Published in final edited form as:

Electrophoresis. 2011 September ; 32(17): 2314–2322. doi:10.1002/elps.201100037.

Immunoglobulin G and Bovine Serum Albumin Streaming Dielectrophoresis in a Microfluidic Device

Asuka Nakano, Tzu-Chiao Chao, Fernanda Camacho-Alanis, and Alexandra Ros

Department of Chemistry and Biochemistry, Arizona State University, Tempe AZ, 85287-1604

Abstract

Dielectrophoresis (DEP) has demonstrated to be a versatile tool to manipulate micro- and nanoparticles with applications for positioning, separation and fractionation. Recent developments of DEP have also shown that DEP can be used for the manipulation of biomolecules, such as DNA. Here, we focus on the manipulation of proteins using insulator-based dielectrophoresis (iDEP). We designed suitable post arrays in a microfluidic channel and use numerical simulations to calculate the electric field distribution as well as concentration of proteins according to a convection-diffusion model for both negative and positive DEP. Experimentally, we find DEP trapping of mainly protein aggregates in phosphate buffer. However, when adding a charged zwitterionic detergent, we observed DEP streamlining of immunoglobulin G (IgG) and bovine serum albumin (BSA). Our experimental observations are in excellent agreement with numerical simulations and indicate positive DEP behavior of IgG and BSA under the employed experimental conditions. Our results demonstrate DEP streaming of proteins in an iDEP device for the first time and indicate the potential of protein DEP for separation and fractionation.

Keywords

BSA; IgG; insulator-based; dielectrophoresis; microfluidic

Introduction

The reliable and rapid separation of proteins is an essential analytical tool for biomedical analyses and biological research. Standard chromatography and electrophoresis based techniques are well established routine methods for the separation of proteins and peptides. However, conventional separation techniques reach their limitations for increased sample complexity demanding for the analysis of relevant disease markers in extremely small concentration and within a huge background. Challenges further arise for time critical samples, i.e. when rapid answers are required, such as for samples with temporally degrading or altering composition or when rapid diagnosis is essential, such as during surgery.

Microfluidic platforms or miniaturized lab-on-a-chip systems incorporate several advantages desirable to address these analytical challenges such as speed, efficiency and high throughput [1]. Apart from downscaling existing technology, miniaturized devices open novel routes for exploiting exciting new phenomena at the interplay of micro- and nanostructures with intrinsic biomolecule properties eventually leading to novel fractionation and separation mechanisms. Previous examples include micro-[2,3] and

nanopillar [4] arrays, magnetic bead [5,6] or nanosphere [7] matrices, entropic traps [8–10] and continuous flow DNA separation [11], which significantly reduce analysis times within pL-nL samples. However, only few examples for novel protein separation mechanisms realized in nanostructured filters have been demonstrated [12,13].

In this contribution, we focus on the manipulation of proteins by dielectrophoresis to exploit its potential for bioanalytical applications. Dielectrophoresis (DEP) is a relatively novel method for the manipulation and separation of biomolecules. Its potential arises from the manipulation of biomolecules in gel free environment as well as rapid separation and pre-concentration capability. In DEP, electric field gradients evoke forces on polarizable objects leading to migration and/or trapping [14,15]. This technique thus contrasts electrophoresis, in which migration of charged species arises in a homogeneous electric field. Based on the dielectric properties of the analytes, a broad range of intrinsic characteristics, such as size, shape, charge, charge distribution and charge mobility, permittivity and deformability can be probed with DEP [16]. It thus provides a large parameter space, which allows highly specific probing of analytes in complex samples.

Microfluidic devices have demonstrated to provide excellent platforms for DEP manipulations. Non-uniform electric fields can be established via integrated metal microelectrodes [17]. A second method termed electrodeless DEP or insulator-based DEP (iDEP) represents an elegant way of creating non-uniform electric fields in microfluidic channels [18–21]. In this case, non-conducting obstacles force the electric field through the created constrictions when an electric field is applied via external electrodes submerged in the microchannel reservoirs. A number of different geometries have been used as insulating elements to create the desired inhomogeneous electric fields. This includes sawtooth geometries [16,22], oil barriers [23], membrane pores [24], filters [25], hurdles and constriction in the channel [26–28] and post arrays [18]. Insulator-based DEP prevents complicated processing steps such as integrating metal electrodes in a microfluidic pathway and, more striking provides the non-uniform field over the entire depth of the microchannel as compared to flat metal electrodes.

