

Scanning Electrochemical Microscopy Monitoring in Microcantilever Platforms.

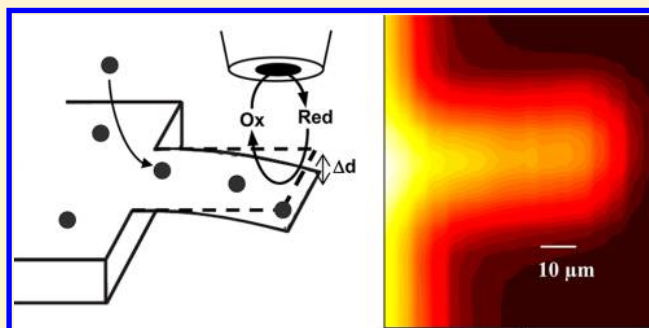
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S Supporting Information

ABSTRACT: The deflection of cantilever systems may be performed by an indirect electrochemical method that consists of measuring the local cantilever activity and deflection in a feedback generation-collection configuration of the SECM. This is illustrated during the electrochemically assisted adsorption of Br onto a gold-coated cantilever, either in its pristine state or previously coated with a thin organic barrier. It is further extended to the adsorption of an antibody in a heterogeneous immunoassay at an allergen-coated microcantilever platform. In both reactions, the cantilever deflection is qualitatively detected from the SECM tip current measurement and a quantitative estimate is obtained through modeling. This electroanalytical strategy provides an alternative approach to standard optical detection. It can overcome some limitations of the optical method by allowing electrochemical characterization of nonconductive cantilevers and appropriate use for closed systems.



The objective is to detect in situ mechanical displacements of various platforms such as arrays of gold coated (Au) microcantilevers. The latter are promising mechanical objects, that were classically developed for the AFM and used as point-end tips to measure ultrasmall forces on particles with size of one atom.¹ Considering their specific mechanical properties characterized by their horizontal deflection or shift in resonance frequency as a result of different local changes in mass, temperature, charge or intermolecular interactions, their largest application is accepted as sensing microdevices. Their ability to be used as physical, chemical and biological sensors is described in several review articles.^{2–4} When operated in a quasi-static mode, microcantilever platforms are used for antibody–antigen assays,^{5,6} DNA hybridization,^{7,8} detection of toxic heavy metal ions,^{9,10} or of any electrochemically induced surface charge accumulation.^{11,12} Then, the cantilever deflection is measured as the result of the interaction of an analyte with the sensing coating deposited onto one of the cantilever faces. The cantilever mechanical deformations are revealed optically by focusing a laser beam at the cantilever end, and measuring the displacement of a reflected spot thanks to a position sensitive detector. This type of detection is well adapted for cantilever arrays, as it allows detecting specifically target species in a mixture.¹³ The cantilever bending measured in this way can reach a subnanometer resolution.

When microcantilever platforms are coated with a conductive gold layer, as presented in Figure 1a, the optical detection can be combined with the electrochemical actuation and/or

detection. On one hand, an electrochemical detection allows an increased robustness of the sensing platform and submonolayer redox state changes can be addressed.¹⁴ On the other hand, an electrochemical actuation may be used to enforce a mechanical deflection of the cantilever driven by its charge^{15–17} or by the molecular reorganization of the moieties assembled on its surface.^{14,18} This strategy is particularly appealing to decipher heterogeneous surface effects,¹⁵ especially when combined with full-field optical detection, to get insight into the mechanical behavior of microcantilevers.^{15,17}

Actually, special care must be taken when optical detection is performed in the course of the electrochemical actuation of the cantilever. Indeed, ions concentration gradients in the double layer induced by the electrochemical actuation may affect the optical response, for example by mirage effects through the local change in refractive index.^{19,20} The objective of this contribution is to propose an alternative detection mode of cantilever deflection based on remote electrochemical measurements. It consists of replacing the local optical probe of the cantilever movement by a local electrochemical probe. Different strategies may be proposed based on dual-electrode systems working in the generation/collection (GC) mode. Such configuration based on redox cycling between a generator and a collector is a widely used electroanalytical technique.²¹ It

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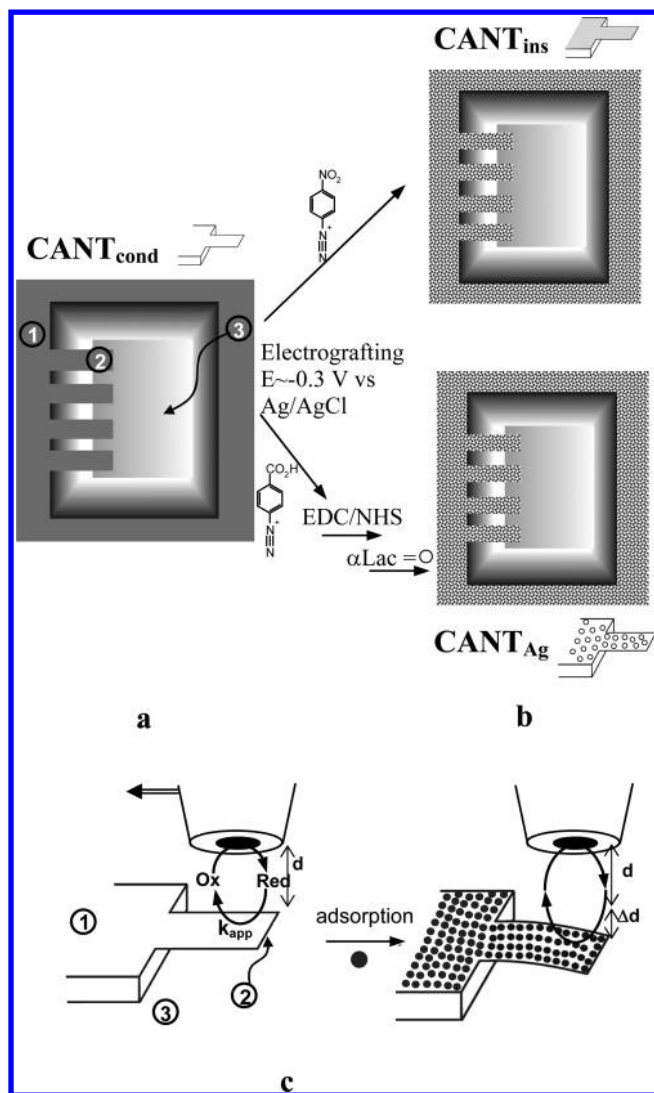


