$1/f^{\alpha}$ Noise in the Dynamic Force Spectroscopy Curves Signals the Occurrence of Biorecognition

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Biorecognition leads to the formation of a specific complex between a couple of biological partners to accomplish a functional task. Its occurrence can be inferred *a posteriori* by analyzing the unbinding force curves in a dynamic force spectroscopy experiment. Because of nonspecific interactions, the method is not, however, exempt from ambiguities and subjectivity. A fingerprint of the partner recruitment in the complex has been disclosed in the fluctuations of the atomic force microscopy cantilever. We demonstrate that the formation of the biotin-avidin specific complex strongly correlates with a $1/f^{\alpha}$ noise in the force curve fluctuations.

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Interactions between biological molecules drive a large variety of cellular processes and span a wide range of strengths and complexity. Upon specific recognition mechanisms, biomolecules give rise to associations with very different properties and functions [1]. The ability of biological molecules to undergo highly controlled and hierarchical processes is regulated by forces at molecular scale, based on a combination of noncovalent interactions [2]. The study of biorecognition at single molecule level allows us to both gather details on the underlying molecular mechanisms and disclose subtle phenomena usually hidden in ensemble measurements [3]. Among the vast repertoire of single molecule techniques, a prominent position is held by dynamic force spectroscopy (DFS), in which a biomolecular complex is forced to unbind upon application of a pulling force at nanoscale [4]. From these data, obtained at inherently nonequilibrium conditions, information on the equilibrium state can be extracted with the help of suitable theoretical models [5,6]. A DFS experiment is carried out by atomic force microscopy equipment, where a tip on the cantilever is functionalized with one partner and is moved toward the substrate covered with the other partner, being the formation of a complex between the two biomolecules eventually promoted. Successively the tip is brought away from the substrate and the complex unbinding events are analyzed [7]. Owing to the stochastic character of single molecule processes, the DFS force curves may exhibit markedly different features due to adhesions, multiple events, molecular stretching, jump-off, etc., [7]. The wide variability of the force curves, acquired in sequence for a statistical significance, makes it difficult to discriminate among force curves related to specific events (i.e., indicative of a biorecognition process between the partners) and to unspecific ones (due to adhesion, or no interaction). Although there is a plethora of different developed strategies and criteria, such a task is so far not exempt from ambiguities and subjectivity [8]. By viewing a biorecognition event as a diffusionlike process in which the individual biomolecules thermally explore the energy hypersurface to selectively find out their final binding state, it could be reasonable to imagine that the related biomolecular interactions might be modulated in time [9]. Indeed, the exploration of the local energy landscape has been found to be connected with force fluctuations, responsible for multiple successive bonds [10,11]. Accordingly, a successful searching process leading to specific complex formation could leave a fingerprint in the fluctuations of the forces acting between the biomolecular partners. By keeping this in mind, we have carefully analyzed the temporal fluctuations of the cantilever, whose tip was functionalized with biotin, during its approach to the substrate covered with its biological partner, the tetrameric protein avidin [12]. The biotin-avidin complex is one of the strongest noncovalent interactions in nature and represents a benchmark for investigating the mechanisms regulating the formation of biomolecular complexes [13]. Remarkably, a power spectrum analysis of the temporal cantilever fluctuations allowed us to disclose a $1/f$ fingerprint of the partner recruitment in the complex formation.

At the beginning of the DFS experiment (for details see Ref. [12]), the tip charged with biotin is far from the substrate onto which avidin molecules are immobilized [Fig. 1(a), right sketch]. The tip is then vertically approached to the substrate (gray lines). The approach curve is flat with small, fast fluctuations around the zero force value until the tip contacts the substrate [Fig. 1(b), at the arrow]. From this point on, the repulsive forces between the biomolecules yield an upward deflection of the cantilever. While the approach curves display almost the same shape in all the cases, different trends have been, instead, observed during the retraction, three representative curves being shown in Fig. 1 (black lines). The retraction curve shown in Fig. 1(a) exhibits, after the contact point, a downward cantilever deflection (due to attractive forces) with a continuous, linear trend, this being due to nonspecific adhesion forces between the functionalized tip and the substrate [7]. Then a jump-off to the baseline occurs, due to a detachment of the tip from the substrate upon retraction of

