on silicon MCs are reported in Ref. [22].

2.2.1. *Procedures: static experiments*

The MC arrays were cleaned in a UV-ozone cleaner (Novascan, US) for 5 min at 30 °C and then immediately coated with DNM following the protocol described in [22].

Solutions of oligonucleotides at 5 μM concentration were printed on DNM coated MCs using a spotter by Scienion GmbH, Germany under controlled conditions of humidity (50%) and temperature (22 °C). In each array, four MCs were printed with COCU8, while the other four with COCU9. All MCs were covered from the basement to the tip with 16 subsequent spots of about 400 pL. Printed arrays were then placed in an uncovered storage box placed in a sealed chamber, saturated with NaCl, and incubated at room temperature overnight. The residual reactive groups of the coating were blocked by dipping the arrays in 50 mM ethanolamine/0.1% SDS/0.1 M Tris for 30 min. After discarding the blocking solution, the arrays were rinsed with water and washed for 30 min in 4 × SSC/0.1% SDS buffer.

The MCs were actuated with a Cantisens Research platform by Concentris GmbH (Basel, CH). The instrument is equipped with a temperature-controlled measurement cell of 5 μL and a multiple laser lever system for individual MC deflection measurement.

The DNA hybridization detection was performed at 25 °C in hybridization buffer (10 mM Tris, 1 M NaCl, 0.005% Tween 20) at a constant flow rate of 0.83 $\mu L\,s^{-1}$. After the stabilization of the MCs signals under flow for about 90 min, 250 μL of the solution of the target oligonucleotides complementary to the COCU8 probes (1 μM in 2 × SSC/0.1% SDS buffer) were injected into the chamber. The signal of the MC deflection was monitored under constant flow at the rate of 0.83 $\mu L\,s^{-1}$, but also stopping the buffer flow after the target injection.

The hybridization signal was calculated as the difference between the mean signal of the four MCs functionalized with COCU8 and the mean signal of the four reference MCs (functionalized with COCU9, 100% non complementary to the targets). In the case of re-use of the arrays, hybridized oligonucleotides were chemically denatured by purging the cell with 30% urea salts in buffer.

Fig. 1A shows a scheme of the surface of the sensor after the polymer functionalization (top) and the DNA probe immobilization and after the DNA detection (bottom).

In order to have a cross-check of DNA hybridization, all the arrays were observed after the MC experiment with a fluorescence scanner (ScanArray Lite, Perkin Elmer, MA, USA).

2.2.2. *Procedures: dynamic experiments*

The MC arrays were soaked into HF aqueous solution (0.4% v/v) for 5 min, exposed to air plasma (RF Power = 25 W, Air Pressure = 0.4 Torr) for 10 min and polymer coated following the protocol reported in [22].

Binding of rabbit IgG to the polymer coated MCs was carried out by spotting 30 μL of protein solution in PBS (0.5 $mg\,mL^{-1}$). MCs were then placed in an uncovered storage box placed in a sealed chamber, saturated with NaCl, and incubated at room temperature overnight. After washes with PBS (20 min) and water (10 min), DNM free reactive sites were then blocked with BSA (2%, w/v) in a phosphate buffer (50 mM, pH 7.2) for 1 h, and rinsed with PBS (20 min) and water (10 min). MCs were then incubated for 1 h with goat IgG, dissolved in the hybridization buffer (Tris–HCl, 0.1 M, pH 8; 0.1 M NaCl; 1% (w/v) BSA; 0.02% (w/v) Tween 20) at a concentration of 0.05 $g\,mL^{-1}$. After incubation, MCs were washed with Tris–HCl (0.05 M, pH 9), 0.25 M NaCl, 0.05% Tween 20, PBS, and water.

Fig. 1B shows a scheme of the surface of a MC after the polymer functionalization and the rabbit IgG immobilization (top) and after the goat IgG detection (bottom).