DEP has been extensively studied for biological objects such as cells [29–31] or viruses [32] with applications in cytometry [33], field flow fractionation [34–36] as well as separation [37,38]. The DEP mechanism for these biological objects is mostly understood [39] in contrast to the underlying polarization mechanism for most biomolecules. A major challenge in biomolecular applications of DEP is our limited knowledge on the origin and mechanism of biomolecule polarizability in electrolyte solutions. Only recently, DEP manipulations of biomolecules were reported. Although relatively high electric fields are anticipated to manipulate biomolecules by DEP, DNA in size ranges from several bp to several hundred kbp could be manipulated using DEP [20,40–44]. Only recently, experimental studies have successfully demonstrated protein trapping [45–53]. Those include DEP of proteins with molecular weights smaller than 100 kDa successfully accumulated on electrodes [45,52], carbon nanotubes [51] or within droplets [53]. Typically, field strengths of 10^6 V/m were generated on μm -sized electrode gaps and protein accumulation due to DEP was observed in a frequency range from 1 kHz – 1 MHz. Hoelzel *et al.* demonstrated the trapping of the multimeric protein R-phycoerythrin at gold electrode gaps [50]. Most recently, Lapizco-Encinas *et al.* were able to demonstrate dielectrophoretic trapping of protein particles in an iDEP device [46].

Here, we further develop an iDEP device to study the dielectrophoretic behavior of proteins, namely bovine serum albumin (BSA) and immunoglobulin G (IgG) molecules in DC mode. In contrast to previous studies [46], we observe the influence of protein aggregation on DEP behavior and downscale the size of the post arrays to sizes smaller than $20\ \mu\text{m}$ in an

elastomeric platform. We further employ novel triangular and elliptical posts designs to improve the electric field gradient formation. Our experimental results demonstrate streaming DEP behavior for both proteins. In addition we show that numerical modeling is in excellent agreement with our experimental observations. Positive DEP results in streaming DEP around the posts and along the flow direction, while negative DEP results in streaming flow in the interspace between the posts. Even though DEP for IgG and BSA is not strong enough to completely trap these biomolecules, we are able to concentrate them along the streamlines in accordance with positive DEP. To the best of our knowledge, this is the first detailed study on IgG and BSA DEP streamline concentration in a microfluidic iDEP device. This study thus points towards the potential of protein DEP for future analytical applications such as separation or pre-concentration.

Theory and Modeling

The anticipated DEP forces acting on biomolecules, i.e. proteins in our case represent an important aspect in insulator-based DEP devices. Little is known on the mechanisms involved in protein DEP up to date and a detailed model is lacking. Therefore, we relate involved trapping forces to well studied theories for solid particles. We thus estimated the anticipated trapping forces in accordance to micro- and nanoparticles. Clarke et al. [54] previously related the trapping forces for protein DEP to an ellipsoid model, assuming an overall prolate elliptic shape of proteins in solution. This seems reasonable in our case considering reported ellipsoid shaped IgG molecules [55]. In the case of DC DEP, the DEP force acting on a prolate ellipsoid is given by [54,56]:

$$\mathbf{F} = \frac{4}{3} \pi abc \epsilon_m \left(\frac{\sigma_p - \sigma_m}{Z\sigma_p + (1-Z)\sigma_m} \right) \nabla E^2 \quad \text{eq. (1)}$$

where a , b , and c (with $a > b = c$) are the radii of the ellipsoid along x , y , and z axis, ϵ_m the medium, i.e. surrounding buffer, permittivity, $\mathbf{E}$ the electric field and σ_m and σ_p the medium and particle conductivity, respectively. Z indicates the geometrical polarization relaxation factor:

$$Z = \frac{bc}{2a^2 e^3} \left[\ln \left(\frac{1+e}{1-e} \right) - 2e \right] \quad \text{eq. (2)}$$

where e denotes the eccentricity:

$$e = \sqrt{1 - (bc/a^2)} \quad \text{eq. (3)}$$

The expression

$$f_{MCM} = \frac{\sigma_p - \sigma_m}{Z\sigma_p + (1-Z)\sigma_m} \quad \text{eq. (4)}$$

denotes the modified Clausius-Mossotti factor arising due to the elliptic model. The magnitude and sign of f_{MCM} governs the DEP behavior and is responsible for DEP migration either to the regions of high field gradients (positive DEP) or away from these regions (negative DEP). Using eq. (4) thus allows predicting protein DEP behavior with the elliptic radii chosen as previously reported for IgG ($a = 5$ nm, $b = c = 2.5$ nm [55]). An experimentally derived value for σ_p of proteins in solution was reported by Clarke et al. [54] for yellow fluorescent protein. We thus use this value for all calculations and a medium

conductivity of 0.01 S/m adapted to our experimental conditions. The corresponding values are indicated in Table 1.