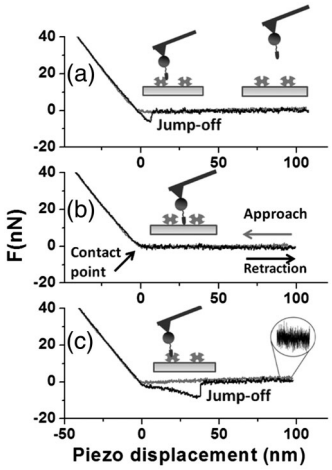

FIG. 1. Representative approach (gray line) and retraction (black line) force curves for a biotin–functionalized tip over a glass substrate covered by an avidin monolayer. The retraction curve features correspond to: (a) adhesions, (b) no events, and (c) specific unbinding events.

the piezo actuator. In Fig. 1(b), the retraction curve practically coincides with the approach one, indicating that no binding events have taken place. A nonlinear downward deflection is observed, instead, in the retraction curve shown in Fig. 1(c); this being generally attributed to some stretching of the molecules which have formed a specific complex [8]. The subsequent jump-off to the baseline reflects the rupture of the complex and allows us to estimate the corresponding unbinding force. During the approach phase, the curves do not apparently show any evidence of a complex formation between the partners. Indeed, only in a few ordered systems, a jump-on event has been observed near the contact point [14]. An *ad hoc* inspection of the retraction curves is generally required to assess if a matching of the partners has occurred. It would be quite challenging to search if a trace of a favorable partner recruitment was left in the temporal course of the atomic force microscopy cantilever fluctuations. We then performed a statistical analysis of the approach curve fluctuations by focusing our attention on both their intensity and spectral content. The fluctuation amplitude has been found to be $(1.05 \pm 0.05) \times 10^{-2}$ nN in the flat part of all the force curve regions. This value agrees with that found for a free cantilever and it is generally ascribed to several causes, such as thermally induced fluctuations, high frequency fluctuations from the force feedback system, mechanical vibrations, drift effects, etc., [15]. Progressively lower values of the cantilever fluctuations have been instead observed as far as the cantilever starts to deflect, consistently with its reduced force sensitivity upon deflection.

The spectral content of the force fluctuations has been evaluated by analyzing a 10 nm-wide region, located at different positions of the approach curves, through the expression:

$$S(f) = \int_0^T \langle F(0)F(t)\rangle e^{2\pi i f t} dt, \quad (1)$$

where f is the frequency, t the time, T the integration time interval, and $F(t)$ is the measured force expressed as a function of time. Indeed, the force is registered as a function of the piezo displacement, x, which in turns depends on time through the relationship $x = vt$, where v is the approaching or retraction speed set in most experiments at 49.8 nm/s. Under these conditions, the integration step of 1.1×10^{-4} s and $T = 0.2$ s have been used [12].

We have found that the power spectra from all the force curve regions, located in the flat part and far away from the contact point (by more than 50 nm), are similar to those shown in the inset of Fig. 2(a). This spectrum shows a plateau at frequencies below a cutoff value at about 5 kHz, this being indicative of white noise related to a δ-correlation function. At higher frequencies, it displays a linear trend with a slope close to 2 (i.e., a $1/f^2$ frequency dependence), reflecting red noise with a constant correlation function [16]. This trend reminds us of the Lorentzian function which is expected to arise from thermally driven cantilever oscillations, z, as described by the Langevin equation [15]:

$$m\frac{d^2z}{dt^2} + \gamma\frac{dz}{dt} + k_c z = F(t), \quad (2)$$

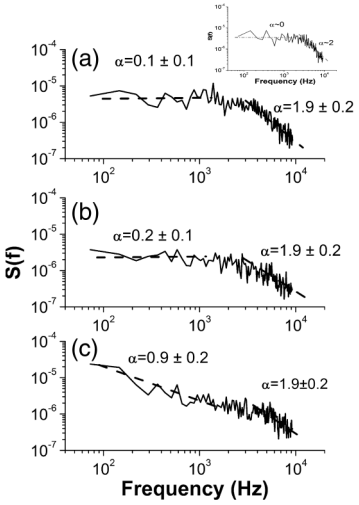

FIG. 2. Power spectra of the cantilever fluctuations as calculated by Eq. (1), for a 10 nm-wide region located just before the contact point of the approach force curve whose successive retraction is indicative of: (a) adhesions, (b) no events, and (c) specific unbinding events (see also Fig. 1). The dashed lines show the best fits with a $1/f^{\alpha}$ dependence; the α values and the corresponding standard deviations having been extracted from a fit of 30 regions. Inset: Power spectrum of the cantilever fluctuations for a 10 nm-wide region located in the flat part of the approach force curve, far from the contact point [see Fig. 1(c)].

where m is the effective mass of the cantilever, γ is the damping coefficient, k_c is the cantilever spring, and $F(t)$ includes the net tip-surface interaction and the contribution from the electronic, thermal noise etc., the characteristic frequency of the cantilever (corresponding to the cut-off frequency) being given by $f_0 = [(k_c/m) - \gamma^2/(4m^2)]^{1/2}$. The found cutoff frequency is remarkably in good agreement with the characteristic frequency (about 5 kHz) estimated by the dynamic method [8].

We then analyzed the noise power spectrum of the approach force curve regions just before the contact point where biorecognition is expected to take place. The power spectra from force curves corresponding to bare adhesion and no events are shown in Figs. 2(a) and 2(b), respectively. Both power spectra show a plateau followed by a linear regime with a slope of about 2, with a cutoff frequency at about 5 kHz, as that shown in the inset of Fig. 2(a). Instead, the plateau is replaced by a $1/f^{\alpha}$ trend with $\alpha = (0.9 \pm 0.2)$, in the power spectrum from curves corresponding to specific biorecognition events, the $1/f^2$ trend at higher frequency not being affected [see Fig. 2(c)]. Then, at low frequencies, a $1/f$ noise is now overimposed on the Lorentzian function arising from the free cantilever noise [15]. We have obtained similar results up to an approaching speed of 200 nm/s [12].

The appearance of $1/f$ noise over the Lorentzian shape has been recently observed in ultrasensitive detection of biomolecular recognition by using a field-effect transistor [17]. A $1/f^{\alpha}$ with a α exponent close to 1 (pink or flicker noise), is generally associated with a slowly decreasing correlation function and is a fingerprint of a complex temporal behavior in systems exhibiting multiple time scale processes [16,18]. Indeed, $1/f$ noise is a ubiquitous feature of many different processes occurring in electronic devices, membrane channel conduction, protein dynamics, economic processes, and so on. In protein systems, $1/f$ noise has been attributed to the trapping and escape from local minima of the energy landscape generated by slightly different conformations, likely involved in the regulation of some biological processes, such as catalysis, exchange of ligands, folding, and even biorecognition [9,19–21]. On such a basis, we can hypothesize that during a successful approach, the two partners could diffusionally explore their relative energy landscape with a continuous trapping and escape from shallow minima, leading to the final binding state with minimum energy. Such a diffusive exploration may result in a characteristic fluctuation of the interaction force involving γ and/or $F(t)$ in Eq. (2), which modulate the cantilever noise spectral content. Therefore, the superposition of the $1/f$ noise on the power spectrum, observed only when a specific interaction between the partners occurs, could be indicative of the formation of a specific complex between the partners.

To further support the above results, we have carried out a DFS experiment and the related spectral analysis on another complex of biomedical interest (Mdm2-p53) [22]. Remarkably, we have disclosed the same $1/f$ noise fingerprint only when specific biorecognition events take place [12]. Additionally, we have performed a control experiment on the interaction of two biomolecules which are known not to form a specific complex (bovine serum albumin and avidin) [8]. In this case, all the power spectra were found to be very similar to those shown in Figs. 2(a) and 2(b) (no $1/f$ fingerprint) as expected for noninteracting biomolecules [12]. All these results confirm that our power spectrum analysis represents a valuable, selective tool for sensing specific biomolecular recognition.