After each binding step, the first and second flexural modes of the MCs were measured in vacuum (base pressure 10^{-5} Torr) by means of an optical lever set-up, already described elsewhere [24].

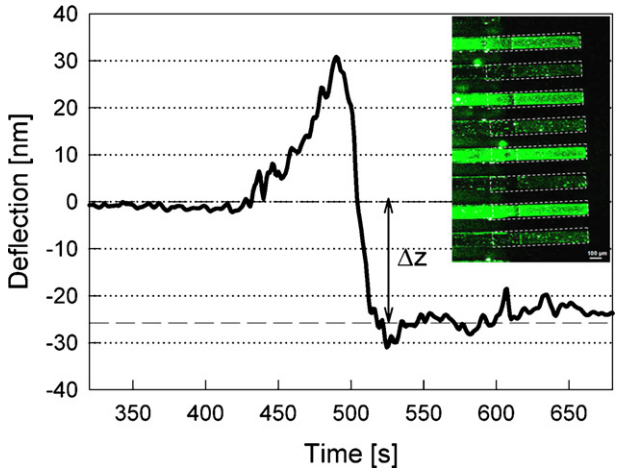

Fig. 2 – Differential signal during the under flow static experiment for DNA detection (1 μM concentration of COCU8 complementary targets). In the inset an image of an array after the target solution injection is shown: a clear fluorescence signal is visible on the COCU8 immobilized MCs.

3. Results and discussion

3.1. *Static experiments*

In order to demonstrate the effectiveness of DNM coated MCs as static biosensors, we chose the detection of DNA hybridization as a model experiment, because the binding reaction as well as its thermodynamic data are well known.

Arrays of eight MCs, functionalized following the polymer coating and the DNA-probe immobilization protocols described in the previous section, were incubated in a solution of target oligonucleotides complementary to COCU8. The difference between the mean signal of the four MCs functionalized with COCU8 and the mean signal of the four reference MCs (functionalized with COCU9) was monitored. This differential deflection provides a signal of the specific hybridization reactions purged from secondary effects such as saline concentration gradients, fluidic and temperature drifts and unspecific interactions between the targets and the non-complementary probes. The measurement was performed under flow of the hybridization buffer as well as stopping the buffer flow after the target injection.

After the deflection measurements, the probe hybridization on COCU8 functionalized MCs was immediately cross-checked by imaging the arrays with a fluorescence scanner.

Fig. 2 shows one of the measured differential signals obtained monitoring the MC deflections, while flowing the target solution into the chamber. In the selected reference system, a positive deflection ($\Delta z > 0$) corresponds to the upward bending of the cantilever due to tensile surface stress (with respect to the cantilever top face), and a negative deflection ($\Delta z < 0$) to a downward bending of the cantilever due to compressive surface stress. The differential deflection diverts to a negative value after an initial positive response during the first seconds after the target injection. This deflection trend was measured for all the performed static experiments and it may be generated by preliminary interactions between the injected targets and the immobilized probes, generating a positive differential deflection corresponding to a tensile stress upon the COCU8 functionalized MCs. After a settling down period of the interface interactions, the hybridization of the complementary DNA strands generates a stable compressive surface stress on these MCs.

A fluorescence image of an array after the molecular recognition experiment is reported in the inset of Fig. 2.

Fig. 3 displays the differential signal obtained monitoring the MC deflections, while letting the target solution to sit in the measurement chamber overnight. The trend of the signal demonstrates the extraordinary stability of DNM coated MCs in aqueous buffers even during very long observation time (14 h) in comparison with most of the experiments published in the literature [1–4]. The fluctuations of the signal during the first 10,000 s, enhanced by the x-scaling, may be generated by the change of the flow conditions and by chemical and physical in-homogeneities of the buffer solution due to salt and detergent particles precipitation and motion in the chamber.

