Trapping Single Molecules by Dielectrophoresis

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We have trapped single protein molecules of *R*-phycoerythrin in an aqueous solution by an alternating electric field. A radio frequency voltage is applied to sharp nanoelectrodes and hence produces a strong electric field gradient. The resulting dielectrophoretic forces attract freely diffusing protein molecules. Trapping takes place at the electrode tips. Switching off the field immediately releases the molecules. The electric field distribution is computed, and from this the dielectrophoretic response of the molecules is calculated using a standard polarization model. The resulting forces are compared to the impact of Brownian motion. Finally, we discuss the experimental observations on the basis of the model calculations.

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The confinement of a single or a few molecules in field cages such the Paul trap [1] is a standard method in physics. However, it is not applicable to objects in fluids such as molecules in solution. Trapping within transparent fluids can be achieved by strongly converging light beams (''laser tweezer'') [2]. Because of differences in the index of refraction between a particle and the suspending medium, lateral forces are exerted on the particle towards the optical focus. However, refraction laws and Brownian motion prevent the use of optical tweezers onto molecules directly. Instead such objects have to be immobilized on the surface of micrometer sized latex spheres [3]. Here we report on the reversible trapping of single molecules in an aqueous environment by the use of strongly divergent rf electric fields.

Alternating (ac) electric fields are routinely exploited for the spatial manipulation of biological cells [4]. Depending on the temporal as well as geometrical field distribution, microscopical particles such as cells or microbeads can be moved, attracted, trapped, oriented, rotated, or stretched. Quantitative models of these electrokinetic phenomena are well established [5]. In most instances inhomogeneous fields are applied, which lead to the attraction of polarizable material towards the regions of highest field strength. This so-called dielectrophoresis (DEP) can lead to particle movement towards electrode edges (positive DEP), but also to apparent repulsion (negative DEP), depending on the—frequency dependent—electric properties of both the object and the surrounding solution. In recent years these methods have been extended to submicroscopical particles such as viruses and polystyrene beads [6]. This has become possible by the development of electrodes with feature dimensions around 1 μm and is motivated by the growing interest in lab-on-a-chip systems. However, since the attracting gradient forces scale with the object's volume, it has been doubted that DEP forces could in practice overcome the impact of Brownian motion at room temperature [7]. DEP forces are proportional to the square of the field strength, so stronger fields usually lead to increased attraction forces. However, other, mostly disturbing, effects also become more pronounced. For planar, interdigitated, parallel electrodes it has been shown experimentally [8] as well as by theoretical work [9] that controlled manipulation of nanometer sized objects by DEP is significantly disturbed by convection flow due to heating and by electrohydrodynamic effects. The smallest objects having been observed in dielectrophoretic field cages so far are fluorescently labeled polystyrene beads of 93 nm diameter [10]. Metal particles of about 20 nm size have been trapped irreversibly, meaning immobilized, by inhomogeneous dc electric fields and observed after drying [11]. In the same manner organic molecules of 5 nm length have been immobilized in an electrically insulating surrounding [11] as well as DNA molecules of 10 nm length in a buffer solution [12]. Very recently, dielectrophoretic immobilization of 20 nm polystyrene nanobeads and 2 to 30 nm gold particles along carbon nanotubes has been demonstrated [13]. Macromolecules as small as albumin and insulin have been found to be affected by ac fields, however, only in experiments on large ensembles of molecules [14]. Positive as well as negative dielectrophoresis of proteins have been analyzed recently [15]. Single molecules of DNA have been observed microscopically to be attracted and aligned between straight interdigitated electrodes. Still, their contour length ranged from 2 [16] to 17 μm [17], allowing for field conditions similar to those used for μm sized objects. Here we report on the observation of the reversible trapping of single, compact protein molecules by an ac electric field in water.