Figure 1. Schematic representation of the platform: (a) bare Au cantilever array, CANT_{cond}; (b) cantilever array grafted with: a thin organic film, CANT_{ins}, or a biochemical layer of an antigen (Ag: α -lactalbumin: α -Lac), CANT_{Ag}; (c) SECM inspection of a species ($\bullet$ = Br⁻ or anti- α Lac) adsorption on the cantilever platform based on electrochemical measurement in the feedback mode. (1) plain substrate, (2) cantilever free end, and (3) carved pool (90 μ m deep).

is the basic principle of the scanning electrochemical microscope (SECM),^{22,23} which allows handling microelectrodes within micro- or submicrometer separation distances.^{24,25} This principle has also been more recently miniaturized in microfluidic systems as electrochemical detection tools²⁶ or in electrochemical nanogaps to produce powerful mechanistic²⁷ and biosensing²⁸ platforms with single molecule detection capabilities. The transposition of the feedback GC mode with microcantilever platforms can be achieved by holding a small electrochemical probe (Generator) in the vicinity of a cantilever electrode (Collector). This is easily achieved in the SECM configuration (Figure 1c). To our knowledge, the combination of microcantilevers and SECM has only been described when microcantilevers are used as SECM tips for simultaneous topographical and electrochemical imaging of a sample surface with enhanced spatial resolution.^{29–31}

Here, we propose to use a standard microelectrode (a tip) to interrogate the microcantilever electrochemical and mechanical

activity. The SECM is used to image and quantify the electrochemical reactivity of gold coated microcantilever platforms. Such micromechanical cantilevers have been used as chemo-sensors or electrochemo-sensors once an appropriate sensing target has been anchored on their surfaces.³ Usually, the coating of an organic layer on an electrode alters the electron transfer properties and results in the lowering of the Au surface conductivity.^{32,33} Here, two types of cantilever platforms are considered: (i) a pristine Au-coated cantilever, CANT_{cond}, which will mimic an electrochemical actuating platform (Figure 1a) and (ii) an Au-cantilever coated with a thin organic layer which will mimic a sensing layer coated cantilever (Figure 1b). An example of the latter platform is illustrated by the case of a film with a few nanometers thickness obtained through the electrografting of a diazonium salt (CANT_{ins}, upper part of Figure 1b); this is a promising, fast and efficient strategy for the coating of conducting surfaces with functional organic layers.³⁴ Indeed, a -COOH terminated thin film obtained via diazonium chemistry can be used to covalently bind a biochemical entity, an α -lactalbumin antigen (lower part of Figure 1b). This sensing platform, CANT_{Ag}, is capable of sensing in a heterogeneous immunoassay the corresponding antibody present in solution via a receptor–ligand interaction.

RESULTS AND DISCUSSION

Electrochemical Inspection of Conducting and Coated Cantilever Platforms. As sketched in Figure 1c, the SECM was used to address and image the electrochemical reactivity of both types (conductive and insulating) of cantilever platforms held in a solution of a redox probe (2 mM Ru(NH₃)₆³⁺ in 0.1 M KBr) with a 12.5 μ m radius Pt electrode (RG = 7) used as the tip. The SECM also allows positioning the tip above either i) the plain substrate or ii) the free end of one cantilever standing over a carved pool (Figure 1). These SECM procedures were achieved in the feedback mode by reducing Ru(NH₃)₆³⁺ at the tip. The SECM approach curves of the unbiased plain, CANT_{cond}, and grafted, CANT_{ins}, substrates are presented in Figure 2a as red and green curves respectively. The experimental (open symbols) curves are compared to theoretical ones (lines). The CANT_{cond} substrate behaves as a conducting surface as its SECM approach curve shows a positive feedback response, while CANT_{ins} presents a negative feedback (lowest curve in Figure 2a). This shows that the conducting behavior of Au is partially blocked by the presence of the organic layer. However, the layer is not completely blocking and allows some charge transfer occurring at an heterogeneous rate constant, $k_{app,ins} = 3.4 \times 10^{-4}$ cm/s, estimated from fitting to theory. Conversely, the electron transfer (ET) rate for the same reaction of the tip electro-generated Ru(NH₃)₆²⁺ at the CANT_{cond} substrate is $k_{app,cond} > 0.02$ cm/s.

SECM Imaging of Cantilever Platforms. The contrast in the electrochemical reactivity of both substrates is further confirmed by SECM imaging of a 70 μ m long and 20 μ m wide microcantilever by a SECM tip rastered at a constant 13 μ m distance above the same pristine and grafted substrates. The inserts b and c in Figure 2 present the SECM images of CANT_{cond} and CANT_{ins} respectively. For CANT_{cond} (Figure 2b), the currents recorded at the lowest corners of the image are representative of the infinite distance current, $i_{T,inf}$, as the tip is held above the deep reservoir of solution; the substrate and cantilever are then detected as regions of tip current, i_T , higher than $i_{T,inf}$. The tip current at the cantilever edge is lower