It has to be said that during the deflection signal stabilization performed before the measurements, the DNM coated MCs demonstrated to be subject to low thermal and fluidic drifts and to possess excellent stability in saline buffers. In particular, a stable signal is obtained after 90 min of buffer flow inside the fluidic chamber with drifts of few tens of nanometers, lower values with respect to gold–thiol functionalized MCs [1–4].

Six molecular recognition experiments were performed on three different arrays of MCs that were regenerated for multiple measurements by removing the target in denaturing conditions. Five experiments were performed flowing the target solution on two new and regenerated arrays, while the remaining one consisted of an overnight experiment. The five under flow experiments showed a mean differential downward signal of 24 nm with an error of 3 nm. The low value of the error demonstrates not only the repeatability of the measurements but also the stability of the coating, even after multiple re-uses of the arrays (after de-hybridization washes). The

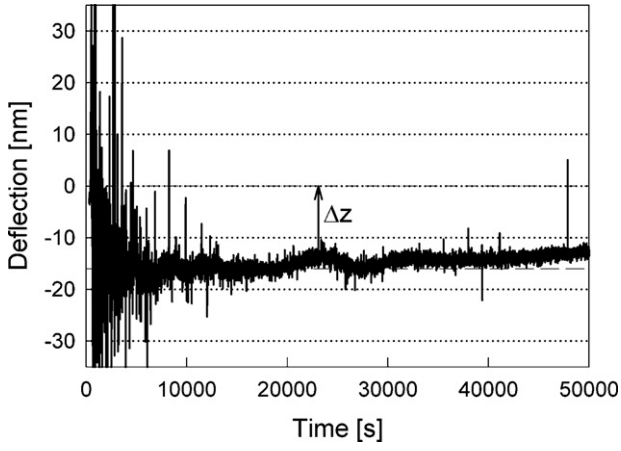

Fig. 3 – Differential signal during the overnight static experiment for DNA detection (1 μM concentration of COCU8 complementary targets).

downward differential signal measured during the overnight experiment resulted to be 16.5 nm.

The applied surface stress was calculated from the measured differential signal using the corrected Stoney equation [25–28]:

$$\Delta\sigma = \frac{1}{4} s \frac{\Delta z E t^2}{L^2(1-\nu)} \quad (1)$$

where $\Delta\sigma$ is the layer stress, Δz is the cantilever deflection, E is the cantilever Young's modulus, t is the cantilever thickness, L is the cantilever length, ν is the cantilever Poisson's ratio and s is the Sader correction factor [28]. $\Delta\sigma$ (which is expressed in $N\,m^{-1}$ and is also called film stress) is related to the properties of the stress inducer, whether it is an applied film or, as in our case, a solid–liquid reacting interface. The compressive stress resulted to be about 5.1 mN m^{-1} and 3.6 mN m^{-1} respectively for the under flow and the overnight experiments, values of the same order of magnitude of the surface stress exerted by DNA duplex formation on MCs when they are functionalized with the "gold–thiol bond" method [1–4].

The results of the above experiments are accurately accounted by the thermodynamic model presented in [5]. The model describes the chemical equilibrium of a receptor/ligand binding reaction confined at the solid–liquid interface placed between the MC surface and the target ssDNA solution. It says that, the standard molar Gibbs free energy of the reaction splits into a chemical equilibrium contribution and a mechanical work contribution according to the equation:

$$\Delta_r G^\circ = \frac{\Delta\sigma}{\alpha(\Gamma_R)_{initial}} + RT \ln\left[c_L\left(\frac{1}{\alpha - 1}\right)\right], \quad (2)$$

where $\Delta\sigma$ is the surface stress given by Eq. (1), α is the receptor/ligand binding efficiency, Γ_R is the density of the immobilized receptor molecules and c_L is the ligand concentration in the buffer solution.