Based on these calculations, positive DEP is predicted for IgG according to a positive f_{MCM} value. It is important to note that these equations were deducted for a prolate ellipsoid particle. While it is a reasonable assumption, the actual polarization behavior of the considered proteins may deviate from these values. However, we can use this model to reasonably estimate the involved trapping forces for such nanoscale objects. This is very useful for the design of insulator-based DEP devices and for the understanding of future DEP based separation mechanisms. We will further show in the results sections that this model is in excellent agreement with our experimental observations. Note, that we purposefully define a negative DEP case for each considered geometry by assigning a particle conductivity of zero. We will discuss the differences in positive and negative DEP in detail in the results section in relation to experimental results.

Furthermore, we consider convection due to electrokinetic forces. We thus assign an electrokinetic velocity

$$\mathbf{u}_{ek} = \mu_{ek} \mathbf{E} = (\mu_{eo} - \mu_{ep}) \mathbf{E} = \mathbf{u}_{eo} + \mathbf{u}_{ep} \quad \text{eq. (5)}$$

where μ_{eo} and μ_{ep} are the electroosmotic and electrophoretic mobility, respectively. IgG and BSA proteins are negatively charged at the employed pH of ~ 8 ; hence, electrophoretic transport of these proteins is in opposite direction to the electroosmotic bulk flow. Depending on the magnitude of μ_{ep} relative to μ_{eo} this could considerably decrease the overall electrokinetic force or result in an overall reversed migration direction (for $\mu_{ep} > \mu_{eo}$ and in the case of opposite sign). In our study, we consider that μ_{ep} is considerably smaller than μ_{eo} and of opposite sign because our experimental observations reflected a strong cathodic electroosmotic flow, confirming that electrophoresis counteracts electroosmosis only to a marginal extent. Therefore, we conducted our simulations using an overall electrokinetic mobility reflecting the actual flow direction. We thus use $\mu_{eo} = 1.5 \times 10^{-8} \text{ m}^2/\text{Vs}$ as previously reported under similar experimental conditions in PDMS microfluidic channels [57].

Similarly to the case of electrokinesis, one can assign a dielectrophoretic mobility μ_{dep} to a particle traveling in a liquid due to dielectrophoresis. For a prolate spheroid, with which an IgG molecule can be approximated, the drag force is then given by [58]:

$$\mathbf{F}_{drag} = 6 \pi \eta \bar{R} \mathbf{u} \quad \text{eq. (6)}$$

where η is the medium viscosity and $\mathbf{u}$ the particle velocity. The mean translational

coefficient is: $\bar{R} = \frac{2}{S}$ with

$$S = 2(a^2 - b^2)^{-\frac{1}{2}} \ln \left[\frac{a + (a^2 - b^2)^{\frac{1}{2}}}{b} \right] \quad \text{eq. (7)}$$

The drag force is balanced by the DEP force given in eq. (1). We can thus write for the DEP velocity

$$\mathbf{u}_{DEP} = \mu_{DEP} * \nabla E^2 \quad \text{eq. (8)}$$

and assign

$$\mu_{DEP} = \frac{2 a b c \varepsilon_m f_{MCM}}{9 \eta \bar{R}} \quad \text{eq. (9)}$$

Similarly to the early works on streaming DEP, we employ a convection-diffusion model to describe streaming DEP [18,59]. We consider electroosmosis the convection component interplaying with diffusion and dielectrophoresis. The total flux in our model can thus be expressed as:

$$\mathbf{J} = -D \nabla c + c[u_{ek} + u_{dep}] \quad \text{eq. (10)}$$

where D is the diffusion constant and c the concentration of the particles. We do not account for any pressure drop across the microchannels and can thus neglect the non-electrokinetic component of velocity in the COMSOL model. At steady state:

$$\frac{\partial c}{\partial t} = \nabla \cdot \mathbf{J} = 0 \quad \text{eq. (11)}$$

To solve this model, we used COMSOL Multiphysics 4.1 software. In particular, we used the *transport of diluted species* module to solve eq. (10) and plot the concentration profile. The *electric current* module was used to model the electric field in the channel, whereas the *creeping flow* model was used to solve the electroosmotic flow component. In accordance with our experiments, an applied electric field of 3000 V/cm along the microchannel, a conductivity of 0.01 S/m and the permittivity of water were selected for modeling (see also Table 1 for simulation input parameters).