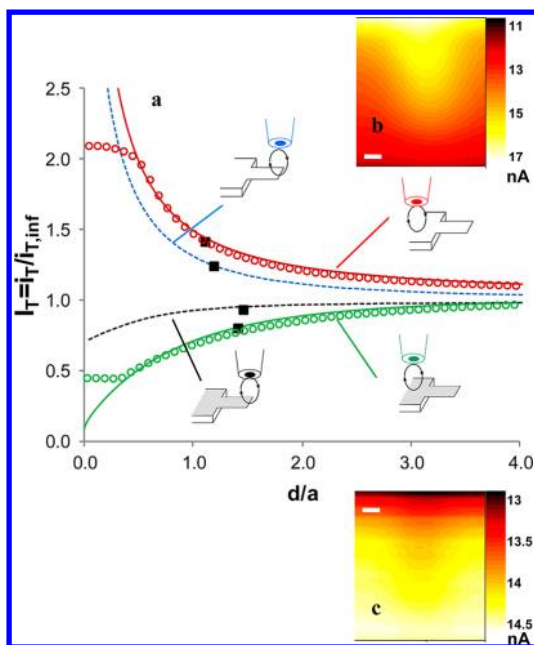


Figure 2. SECM approach curves (a) and images (b, c) with a $12.5\ \mu\text{m}$ radius tip of unbiased bare cantilever substrate $\text{CANT}_{\text{cond}}$ (red open circles, experimental curve; red line, theory) and grafted cantilever substrate covered with nitrobenzene film, CANT_{ins} (green open circles, experimental curve; green line, theory). (c) Redox probe: $2\ \text{mM}\ \text{Ru}(\text{NH}_3)_6^{3+}$ in $0.1\ \text{M}\ \text{KBr}$, $E_{\text{tip}} = -0.3\ \text{V}$ vs Ag/AgCl . Dashed lines: theoretical approach curves of the cantilever free end (same reactivity as the plain substrate, bare, dashed blue; grafted, dashed black). ■: Currents measured above plain substrate and cantilever free end during SECM images capture in b and c performed at constant height d (measured above the plain substrate) of $\text{CANT}_{\text{cond}}$ with $d = 13.5$ and of CANT_{ins} , $d = 16.5\ \mu\text{m}$. d measured above the plain substrate. Scale bar = $10\ \mu\text{m}$. Tip currents are presented in their absolute values (for $\text{Ru}(\text{NH}_3)_6^{3+}$ reduction, a negative cathodic current is detected experimentally).

than that above the substrate due to a lesser contribution in the regeneration process from the small microcantilever object. Conversely, in the image of the coated CANT_{ins} (Figure 2c), the substrate and cantilever are detected as regions of tip current, i_T , lower than $i_{T,\text{inf}}$, as a result of diffusion hindrance by the partly blocking substrate. The tip current at the cantilever edge is higher than that above the substrate, due to lesser diffusion hindrance by the small object in regard to the tip.

The 3D modeling of the SECM tip current – distance curve (approach curve) above the free end of a cantilever for both platforms is presented as dashed lines, in Figure 2, using the electrochemical reactivities, k_{app} , estimated on the plain substrates (see above). Such modeling confirms that the cantilever is described by less contrasted current than the plain substrate. It also shows that the tip current values recorded at the cantilever end correspond to slightly higher tip–substrate separation distances, indicating that the cantilever is tilted down (by $<1\ \mu\text{m}$). Finally, a higher contrasted image can be obtained at smaller tip–substrate separation distance (Supporting Information Figure S11 for $\text{CANT}_{\text{cond}}$ imaged at $1.5\ \mu\text{m}$ constant height).

SECM Sensing of Mechanical/Chemical Transformation at Microcantilever. The SECM tip was then used as a local sensor of mechanical or (electro-)chemical changes in microcantilever platforms. The SECM tip positioned either

over one cantilever free end or over the plain substrate is used as an amperometric sensor of the local substrate activity. The tip is then biased at a constant potential for mass-transfer limited transformation of the redox probe. The principle consists of recording the variations in tip current associated to the substrate (electro-)chemical transformation. Such transformation may alter the electrochemical activity of the substrate, which is detected as a variation in feedback or of redox cycling of the redox probe at the substrate. For the microcantilever, the surface transformation may also result in the cantilever deflection corresponding in change in tip–substrate separation distance and also detected as change in tip current.

The principle is tested through the observation of species adsorption on the substrate surface. The same electrolytic solution is used, the tip is now immobile at a close distance, d , from the substrate and biased at $E_T = -0.3\ \text{V}$ vs Ag/AgCl for mass-transfer limited reduction of $\text{Ru}(\text{NH}_3)_6^{3+}$. Meanwhile, the substrate potential, E_S , is scanned at $10\ \text{mV/s}$ toward anodic potentials ($0.1 < E_S < 0.6\ \text{V}$ vs Ag/AgCl). Under these conditions, the substrate does not allow the transformation of the $\text{Ru}(\text{NH}_3)_6^{3+}$ redox probe present in solution but may regenerate it from its reduced form ($\text{Ru}(\text{NH}_3)_6^{2+}$) generated at the tip (Figure 1c). The variations of the feedback current collected at the immobile tip for this regeneration at the substrate during the variations of E_S are presented in Figure 3. The tip position was fixed at $3\ \mu\text{m}$ height over the $\text{CANT}_{\text{cond}}$ substrate. The tip current is recorded simultaneously with the