The $\Delta_r G^\circ$ value of the DNA hybridization reaction featured in the MC experiment was evaluated from Eq. (2) and the experimental data compared with the nominal value calculated from the nearest-neighbour model [29]. Taking into account the experimental physical parameters, $E = 168.5$ GPa, $\nu = 0.25$, $T = 25\,^\circ$C and the binding efficiency and the surface coverage values accurately determined in [20], $\alpha = 80\%$ and $\Gamma_R = 3 \times 10^{12}$ DNA strands, in the case of the overnight experiment (the closest to equilibrium), one obtains $\Delta_r G^\circ = -113.6 \pm 17$ kJ mol^{-1} [30]. This result is consistent with the theoretical value calculated from the nearest-neighbour model: $\Delta_r G^\circ = -115.9$ kJ mol^{-1}. The outcome of this analysis confirms the validity of the model even when the surface is functionalized through polymer films with complex architecture. It follows that polymer coated MCs can be used to evaluate the standard molar Gibbs free energy, and in turn the affinity, of the binding reaction occurring onto MCs (provided that the binding efficiency and the receptor concentration at the surface are known). As a side effect, this result is a further evidence that the thermodynamic approach developed in [5] might be a valid contribution to the open discussion about the molecular mechanism that govern MC transduction [1,31–33].

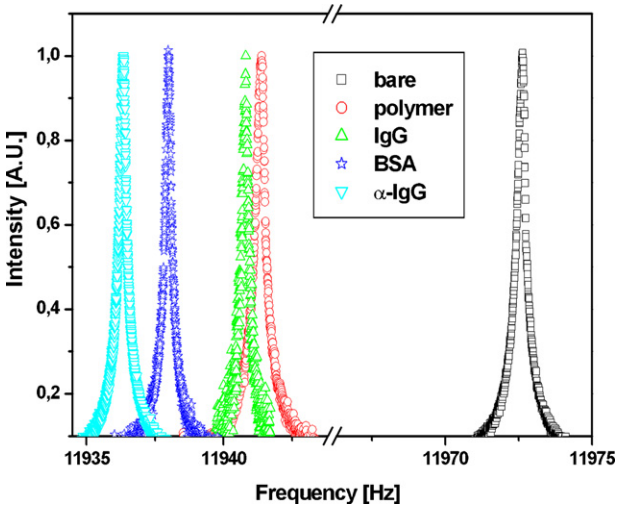

Fig. 4 – Normalized first resonant mode of a MC (nominally 450 μm × 50 μm × 2 μm) after each modification step during the dynamic experiment, i.e. DNM-polymer deposition, immobilization of rabbit IgG, passivation with BSA and recognition of goat IgG.

3.2. *Dynamic experiments*

The effectiveness of DNM coated MCs as dynamic biosensors was assessed through a model experiment aimed at detecting antigen/antibody interactions.

In particular, DNM coated MCs were employed to investigate the recognition reaction between the rabbit IgG (receptor) and the goat anti-rabbit IgG (ligand). The experiments were intended not only to quantify the molecule attachment density, but also to assess the activity of the immobilized antibodies. In fact, the ability of the functional polymeric film to immobilize antibodies with a native conformation of the antigen-binding region is crucial for the effectiveness of the sensor. DNM coated glass slides were successfully used in protein/protein interaction experiments, where we demonstrated that both Fc and Fab domains of a capture antibody were freely accessible once bound to the surface. The polymer coating immobilizes probes in a random conformation and creates an aqueous micro-environment in which epitopes have a good accessibility [34].

The dynamic experiment consisted in monitoring the first and second resonant curves of 10 different sized MCs before and after each modification step (DNM coating, IgG immobilization, BSA passivation and α-IgG interaction) in order to calculate the corresponding mass increments. Figs. 4 and 5 show respectively an example of the normalized peaks of the first and second resonant modes of a MC (nominally 450 μm × 50 μm × 2 μm) after each modification step. The curves were fitted with a Lorentzian function and the first and second resonant frequencies values were calculated (see Table 1).