Materials and Methods

Chemicals

Si wafers were purchased from University Wafer. The negative photoresist SU-8 2007 and the developer were obtained from Microchem (Newton, MA, USA). Sylgard 184, composed of the silicone elastomer base and the curing agent for poly(dimethylsiloxane) (PDMS), was purchased from Dow Corning Corporation (Midland, MI, USA). Potassium phosphate monobasic, sodium phosphate dibasic, poly (ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (F 108), and 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), acetone and isopropanol were purchased from Sigma-Aldrich (St. Louis, MO, USA), Alexa Fluor 488 BSA and Alexa Fluor 488 chicken anti-rabbit IgG conjugates were obtained from Invitrogen (Carlsbad, CA, USA). Glass slides were obtained from Fisher Scientific, and Pt wire was from Alfa Aesar (Ward Hill, MA, USA). Ultrapure water was supplied from a Synergy purification system (Millipore, USA).

Microdevice fabrication

The inverted microstructure was created on a Si wafer via photolithography as previously reported [60]. Briefly, a Si wafer was spin-coated with the negative photoresist SU-8, UV-exposed through a chrome on soda-lime photomask (Photosciences), and developed in a developer bath. This structure served as the master wafer for elastomer molding. The latter step was performed by casting PDMS on the master wafer and curing for 4 h at 85 °C. Subsequently, the structured PDMS slab was peeled off from the wafer. Reservoir holes of 2 mm diameter were punched manually through the PDMS at the end of the channels creating channels of 1 or 2.5 cm length. The structured PDMS and a glass slide were cleaned in a sonication bath with isopropanol followed by water and blow-dried with nitrogen. The

PDMS slab and the glass slide were treated with an oxygen plasma cleaner (PDC-001 Harrick Plasma, Harrick, US) for 1 min at the highest RF-setting and then brought into contact.

A polycarbonate block (60*45*5 mm³) with through holes above the microchip reservoirs was placed on top of the PDMS device to enlarge the reservoirs and to hold the Pt electrodes in position. Before performing experiments, the polycarbonate block was rinsed with deionized water, dried completely, and pressed onto the PDMS chip so that both components adhered tightly.

Microchip operations

The microchannel walls were treated with a solution of 500 μ M F108 in phosphate buffer (2.5 mM, pH 8.1) for approx. 10 hours. Afterwards the buffer was exchanged with a phosphate buffer with the desired conductivity (0.01–0.04 S/m) as assessed with a conductivity meter (ORION 013005MD, Thermo scientific, USA). For the DEP trapping experiments, the reservoirs were filled with 50 μ L phosphate buffer solution. Protein concentrations were 1.7 nM for IgG and 7 nM for BSA. When the zwitterions additive was added, the resulting CHAPS concentration was 1.7 mM for IgG and 4.9 mM for BSA. Pt-electrodes at the assembled microchip were connected to a high voltage power supply HVS448 6000V, LabSmith, Livermore, CA, USA) to apply DC voltages.

Detection/Data analysis

An inverted microscope (IX 71, Olympus, Center Valley, PA USA) with an automated x/y stage (CS 152 EF, Prior Scientific, Rockland, MA, USA), a 40 $\times$ objective (LUCPlan FL N, Olympus, Center Valley, PA USA), a mercury burner (U-RFL-T, Olympus, Center Valley, PA USA) and fluorescence filter set (exciter ET470/40 $\times$, dichroic T495LP, emitter ET525/50m, Olympus USA) were employed for capturing images and video sequences. A CCD camera (Quantum 512SC, Photometrics, Tucson, AZ, USA) was used for data acquisition in conjunction with Micro-Manager software (University of California, San Francisco, USA, version 1.3). Video sequences of the protein trapping were recorded at 0.2 frames/second. Image J software (version 1.43) was used for data analysis. The concentration factor was calculated by subtracting the averaged intensity in the non-streamline regions I_{NT} from the averaged intensity of the concentrated streaming regions I_T and normalizing this difference with I_{NT} .