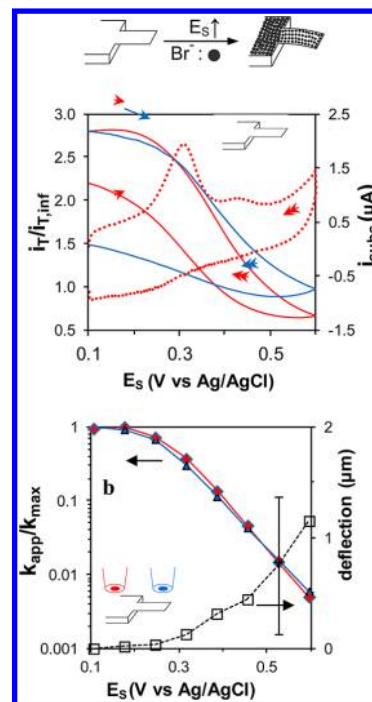


Figure 3. Tip current, i_T , responses normalized to current at infinite distance, $i_{T,\text{inf}}$ in an aqueous $2\ \text{mM}\ \text{Ru}(\text{NH}_3)_6^{3+}$ + $0.1\ \text{M}\ \text{KBr}$ solution. (a) Tip currents measured over $\text{CANT}_{\text{cond}}$ substrate: above the plain substrate (red) or the cantilever free end (blue) assorted with the substrate response (i_{subs} for a $0.36\ \text{cm}^2$ substrate, dashed red in a). (b) ET rate for redox probe regeneration at the substrate estimated from tip current response above (red diamond) plain substrate or (blue triangle) cantilever free end; deflection (open square) estimated from tip response, assuming the cantilever reactivity is the same as that of the substrate (red diamond).

substrate response (dashed line in Figure 3a), whether the tip is positioned over the substrate (red line) or over a cantilever free end (black line); it allows the description of the tip/substrate coupling in the (TG/SC) feedback mode of the SECM.

We have tested the methodology in the case of an electrochemically assisted adsorption on the substrate since this situation mimics what is encountered in chemosensor microcantilever platforms. The adsorption of Br^- was chosen to be tested as it has been extensively characterized^{35–38} and should be compatible with independent SECM interrogation without excessive Au dissolution. Figure 3a illustrates the cyclic voltammetric response of the $\text{CANT}_{\text{cond}}$ substrate in 0.1 M KBr. It presents a main peak at 0.29 V versus Ag/AgCl assigned to the oxidative adsorption of Br^- on Au. It is followed by a smaller peak at 0.45 V representative of the Br adlayer reconstruction³⁶ and then by the onset of Br_2 formation and/or Au dissolution. Reports by Lipkowsky's group^{35–37,39} depict the Br^- adsorption process as a potential dependent coverage of the Au surface with Br. This process starts at the peak onset until a closely packed monolayer is attained at anodic potentials ($E_s > 0.5$ V vs Ag/AgCl).^{35,39} The process, depicted by (1), yields to the formation of a chemisorption bond with partial covalent character between the adsorbed Br^- and the metal surface, as evidenced by thermodynamic analysis of charge density data,^{35–37} second harmonic generation spectroscopy³⁵ and X-ray diffraction studies.³⁸



During this process, the Br surface coverage is proportional to the electrode surface charge density. The integration of the forward substrate CV scan indicates that on $\text{CANT}_{\text{cond}}$, the Br^- adsorption is associated to a flow toward $\text{CANT}_{\text{cond}}$ of a positive charge of $90 \mu\text{C}/\text{cm}^2$, a value in agreement with full electrode coverage.

This oxidative process associated to Br adsorption is recorded simultaneously by the interrogating SECM tip. At potentials more positive than the onset of the electrochemical substrate transformation, the tip current decreases, indicating a decrease of the $\text{Ru}(\text{NH}_3)_6^{3+}$ regeneration at the Au surface during its coverage by the Br layer. The extent of the Au coverage on the ET properties may be seen on the forward and backward potential scan, from both local SECM observation above the plain substrate and the cantilever end. However, a hysteresis is observed and the original surface is not totally recovered (desorption not completed) at the end of the backward CV scan. Desorption is completed during longer polarization times or polarization at more cathodic potentials, but is difficult to quantify in the presence of $\text{Ru}(\text{NH}_3)_6^{3+}$ in the solution. Then, only the forward potential scan is used to analyze the local potential dependency of the adsorption process. This is obtained through the estimate of the local electron transfer rate from the substrate to the tip-generated $\text{Ru}(\text{NH}_3)_6^{2+}$. The ET rate constant, k_{app} in cm s^{-1} , is obtained from available theoretical analytical expressions relating the tip current to finite ET kinetics at the plain substrate,⁴⁰ or, for the cantilever free end, from 3D modeling. For both locations, k_{app} values, normalized by their maximal value, k_{max} are plotted in Figure 4b. They decrease exponentially with the substrate potential with a Tafel coefficient of the order of $\alpha = RT/d \ln k_{\text{app}}/F d E_s = 0.39$ and 0.33 , respectively for the plain substrate and the cantilever end. This shows that the Br surface coverage alters the ET kinetics. Moreover, this electrochemically induced deposition on the cantilever should induce a stress accom-