Planar gold electrodes were prepared on a low *n*-doped silicon substrate by electron beam lithography. Since DEP is proportional to grad$|E|^2$ [4,5], it can be increased by a short electrode distance, a high voltage applied, or by a strong curvature of the electrodes. For example, sawtooth electrodes have been used in DEP experiments on DNA and viruses [18]. In order to achieve a maximum field

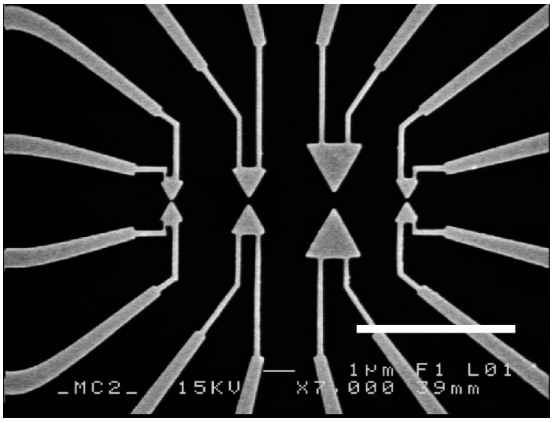

FIG. 1. Electron micrograph of gold electrodes on a silicon substrate. Scale bar 5 μm. The largest electrode pair with 500 nm gap width has been used.

gradient while keeping disturbing effects such as heating and electrohydrodynamic fluid flow to a minimum, a pair of triangular electrodes with a mutual distance of 500 nm was chosen (Fig. 1). The actual tips were produced as sharp as possible with a radius of curvature of less than 60 nm. As a suitable molecule *R*-phycoerythrin (RPE) from red algae was chosen for its intense autofluorescence. It is a 240 kDa protein of disklike shape with a diameter of 11 nm and a thickness of 6 nm [19]. Electrodes were covered by 1.5 μl of an aqueous solution of RPE at a concentration of 1 molecule per 2.5 μm^3 (0.6 nM). To minimize electrical currents, great care was taken to clean all relevant surfaces from ionic contaminations. All solutions were made from freshly prepared ultrapure water. From studies with similar electrodes, which allow for conductivity measurements in such a small volume, an upper limit for the electrical conductivity in the actual experiment can be estimated to 20 μS/cm. Sealing against evaporation was achieved by a thin gasket ($d = 80$ μm), a cover slide, and silicon oil. Observation was done through a fluorescence microscope (Leica DMRB) equipped with a high aperture objective ($\times 100$, numerical aperture $= 1.30$) and a cooled CCD camera (Kappa PS 2C). ac signals were produced by a function generator (Model 193, Wavetek) and monitored with a demodulator probe, a bench multimeter, and a frequency counter. Without electric field no single fluorescence source could be discerned besides an overall background due to motion blur of the dissolved, fluorescing RPE molecules [Fig. 2(a)]. Applying a sinusoidal signal of 10 V (root mean square) at 1 MHz resulted in fluorescing spots appearing independently at both electrode tips within about 10 s [Fig. 2(b)]. Both spots were of identical fluorescence intensity (within 10%) amounting to more than the fourfold of the detection limit. Therefore, one can rule out the possibility that the spots consist of several fluorescing objects whose total fluorescence intensity exceeds the detection threshold. Acquiring images over a period of 8 min at 1 min intervals (data not shown) revealed an increase of fluorescence intensity at the electrode tips

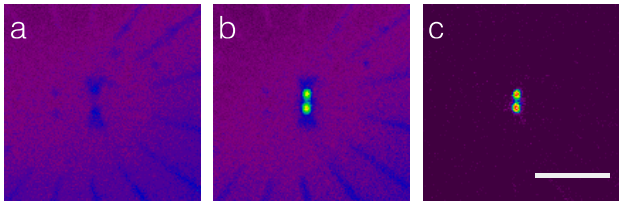

FIG. 2 (color online). Dielectrophoretic trapping of single *R*-phycoerythrin molecules between triangular electrodes. Fluorescence micrographs are in false color representation. (a) Protein solution (0.6 nM) and electrodes before field application. (b) After 10 s field application at 1 MHz and 10 V. (c) Difference of images (b),(c). Scale bar 5 μm.

linearly with time. Switching off the field lead to an immediate disappearance of the fluorescence, obviously due to diffusion. There was no visible adsorbance or coupling to the electrodes. At a field frequency of 0.1 MHz similar attraction was found, while at 5 MHz it was much weaker. Experiments with fluorescently labeled antibody molecules yielded similar results.