Since active areas of different dimensions were used, a range of mass increments will be given, while the calculated average surface density will be used as an index of cover-

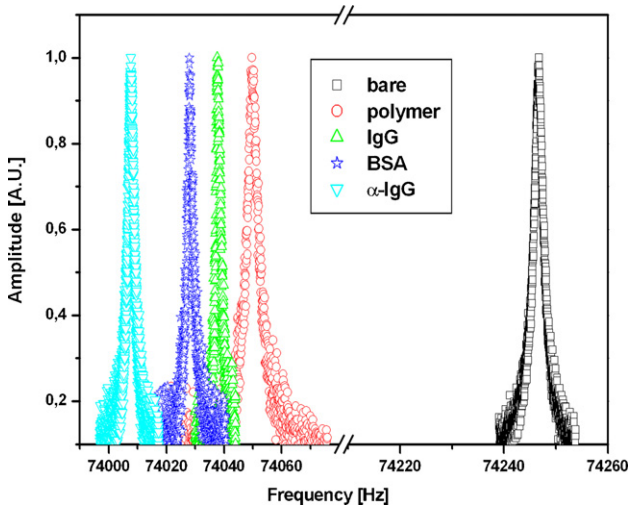

Fig. 5 – Normalized second resonant mode of a MC (nominally 450 μm × 50 μm × 2 μm) after each modification step during the dynamic experiment, i.e. DNM-polymer deposition, immobilization of rabbit IgG, passivation with BSA and recognition of goat IgG.

age. Regarding the entire set, the relative uncertainty of such measurements was found to be around 10%.

We monitored both the first and the second flexural modes, because an improvement of sensitivity is expected when analyzing higher modes with respect to the fundamental one [35–37].

The shift of the eigenfrequency value Δf was related to the deposited mass variation Δm with the following equation [22]:

$$\frac{\Delta f}{f_0} = -\frac{1}{2}\frac{\Delta m}{m_0} \quad (3)$$

where f_0 is the resonant frequency and m_0 is the mass of the MC before molecule binding. This equation is effective when the beam spring constant (k) remains substantially constant after molecule binding and if the added mass is uniformly deposited on the surfaces. This assumption is overall well accepted in microcantilever-based biosensing, in which the adsorbate properties such as thickness, stiffness and surface stress, play a minor rule on the vibrational characteristics of the Si beam [38,39]. Then we defined the mass responsivity as $R \approx 2\pi\,\Delta f_n/\Delta m$ and the intrinsic sensitivity of each flexural mode as $\delta m_n \propto R^{-1}\sqrt{1/2\pi f_n Q_n}$, where f_n is the n-mode resonant frequency, Δm is the deposited mass and Q_n is the quality factor.

Table 1 – The first and the second resonant frequencies of a MC (nominally 450 μm × 50 μm × 2 μm) after each modification step of the dynamic mode experiment. The data are reported with the related instrumental uncertainty.

	1st resonance frequency (Hz)	2nd resonance frequency (Hz)
Bare MC	11972.6 ± 0.2	74246.7 ± 0.7
DNM coating	11941.3 ± 0.2	74050.0 ± 0.7
Rabbit IgG	11940.8 ± 0.2	74038.0 ± 0.7
BSA passivation	11938.0 ± 0.2	74028.2 ± 0.7
Goat IgG	11936.3 ± 0.2	74007.7 ± 0.7

The ratio between the sensitivities of the two flexural modes ($\delta m_1/\delta m_2$) and the average mass responsivity R were calculated. $\delta m_1/\delta m_2$ resulted to be around 15, while R was 0.4 Hz/pg for the first resonant mode and 2.5 Hz/pg for the second one.

Rabbit IgG immobilization induced mass increments in the range of 9–60 pg, which in terms of molecules per surface unit leads to an average value of 5×10^{11} molecules cm^{-2}. This attached protein density is slightly lower but comparable with the theoretical maximum density estimated from the known size of an Ab IgG molecule (23.5 nm × 2.5 nm), which is 17×10^{11} molecules cm^{-2} [40].