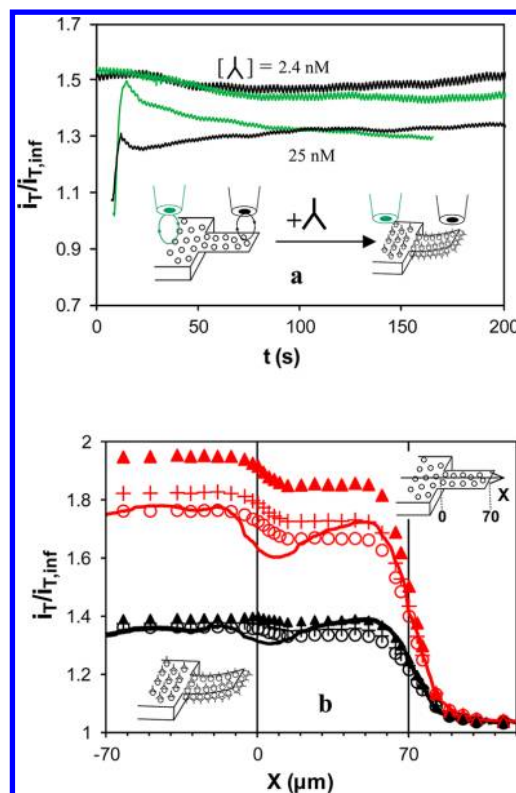


Figure 4. Interaction of Ab with Ag (α -lac) immobilized on an Au microcantilever platform, CANT_{Ag} . (a) Transient tip responses recorded during Ab adsorption on the CANT_{Ag} platform, $d = 5 \mu\text{m}$, above (green) plain substrate or (black) cantilever free end; injection at $t = 10$ s of 2.4 or 25 nM of Ab. (b) Experimental (lines) and theoretical (symbols) linescans tip response while rastered above, $X < 0$, the plain substrate and, $0 < X < 70 \mu\text{m}$, the cantilever free end, after (black) Ab adsorption followed by (red) Ab desorption in the presence of glycine; theoretical responses for tip-object separation distances from top to bottom $d = 4, 4.9, 5.6 \mu\text{m}$: red symbols, $k_{\text{app,Ag}} = 0.02$; black symbols, $k_{\text{app,AgAb}} = 0.008 \text{ cm/s}$. Redox probe: 2 mM $\text{Fe}(\text{CN})_6^{4-}$ in PBS. $E_{\text{tip}} = 0.4$ V vs Ag/AgCl, substrate unbiased.

panied by the cantilever deflection and therefore by a change in the tip-cantilever separation distance. Indeed, the Br adsorption has a compressive effect on Au(111), associated to a decrease of the interfacial energy of 0.85 N/m/V , estimated from electrocapillary curves.³⁷ The extrapolation of these electrocapillary data obtained in a diluted KBr solution yields a considerable compressive surface stress of $\Delta\gamma \approx -0.5 \text{ N/m}$ for the Br adsorption at $E_s = 0.6$ V on Au(111). This suggests an important deflection of the cantilever. Its contribution on the tip current may be appreciated from the $k_{\text{app}} = f(E_s)$ relationship observed at the cantilever end. Assuming that the electrochemical activity, $k_{\text{app}} = f(E_s)$, of the cantilever follows that of the substrate (meaning at each E_s , $k_{\text{app,plain}} \approx k_{\text{app,cantilever}}$), the smoother potential variations of the tip current above the cantilever end can be interpreted as a variation of the tip-cantilever separation distance, Δd in Figure 1c.

Qualitatively, the variation of the tip-cantilever separation distance can be discussed from a general inspection of approach curves (variations of I_T with d/a). Reactive substrates are characterized by positive feedback behaviors ($I_T > 1.2$) and a decrease of the tip current is associated with an increase of the tip-substrate separation distance d/a . Conversely, at low substrate reactivity, negative feedback is observed ($I_T < 1$) and an increase of d/a is associated with an increase of the tip

current. Finally, in a limited range of $k_{\text{app}} \approx D/a$ (D is the diffusion coefficient of the redox probe), the approach curve is almost independent of d/a and $I_T \approx 1$; therefore, no information may be obtained on the tip–substrate separation distance.

These three distinct behaviors qualitatively fit with the potential-dependent behaviors observed experimentally in Figure 3a,b. For $0.3 < E_s < 0.5$ V, the reactivity of the substrate is high ($I_T > 1.3$ in Figure 3a), and the cantilever response shows lower $k_{\text{app}}/k_{\text{max}}$ values than the plain substrate, indicating a larger d above the cantilever than above the plain substrate (downward cantilever deflection). For $E_s > 0.52$ V, the substrate reactivity is in the negative feedback region ($I_T < 1$ for the plain substrate in Figure 3a) and $k_{\text{app}}/k_{\text{max}}$ is higher on the cantilever than on the plain substrate, also indicating a larger d above the cantilever (downward deflection). The identical reactivity and $I_T \approx 1$ correspond to the region with no distance effect and no information can be obtained on the cantilever deflection.

A quantitative description of the cantilever deflection ($\square$ in Figure 3b) is obtained from 3D modeling, knowing the experimental tip current values (provided in Figure 3a) and the k_{app} extrapolated from the plain substrate values (red diamond in Figure 3b). It confirms that the cantilever bends down during the positive charge accumulation and Br adsorption, in agreement with a compressive surface stress. The electrochemically induced electrode coverage induces a bending of the cantilever by ~ 1.2 μm at $E_s = 0.6$ V while submicrometric deflections can be detected at lower potentials. The values of the deflection may vary by less than 20% because of uncertainties on the position (x, y, z) of the tip above the cantilever end. The approximate μm deflection is supported by the mechanical properties of the microcantilever. Indeed, it is typically the magnitude of deflection detected by independent interferometric measurement in air upon a 10 °C temperature change.

Similarly, the tip response above the CANT_{ins} platform is addressed in Supporting Information (Figure SI2). Briefly, different behaviors are observed above the cantilever free end or the plain CANT_{ins} substrate, suggesting qualitatively the mechanical contribution of the cantilever. However, it also suggests differences in local electrochemical reactivity. It is difficult to deconvolute properly from the tip responses, obtained at a single tip height, the contributions of reactivity and cantilever deflection.

SECM Sensing of Receptor–Ligand Reaction. Once the potentiality of the SECM sensing of cantilever activity has been demonstrated on electrochemically actuated systems, it was tested for heterogeneous immunoassays. We have followed the same electrochemical approach to describe the formation of an immune complex at a microcantilever platform surface. Here, the SECM tip is used to follow, in the feedback mode, a model immunological reaction occurring heterogeneously between the cantilever platform, CANT_{Ag}, on which an antigen ($\text{Ag} = \alpha$ -lactalbumin) has been immobilized and its corresponding antibody (Ab) present in the solution. The Ag–Ab interaction is probed upon Ab injection from the current variations detected at the tip for the oxidation of a redox probe (2 mM $\text{Fe}(\text{CN})_6^{4-}$ in PBS), with the tip held at 5–6 μm from the CANT_{Ag} surface.