A $1/f$ noise in the power spectra of many systems is often associated with interesting peculiarities in the related temporal series, such as asymptotic power law decay, Lévy statistics, and so on [23,24]. Accordingly, we have analyzed the cantilever fluctuations in terms of a two-state, binary process during which the tip switches from a state close to the substrate, to the other far from it. In particular, we have defined τ_{on} as the time during which the tip is closer to the substrate with respect to a threshold, while τ_{off} is the time during which the tip is farther from the substrate. The threshold has been chosen as the average cantilever deflection in the analyzed interval, roughly corresponding to the baseline. We have calculated the distributions of both τ_{on} and τ_{off} for 10 nm-wide regions of the approach curves. The two distributions are very similar; in Fig. 3 we only show the τ_{on} distributions corresponding to force curve regions located just before the contact point. We remark that the distributions from

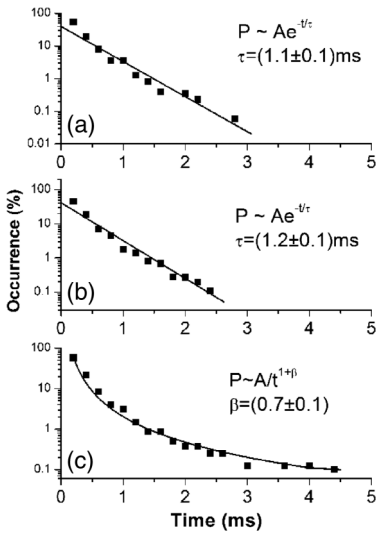

FIG. 3. τ_{on} distributions extracted from 10 nm-wide regions of the approach force curves located just before the contact point of the approach force curve whose successive retraction is indicative of: (a) adhesions, (b) no events, and (c) specific unbinding events. Each distribution has been obtained from a collection of 30 force curves. Continuous lines are the best fits by the given expressions; the fitting parameters having been also reported.

force curves characterized by adhesion and no event in the retraction phase, show an almost linear trend in a semi-log plot [Figs. 3(a) and 3(b)]. A fitting with an exponential function $P \sim Ae^{-t/\tau}$, provided a τ of about 1.1 ms for both the cases [continuous lines in Figs. 3(a) and 3(b)]. Notably, a similar trend has been observed for the τ_{on} distributions from regions of the approach curve located far away from the contact point. At variance, the τ_{on} distribution from the curves exhibiting specific events in the retraction phase, significantly deviates from a linear regime, with the appearance of a tail at long times [see Fig. 3(c)]. This distribution is best fitted by a power law $P \sim \frac{A}{\tau^{1+\beta}}$, with a β exponent of (0.7 ± 0.2) [continuous line in Fig. 3(c)]. The occurrence of a power law distribution with an exponent between 1 and 2 (i.e., $0 < \beta < 1$) has been observed in many different complex phenomena and it reflects the existence of processes covering different time scales [25]. We note that a $1/f$ noise in the power spectrum and a concomitant power law in the temporal series of the system could arise from a superposition of independent stochastic signals, as recently demonstrated [26]. In this respect, the presence of a long tail in the τ_{on} distributions could also be due to the diffusive process to which the biological partners undergo, by exploring the rough energy landscape during the biorecognition [27]. Indeed, in the framework of the Zwanzig's model, the diffusion coefficient of a particle moving on a rugged energy surface is lowered according to the expression $D = D_0 e^{-(\epsilon/kT)}$ where ϵ is the average energy roughness [28]. Therefore, if we take into account that the energy roughness of the biotin-avidin complex has been estimated to be about $5k_BT$ [29], a drastic slowing down of the diffusion coefficient is expected and this might be responsible for the long tail in the τ_{on} distribution.

In summary, our results show that the cantilever fluctuations contain a fingerprint of the biorecognition process to which the biological partners undergo during their approaching, which finally leads to a specific complex formation. These findings offer, on one hand, a new perspective for the study of the energy landscape regulating the biorecognition dynamics and, on the other, a valuable tool to reliably discriminate the DFS curves corresponding to biorecognition events from those related to nonspecific interactions. The latter aspect could be, moreover, of significant help for DFS data analysis and may lead to implementation of dedicated software facility on commercial instruments.

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