The passivation of the free active sites of DNM with BSA leaded to a mass shift in the range of 20–120 pg, which in terms of molecules' density corresponds to an average value of 12×10^{11} molecules cm^{-2}. Since planar size of BSA (14 nm × 4 nm) [40] is very close to Ab IgG one, we can conclude that nearly all the polymer reactive sites were successfully saturated: 5×10^{11} molecules cm^{-2} of rabbit IgG plus 12×10^{11} molecules cm^{-2} of BSA is equal to 17×10^{11} molecules cm^{-2}, i.e. the theoretical maximum density for both the proteins.

The mass increments due to goat IgG interactions induced mass increments in the range of 30–270 pg, which in terms of molecules per surface unit leads to an average value of 15×10^{11} molecules cm^{-2}. The higher binding density for the secondary antibody was expected, as secondary antibody are able to recognize more than one site of the receptor protein.

4. Conclusions

The effectiveness of polymer (DNM) coated silicon MCs for label free recognition of DNA oligonucleotides and proteins was demonstrated.

DNA hybridization experiments were performed in static mode. Differential deflections of 23.5 nm and of 16.5 nm were measured for the under flow and for the no-flow overnight experiments, respectively. It turned out that in all the experiments the surface stress exerted by DNA duplex formation was in the order of some mN m^{-1}, that is the same order of magnitude of the surface stress exerted on MCs when they are functionalized by the "gold–thiol bond" method (the most adopted approach). We showed that DNM coated MCs have excellent stability in saline buffers and are subject to lower thermal and fluidic drifts in comparison with gold–thiol functionalized MCs. They can also be regenerated at least for three times by removing the target oligonucleotides in denaturing conditions, allowing for a fair reuse. Finally, the experimental results are consistently described by the thermodynamic model presented in Ref. [5], demonstrating that the polymer coated MCs (actuated in static mode) are a reliable tool for evaluating ligand-receptor energetics.

DNM coated MCs were also employed to investigate the recognition reaction between the rabbit IgG and the goat anti-rabbit IgG. In this case MCs were actuated in the dynamic mode. The first and second flexural modes were monitored

after DNM coating, IgG immobilization, BSA passivation and α-IgG interaction, in order to calculate the corresponding mass increments. The calculated mass increments were of 9–60 pg for the IgG immobilization, that in terms of molecules per surface unit leads to 5×10^{11} molecules cm^{-2}, while for the BSA passivation we calculated a 20–120 pg mass shift, corresponding to 12×10^{11} molecules cm^{-2}. Finally, the goat anti-rabbit IgG recognition induced a mass increment of 30–270 pg, corresponding to 15×10^{11} molecules cm^{-2}. The obtained results are in good agreement with the literature data as well as with theoretical calculations. The average mass responsivity was 0.4 Hz/pg for the first resonant mode and 2.5 Hz/pg for the second one. This data prove the effectiveness of DNM coated MCs also in the dynamic mode of operation, and in particular for detection of antigen/antibody interactions.

In conclusion, we demonstrated that the DNM polymer provides a valid innovative and versatile MC functionalization approach, which features both static and dynamic modes of actuation and also suits both DNA hybridization and protein/protein interactions. The ability to bind a wide range of biomolecules with high density, the stability of the DNM coated MCs in aqueous buffers, the re-usability of the arrays, added to the easiness, fastness and cheapness of the coating procedure, represent a great improvement with respect to specific and more time consuming functionalization methods commonly adopted in the literature. Therefore DNM coated MCs candidate themselves as an efficient analytical platform for both DNA and protein recognition and as a reliable tool for fundamental studies on biomolecules interactions.

Experiments devoted to the determination of the sensitivity and selectivity of DNM coated MC biosensors and to the application of this technique to other classes of biosensors based on Micro–Electro–Mecahnical Systems (MEMS), including Quartz Crystal Microbalances (QCMs), are underway.

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