Except from the initial current transient spike due to solution injection, the Ab adsorption on the plain substrate (Figure 4a, green lines) results in a decrease of the tip response. The

current decrease indicates that upon the Ag–Ab complex formation, the ET from the Au surface to the redox probe is attenuated. Here, it results from the ET attenuation upon the surface immobilization associated to the coverage of the CANT_{Ag} platform surface with a more compact biomolecular layer. This is corroborated by the current increase detected upon desorption of the Ab during glycine injection (not shown), which causes the dissociation of the immune complex from the surface. These observations are in line with the decrease of the efficiency of cycling of redox probes detected during the specific capture of biochemical targets.^{28,41–44} A similar indirect electrochemical strategy for label-free detection of biochemical capture has been originally proposed for DNA hybridization^{41,42} and applied more recently for Ab detection, either through Ag–Ab interaction at screen-printed electrodes⁴³ or for the detection of coumarin-antibody on a coumarin-derivatized microelectrode in molecular addressable libraries.⁴⁴ Figure 4a also suggests that the sensitivity of such electrochemical method based on ET attenuation upon Ag–Ab association is approximately nanomolar, a value ~ 10 times higher than that attained for α -lac in a magnetic nanoparticles-based immunoassay⁴⁵ or in surface plasmon resonance biosensing.⁴⁶

Conversely, above the cantilever free end (Figure 4a, black lines), the Ab immobilization is associated to a first current decrease followed by a current increase, in favor of both ET attenuation and tip-cantilever distance change. Such opposite current variations then indicate that during the Ab association, the cantilever is approaching the tip.

The upward (or downward) deflection associated to the biomolecular adsorption (or desorption) on the cantilever is also confirmed from linescans of the tip current recorded while it is rastered slowly at 5 $\mu\text{m/s}$, to prevent convective perturbation,⁴⁷ along the cantilever symmetry axis after the adsorption and desorption processes (Figure 4b). It can be seen from Figure 4b that the substrate response ($x < 0$), or the cantilever one ($0 < x < 70$ μm) is heterogeneous and varies locally by $\sim 15\%$. However, the value of the tip current above the end of the cantilever ($X \approx 70$ μm) is slightly lower (by $\sim 5\%$) than that recorded above the substrate when the Ab has been adsorbed (black line in Figure 4b). Conversely, after desorption of the immune complex, a linescan is recorded in a solution of the redox probe in the presence of 0.1 M glycine (red line in Figure 4b). The tip response above the cantilever end is lower by 20% compared to that on the substrate. Within the condition of positive feedback ($I_T > 1.2$), such difference indicates the relative downward deflection of the cantilever associated to the desorption process. These linescans can be compared to theoretical tip responses obtained from 3D modeling. They are presented in Figure 4b as symbols for respectively tip-object separation distances $d = 4, 4.9$, and 5.6 μm and for apparent ET rate constants, $k_{\text{app,Ag}}$ and $k_{\text{app,AgAb}} = 0.02$ and 0.008 cm/s respectively. A good fit is observed above the plain substrate with the same $d = 5.6$ μm (circles) before or after Ag–Ab formation with the respective k_{app} values. Meanwhile, above the cantilever end ($50 < x < 70$ μm), the best fit is obtained, with the same k_{app} values, for $d = 4.9$ μm or $d = 4$ μm respectively before and after Ag–Ab formation. This difference indicates that the Ag–Ab interaction is associated with a 0.9 μm upward deflection of the cantilever free end.

Even though the magnitude of the deflection is comparable, the upward deflection during Ab adsorption is at odd with the downward deflection observed during Br adsorption. However,

both systems may behave differently. Indeed, the Br adsorption is actuated electrochemically and then only occurs on the gold coated face of the cantilever, while the Ab adsorption may occur on both faces of the cantilever. The upward deflection of the cantilever during Ab immobilization then indicates that the upper gold side of the cantilever is less coated by Ag than the lower silica side. It is not surprising, owing to the Ag immobilization procedure on the Au surface: the seeding of the Au surface with the $-\text{COOH}$ anchoring group was obtained by diazonium electrografting and the resulting layer does not completely cover the Au surface as it still shows positive SECM feedback ($I_T > 1.3$).

CONCLUSION

The deflection of cantilever systems may be addressed by an indirect electrochemical method, such as redox cycling between a microelectrode, such as a SECM tip, and a microcantilever. Such alternative to the classical optical detection consists of measuring the local cantilever activity and deflection in a feedback generation-collection configuration of the SECM. This is illustrated in different sensing systems. We first use the electrode nature of the cantilever platform to induce the electrochemically assisted adsorption of Br on an Au cantilever, either in its pristine state or previously coated with a thin organic barrier. The Br adsorption is accompanied by the attenuation of the ET to a redox probe and is associated to the measurable bending of the cantilever. When a thin organic layer coats the Au surface, because of the heterogeneity of the coating layer on the whole platform, the deflection cannot be extracted from the SECM measurements, even though contrasted behaviors qualitatively suggest a mechanical effect at the cantilever. Then, a microcantilever platform is used to sense an immunological reaction between a surface immobilized antigen and its solubilized antibody. The Ab–Ag interaction is associated with a feedback decrease due to the ET attenuation during the recognition event associated to the coverage of the electrode surface by a passivating biomolecular layer. It is also associated to a deflection of the cantilever detected by a local tip current measurement.

The resolution of the electrochemical technique cannot be compared yet to the standard optical one, but some advantages of this approach may be pointed out. First of all, the simplicity of the setup and the possibility of simultaneously measuring the cantilever deflection and characterizing the surface reactivity by SECM can be mentioned. Although the optical detection of cantilever mechanical movements is the most sensitive detection technique, some limitations have been expressed. Indeed, the optical technique is not suited for samples absorbing or scattering light, and also for fluids with suspended particles. The proposed electrochemical technique can overcome some of these limitations, by allowing electrochemical characterization of nonconductive cantilevers and appropriate use for closed systems, where the optical detection is not possible. Moreover, it is obvious that the resolution for the detection of the cantilever mechanical displacement depends on the tip radius. Then, owing to the current possibilities to fabricate submicrometric UMEs, imaging resolution down to the nanometer scale is expected.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and details, SECM image at $1.5\ \mu\text{m}$ height of $\text{CANT}_{\text{cond}}$ and CV response of CANT_{ins} substrate

during Br adsorption. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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Scanning Electrochemical Microscopy Monitoring in Microcantilever Platforms.