In order to clarify whether the dielectrophoretic trap is acting on single molecules or just larger aggregates, additional experiments were performed. A drop of the RPE solution was put on a precleaned glass surface, sealed with a cover slide, and observed under the microscope. Fluorescing spots were found appearing and disappearing at the surface. The intensity histogram of these spots is shown in Fig. 3. It exhibits a single peak similar to the distribution that has been reported in another single molecule study on RPE [19]. From this it can be concluded that there is only one fluorescent molecular species present in the solution.

We further performed fluorescence correlation spectroscopy (FCS) [20] on the original RPE solution (Zeiss Confocor 2). The resulting autocorrelation function $G(t)$ (Fig. 4) again hints to the presence of only one molecular species. However, single color FCS only distinguishes between molecules differing in molecular weight by at least a factor of 4 [21]. Thus aggregates of up to 4 mole-

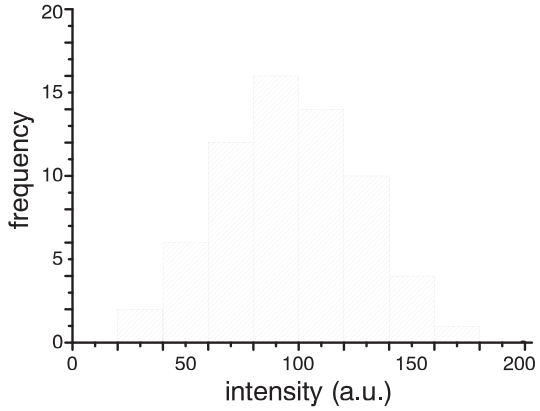

FIG. 3. Fluorescence intensity histogram of *R*-phycoerythrin adsorbed onto a glass surface.

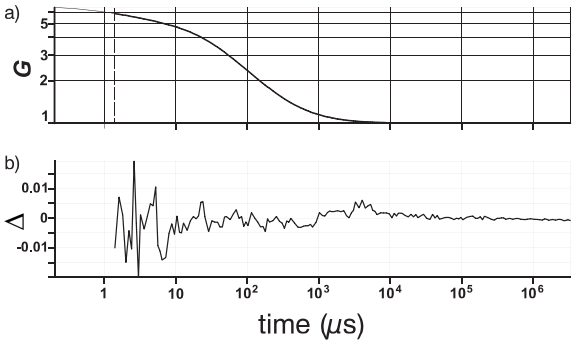

FIG. 4. (a) Fluorescence autocorrelation function $G(t)$ of the R-phycoerythrin solution. (b) Difference between data and numerical simulation assuming a single fluorescent species.

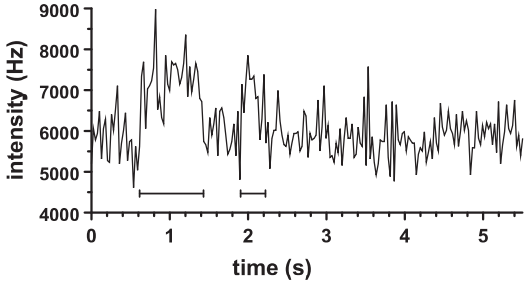

FIG. 5. Temporal course of fluorescence intensity at the electrode tips at 0.7 MHz and 1.6 V. Bars emphasize ''on'' periods.

cules might be present in the solution. The FCS experiment yields a particle concentration of 0.5 nM, which is quite close to the value of 0.7 nM, which results from the photometrical determination. While photometry measures properties averaged over a volume of some μl, FCS determines the properties of single objects. Therefore the agreement in concentration can be explained only by the presence of single RPE molecules, since molecular aggregates would result in photometrical concentration values being multiples of the FCS data.