Results and Discussion

Overall setup

Figure 1 demonstrates an example of a post array geometry used to create iDEP devices. In a microchannel, non conducting post are integrated, so that electric field gradients are created around the posts. We used a linear channel exhibiting such iDEP arrays and applied potential to it via electrodes immersed in the channel reservoirs. We devised two iDEP microfluidic devices with different insulating post geometries. In the first device we created triangular post arrays in which strong gradients are expected between the tips of the triangles facing each other (see Fig. 2 for this case). A second device consisted of elliptical post arrays with a symmetric distribution of electric field gradients. These structures were fabricated using standard photo- and soft-lithographic techniques. The resulting geometries were verified by scanning electron microscopy and we thus adapted numerical simulations of protein DEP in these devices to the actual post dimensions.

Numerical Simulations

We start the discussion on numerical simulations with the triangular post array geometry and IgG. Figure 2 (a) and (b) represent the electric field and ∇E^2 distribution at an applied field of 3000 V/cm. We can see that the vertices facing each other are the areas with the highest electric field (1×10^6 V/m) and strongest ∇E^2 values (1×10^{17} V²/m³). Therefore, in the expected case of positive DEP proteins would be attracted towards the triangular tips facing each other.

We compare our iDEP geometry with previously reported streaming dielectrophoresis for 200 nm latex spheres. In their contributions, Cummings et al. [18,59] demonstrate that streaming behavior and concentration occurs when DEP overcomes diffusion but is too weak to overcome the electrokinetic flow. These findings occurred using 200 nm particles, which are larger in size than IgG or BSA and performed in different iDEP post geometries at applied electric fields of 80–250 V/cm [18]. In our case, we can estimate the arising u_{eo} and u_{dep} via the computed E and ∇E^2 values using eq. (5) and (8), respectively. We thus observe that the DEP force cannot overcome the electrokinetic flow (see Table 1 and theoretical section). Therefore, we would expect DEP streaming behavior for proteins in accordance with previously reported data for nanoparticles [18,59].

To test the assumed DEP behavior, we computed the concentration profile for particles exhibiting positive ($\mu_{dep} = 8.60 \times 10^{-24}$) and negative ($\mu_{dep} = -7.14 \times 10^{-24}$) DEP adapted to the case of IgG in Figure 2c and 2d with an initial concentration of unity. When DEP is positive (Figure 2 c), the maximum concentration is created along the posts. However, when DEP is negative the very same regions become depleted (see Figure 2d).

Similar results were obtained for the case of elliptical posts. Figure 3 a and b depict the electric field and ∇E^2 at 3000 V/cm in this geometry. The highest electric field and ∇E^2 are located at the vertical edges of the ellipse, corresponding to values of 6×10^5 V/m and 2×10^{17} V²/m³ respectively. As above, we computed the concentration distribution for both positive and negative DEP. The simulation results show that for positive DEP the maximum concentration is located along the posts (Figure 3 c) and for negative DEP the maximum concentration is depleted in the same areas (Figures 3 d). Note, that the concentration enhancement in the positive DEP case is more pronounced than the depletion in the negative DEP case. Both geometries therefore demonstrate highly distinct regions for DEP-dependent enhancement (positive DEP) or depletion (negative DEP) that can be used to manipulate proteins within the microfluidic system.

It is important to mention that potential electrolysis or Joule heating effects were not considered in our simulation. However, we have conducted a separate study investigating the creation of pH gradients in iDEP experimentally as well as by numerical simulations [61]. This study showed that the pH changed towards the acidic range in time scales of ~10 min. under similar buffer conductivity and applied potentials. A lower pH can reduce the EOF in PDMS microchannels, thus resulting in enforced DEP versus electroosmotic flow. As a consequence streamline iDEP of proteins is enhanced thus adding positively to streaming iDEP. Indeed, our numerical simulation show lower concentration factors as compared to our experiments (see below) indicating an overestimation of the electroosmotic component in simulations. Although Joule heating can in principle not be neglected in our case, we experimentally found little influence on protein DEP within the course of our experiments, as discussed below.