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Supporting Information

1. Experimental section

1.1. Materials. The microcantilever platforms were fabricated by LAAS (Toulouse, France). They were obtained by i) etching the thermally grown oxide layer on top of a silicon (100) wafer to define arrays of 8 microcantilevers (70 μm long, 20 μm wide, 770 nm thick), ii) release by etching the exposed silicon with KOH and iii) coating the whole wafer forming the cantilever platform with a 50 nm Au layer on top of a 20 nm Ti adhesion layer. Chemicals: $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$, $\text{K}_4\text{Fe}(\text{CN})_6$, 4-nitrobenzene diazonium, 4-carboxybenzene diazonium, KCl, pH 7.5 phosphate buffer saline (PBS) made from (137mM NaCl, 8mM ($\text{KH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4$)), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), ester of N-hydroxysuccinimide NHS, α -lactalbumin, glycine, were used as received from Aldrich, anti- α -lactalbumine was used as received from Genetex.

1.2. Cantilever platform surface derivatizations. The coating of the microcantilever platform with an organic layer was achieved by the reduction at an Au-coated microcantilever platform, $\text{CANT}_{\text{cond}}$, of a 0.1 M NBu_4BF_4 acetonitrile solution containing 5 mM of either 4-nitrobenzene diazonium (CANT_{ins}) or 4-carboxybenzene diazonium (CANT_{Ag}). The reduction was achieved under potentiostatic conditions by applying a potential 0.3 V more negative than

the diazonium reduction potential for 300 s. This procedure leads to the immobilization of a 8 nm thick nitrobenzene multilayer on Au, CANT_{ins}. The thickness was obtained from ellipsometric measurement on the plain substrate surface whose dimension is larger than the ellipsometric laser beam diameter. The –COOH terminated functionalities of CANT_{Ag} were then coupled to α -lactalbumin antigen (Ag, 20 μ M in PBS) through peptidic coupling via EDC/NHS: the substrates were treated with a solution of NHS (60 mM) and EDC (30 mM) in ultrapure water for 90 min, then rinsed in Milli-Q water and dried under a flow of dry nitrogen. The Ag was then immobilized by reacting the substrates with an Ag solution (20 μ M in PBS) for 2 h. After this step, the excess of non immobilized Ag was removed by three repeated washings with PBS. In order to block binding sites not occupied by bioactive species, the Ag derivatized substrates were then immersed for 15 min in a 1-aminoethanol solution (1 M), then washed with PBS and Milli-Q water. To prevent non-specific interaction, the substrates were further immersed for 15 min in 2 % polyvinyl-pyrrolidone solutions, then vigorously rinsed three times with PBS and Milli-Q water. The bioactive surfaces prepared were abbreviated as CANT_{Ag}.

1.3. SECM procedures. The SECM experiments were performed with either a homemade or a CH910B SECM (CH Instruments, Austin, Tx, USA) station equipped with video observation (Navitar long range lenses). Images and approach curves were obtained after tilt adjustment of the Au plain platform versus the SECM probe displacement. The positioning of the tip above the plain substrate or the free end of a microcantilever was assigned from the SECM image, the tip was positioned respectively 100 μ m away from the plain substrate/pool border or 5-10 μ m from the cantilever end. For both the plain substrate and the cantilever free end, distances from the tip, d , are measured above the plain substrate, this means that if the cantilever is tilted, the true distance between the tip and the cantilever free end is either larger (downward deflection) or smaller (upward deflection) than d . Linescans are drawn at tip scan

rate of 5 $\mu\text{m/s}$ to minimize convection influence on the tip current.¹ Images are made as 10 $\mu\text{m/s}$ linescans in the x-direction, the tip is then moved after each x-line 2 μm in the y-direction.

For short-time experiments, such as SECM approach curves (Fig 2a) and tip interrogations (Figs 3 and 4, SI2), the experiments were conducted in a 20-40 μL droplet of electrolytic solution covering the whole 6x6 mm^2 substrate area. Longer experiments, such as SECM images, were obtained in a Teflon electrochemical cell (CH Instruments) filled with 500 μL of electrolytic solution. In both cases the evaporation extent was found negligible. The tip was made from a 25 μm diameter Pt wire embedded into a glass insulating sheath of radius 7 times larger than the tip radius ($\text{RG}=7$). A 250 μm diameter Pt wire was used as the auxiliary and a 250 μm diameter Ag/AgCl wire was used as a pseudo-reference electrode. The tip positioning, imaging and substrate characterization was obtained in an electrolytic solution containing a redox probe ($\text{Ru}(\text{NH}_3)_6\text{Cl}_3$ for $\text{CANT}_{\text{cond}}$ and CANT_{ins} ; $\text{K}_4\text{Fe}(\text{CN})_6$ for CANT_{Ag}). The tip was biased at a potential where the redox probe is transformed under mass transfer control: $E_{\text{tip}} = -0.3 \text{ V}$ vs Ag/AgCl for $\text{Ru}(\text{NH}_3)_6^{3+}$ reduction or $E_{\text{tip}} = 0.4 \text{ V}$ vs Ag/AgCl for $\text{Fe}(\text{CN})_6^{4-}$ oxidation.