Commonly, the presence of single molecules is demonstrated by fluorescence time traces with subsecond resolution exhibiting blinking or single-step photobleaching. For this end the electrode tips were placed at the FCS focus, and fluorescence intensity was monitored with an avalanche photodiode. An electrical signal of 0.7 MHz and 1.6 V was applied to the electrodes. As a consequence of this voltage reduction, as compared to the first experiment, the volume where dielectrophoretic trapping takes place decreases. This avoids capturing several molecules within the time interval in which bleaching occurs. The resulting fluorescence time trace was very noisy (Fig. 5), presumably due to reflections by the metallic electrodes. Still, steplike ''on and off'' behavior is discernible, which is characteristic for single molecule events. From the results of all these experiments, it follows that indeed molecules are attracted by dielectrophoretic forces as single molecules and then are trapped at the electrode tips.

For a better understanding of the effects involved calculations were performed about the forces that act on particles being suspended or dissolved in a fluid. For a successful spatial manipulation by dielectrophoresis electric forces must exceed diffusion and friction. In order to calculate the minimum force that is needed to overcome these effects, one can define an observable deterministic threshold force F_{th} [22]:

$$F_{\mathrm{th}} = \sqrt{\frac{2}{D\Delta t}} k_B T,$$

where D is the molecules's diffusion constant, Δt the experimental time interval, k_B the Boltzmann constant, and T the absolute temperature. After the time interval Δt the molecule's position is given by a normal distribution with the standard deviation Δ. Then F_{th} is the force that is needed to displace the molecule by Δ within the time Δt. This equation holds for 68% of all molecules, i.e., 1 standard deviation, due to the stochastic nature of their movement. Taking the diffusion constant of RPE of 40 $\mu\mathrm{m}^2\,\mathrm{s}^{-1}$ [19] and the experiment's duration of 10 s, one obtains a threshold force of 3×10^{-16} N per molecule.

The dielectrophoretic force is given by $F_{\mathrm{DEP}} = 2\pi\varepsilon_m r^3\,\mathrm{Re}[\tilde{f}_{\mathrm{CM}}]|\,\mathrm{grad}|E_{\mathrm{rms}}|^2|$. ε_m denotes the medium's permittivity, r the particle's radius, $\mathrm{Re}[\tilde{f}_{\mathrm{CM}}]$ the real part of the complex Clausius-Mossotti factor, and $\mathrm{grad}|E_{\mathrm{rms}}|^2$ the gradient of the root mean square of the electric field strength [5]. The Clausius-Mossotti factor is given by $\tilde{f}_{\mathrm{CM}} = (\tilde{\varepsilon}_p - \tilde{\varepsilon}_m)/(\tilde{\varepsilon}_p + 2\tilde{\varepsilon}_m)$, with $\tilde{\varepsilon}_m$ and $\tilde{\varepsilon}_p$ being the complex permittivity of the medium and the particle, respectively. For a spherical object $\mathrm{Re}[\tilde{f}_{\mathrm{CM}}]$ can reach values close to unity. Taking into account the prolate shape of the RPE molecule, one can calculate for $\mathrm{Re}[\tilde{f}_{\mathrm{CM}}]$ a value of 1.1 for a random orientation and a value of 1.3 for an orientation in parallel to the electric field [23]. In our experiments the dielectrophoretic response of RPE was found to be maximal at 0.1 MHz, of similar strength at 1 MHz, and much lower, though still positive, at 5 MHz. From this and the general dependence of $\mathrm{Re}[\tilde{f}_{\mathrm{CM}}]$ on frequency [5,22,23], $\mathrm{Re}[\tilde{f}_{\mathrm{CM}}]$ is expected to be around unity. For r we take the Stokes radius of RPE to be 5.6 nm [19]. The electric potential distribution $U(x, y, z)$ for the applied electrode configuration is calculated using a finite element method (Maze, Field Precision). From this $|\vec{E}(x, y, z)|^2$ and $\mathrm{grad}|E|^2$ are computed in Maple (Waterloo Maple). The highest value of the field gradient is found close to the opposing electrode tips, as expected, where molecular trapping has been observed, with $\mathrm{grad}|E|^2$ exceeding 10^{21} $\mathrm{V}^2\,\mathrm{m}^{-3}$ (Fig. 6, lower part). This translates to holding forces of more than 0.1 pN per molecule.