1.4. Immunoassays. The immunoassay experiments in the SECM configuration were performed in solutions containing the same 2 mM concentration of $\text{Fe}(\text{CN})_6^{4-}$ redox probe in order to avoid the redox probe dilution. A stock solution of 50 nM antibody + 2 mM $\text{K}_4\text{Fe}(\text{CN})_6$ solution was prepared and stored at 4°C. The 2.4 nM antibody measurement was performed by recording the $\text{Fe}(\text{CN})_6^{4-}$ oxidation while adding a 2 μL drop of the Ab stock solution into a 40 μL drop of 2 mM $\text{Fe}(\text{CN})_6^{4-}$ already in contact with the cantilever platform and the SECM tip. The 25 nM experiments were performed by the direct deposition of a 40 μL drop of required solution between the substrate and tip. Ag-Ab dissociation was achieved after successive rinsings of the substrate/tip assembly previously exposed to the Ab solution

with 4x40 μL of MilliQ water, then with 2x40 μL of $\text{Fe}(\text{CN})_6^{4-}$ solution, followed by removal of the latter and deposition of a 40 μL of a 0.1 M glycine + 2 mM $\text{K}_4\text{Fe}(\text{CN})_6$ solution.

1.5. Simulations. Simulated approach curves above the cantilever free end were obtained by the finite elements method with COMSOL 3.4 package. The simulations were computed in 3D geometry for different tip positions above the substrate and different finite electron transfer kinetics at the substrate corresponding to the experimental situations. The simulation consisted, as described elsewhere,^{1,2} of the numerical solution of the diffusion equation under the appropriate boundary conditions and electrode position. The probe current was evaluated using a weak constraint procedure. To generate SECM approach curves, a steady-state simulation was obtained for a given probe-substrate distance, d . $L=d/a$ is the dimensionless probe-substrate distance, it is taken as $L = 20$ (infinite distance) at the beginning of the simulation. This simulation procedure was repeated from a simple MATLAB routine, which iteratively decreases L down to 0.01. The optimized 3D modeling conditions (meshing) were obtained for the tip held above the plain substrate; they correspond to tip responses that reproduce the standard 2D axisymmetric responses. These conditions were used for the simulation of the tip response above the whole cantilever platform.

2. SECM image of $\text{CANT}_{\text{cond}}$ at constant height $d=1.5 \mu\text{m}$.

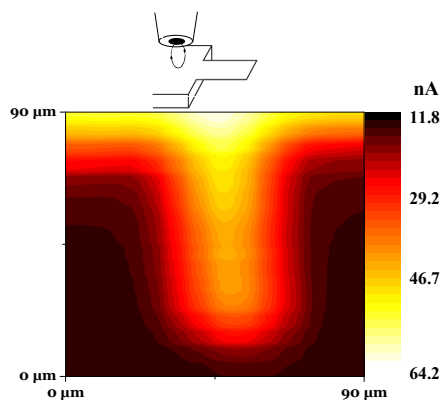


Figure SI1. SECM feedback image acquired with a 12.5 μm radius Pt UME in a 2 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ + 0.1 M KBr solution at constant height $d = 1.5 \mu\text{m}$ (measured above the plain substrate) of a bare Au cantilever $\text{CANT}_{\text{cond}}$. d measured above the plain substrate. $E_{\text{tip}} = -0.3 \text{ V}$ vs Ag/AgCl. Tip currents are presented in their absolute values (for $\text{Ru}(\text{NH}_3)_6^{3+}$ reduction, a negative cathodic current is detected experimentally).

3. Response of CANT_{ins} platform to Br adsorption.

The response of the CANT_{ins} platform is addressed in Figure SI2 when it is covered by a thin diazonium-derived organic layer. The tip was positioned at 5 μm height from the plain CANT_{ins} substrate. First, the substrate coated with the organic layer does not present any more the adsorptive feature related to Br adsorption. Moreover, the tip response shows contrasted behavior, depending on its location above the platform. Above the plain substrate, the regeneration process at the substrate slightly increases with the substrate potential, then it decreases as $E > 0.5 \text{ V}$. However, along the whole potential range, $k_{\text{app,subs}} = 5.3 \pm 0.6 \times 10^{-4} \text{ cm s}^{-1}$. On the contrary, the tip response above the cantilever free end is also constant for $E < 0.47 \text{ V}$, but decreases down by 20% as $E > 0.5 \text{ V}$.

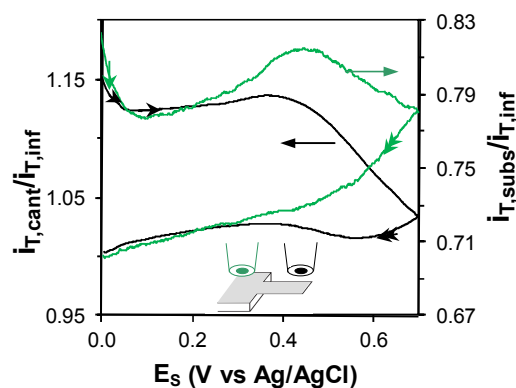


Figure SI2. Tip current, i_T , responses normalized to current at infinite distance, $i_{T,\text{inf}}$ in a 2 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ + 0.1 M KBr solution measured above a grafted substrate CANT_{ins} . Tip currents measured above the plain substrate (green) or the cantilever free end (black).

This contrasted reactivity qualitatively suggests the mechanical contribution of the cantilever. For $E < 0.47$ V, the ET rate at the cantilever free end corresponds to $k_{app,lev} = 2.3 \times 10^{-3} \text{ cm s}^{-1}$, an activity four times higher than on the plain substrate. Such heterogeneity of surface reactivity at the grafted platform indicates that the cantilever region has been less efficiently covered by the aryl layer than the plain substrate. It is then difficult to deconvolute properly from the tip responses, obtained at a single tip height, the contributions of reactivity and cantilever deflection. However, assuming the cantilever is not significantly deflected during the cyclic voltammetry, the decrease of the reactivity for $E > 0.5$ V likely also corresponds to Br adsorption. On the organic-coated cantilever, it is retarded by an ~ 0.35 V overpotential as the organic layer is an efficient barrier to inner-sphere charge transfer.³

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