From the spatial distribution of $\mathrm{grad}|\vec{E}|^2$ and a threshold force F_{th} of 3×10^{-16} N, one can derive the space in which RPE molecules are trapped and drawn towards the electrodes (Fig. 6, upper part). This space is found to resemble half an ellipsoid with a volume of about 20 $\mu\mathrm{m}^3$. With an RPE concentration of 0.6 nM about 7 molecules

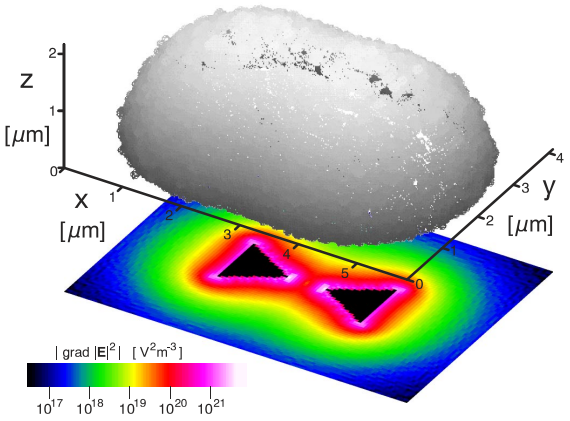

FIG. 6 (color online). Calculation of the electric field gradient grad$|E_{\mathrm{rms}}|^2$. The color coded projection shows the field gradient in the electrode plane (note the logarithmic scale). The gray shaded surface in the upper part encloses the space where the electric gradient force exceeds molecular diffusion ($F > 3 \times 10^{-16}$ N).

are to be expected in this volume. In the actual experiment, however, only 2 molecules have been trapped. This difference is a consequence of a number of reasons. (a) The necessary threshold force has been calculated for only 68% of all molecules. (b) Re$[\tilde{f}_{\mathrm{CM}}]$ has just been estimated. (c) Nonlinear phenomena such as the Wien effect are expected to lower Re$[\tilde{f}_{\mathrm{CM}}]$ at high field strengths. (d) Electrohydrodynamic effects such as ac electro-osmosis and electrothermal fluid flow interfere with dielectrophoresis [8,9]. Taken together, these effects result in a reduction of dielectrophoretic forces. Thus the trapping of 2 molecules within 10 s appears to be quite a realistic outcome.

There might exist also effects which act in favor of dielectrophoresis. Usually the dielectrophoretic response of polystyrene microbeads is explained by their surface conductance [24] being a consequence of charge movements both in the Stern layer and in the diffuse double layer which surround the particles in aqueous solution. At low ionic strengths these layers well exceed 10 nm. Thus the electrically effective size of the RPE molecules might be larger than its reported hydrodynamical diameter of only 5.6 nm and hence experience larger DEP forces.

For a better understanding of the underlying phenomena, further investigations are needed. Systematic variation of field properties such as strength and frequency, variation of the solution's ion composition and pH, and variation of the protein's properties will eventually lead to lab-on-a-chip systems controlling single molecules.

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