IgG iDEP

Next we tested the iDEP designs for protein trapping experimentally. The triangular geometry was first employed to test DEP behavior of IgG. The protein solution was filled in the cathode reservoir and moved towards the post array at low applied potentials via EOF. Upon an increase of the electric potential we observed immobilization of protein within the device (see Figure 4a). This indicates that in the respective trapping areas the DEP component overcame the electrokinetic flow. This was a surprising finding as according to the simulations only streaming DEP was expected. We noticed, however, that once the electric field was turned off, proteins diffused away from the trapping positions as large, cohesive aggregates. Hence, we speculated that under these experimental conditions, large protein aggregates may form, and subsequently become trapped. This could be attributed by Joule heating within the microdevice at higher electric fields potentially leading to protein denaturation and subsequent aggregate formation. This trapping behavior can be explained by the much larger size of the aggregates compared to individual proteins and thus larger DEP forces as can be derived from equation 1.

To prevent aggregate formation, we added the zwitterionic detergent CHAPS to the buffer. CHAPS is known to improve protein solubility [62] in bioanalytical applications and we expected reduced aggregate formation with this buffer additive. Due to the zwitterionic nature of CHAPS the overall conductivity of the buffer was not significantly changed. In subsequent experiments, large aggregates were not detected anymore. While the proteins may still be denatured due to Joule heating the CHAPS clearly prevented the formation of larger aggregates. More importantly, we clearly observed a different behavior of IgG-proteins in the channel upon electric field application. In fact, our results indicated DEP streamline formation at 3000 V/cm as demonstrated in Figure 4b. Interestingly, these streamlines are formed at the same locations as in our simulation in the case of positive DEP (see Figure 2c). This positive DEP behavior is in accordance to DEP manipulation with IgG molecules as reported by Clarke et al. at nanotip electrodes [54]. Our numerical model is thus in excellent qualitative agreement with experimental findings when an agent preventing protein aggregation was added. In addition, we observed a slight increase in fluorescence in the direction of the bulk flow (see Figure 4 b). This was also observed in the simulations with the triangular posts (Figure 2 c) indicating that the concentration effect could be intensified with extended rows of insulating structures.

Furthermore, we investigated the influence of protein DEP in relation to the buffer conductivity. To this aim, we repeated the experiments with IgG in presence of CHAPS, but varied the conductivity of the buffer between 0.01 S/m and 0.04 S/m by changing the salt concentration. As demonstrated in Figure 4 b–e, the intensity of the streamlines decreased with increasing buffer conductivity. This phenomenon was further investigated more quantitatively. Figure 5 shows the concentration factor determined at the streamline positions in relation to applied electric field. We see that the highest concentration increase was achieved in the lowest conductivity case (0.01 S/m). At an applied potential of 3000 V/cm, this resulted in a concentration factor of $\sim 24 (\pm 1) \%$. Interestingly, this corresponds to an order of magnitude higher concentration factor as compared to simulations. We attribute this to an overestimation of the electroosmotic component versus the electrophoretic migration of proteins in opposite direction. In fact when using our numerical model and simulating the case of a reduced electroosmotic flow component, we find higher concentration factors in accordance with experiments (data not shown).

Further, Figure 5 also shows that higher buffer conductivities resulted in an overall lower concentration factor (down to $\sim 2 \%$ at 0.04 S/m and 3000 V/cm). We find an explanation to this behavior in the case of positive DEP from equation 1 and 4. While increasing σ_m , the f_{MCM} factor is reduced, thus leading to lower DEP forces. As a consequence, concentration

of proteins in streaming iDEP is reduced. Overall, the data demonstrated successful DEP streaming manipulation of IgG proteins resulting in enhanced concentration of proteins at defined areas in the chip. It was also shown that aggregation of IgG readily influences iDEP trapping behavior and is in contrast to experiments where aggregate formation is circumvented by the use of a surfactant.

BSA iDEP

We further investigated whether the findings were also applicable to other, smaller, proteins. We thus further studied the DEP behavior of the smaller protein BSA employing the elliptic post array layout. Similarly to the case of IgG, we also observed trapping of approximately μm -sized entities suggesting protein aggregation (see Figure 6a). This indicates that protein aggregation is not limited to IgG, but also occurs with other proteins. In fact it is therefore possible that most iDEP manipulations of proteins may require the control of protein aggregation.

As previously with IgG, we further tested the DEP behavior of BSA with the zwitterionic additive CHAPS. Similar to the larger IgG proteins, we observed streamline DEP once the zwitterionic agent is added (Figure 6b). The concentration of BSA is clearly increased along streamlines developing between the center edge positions of the periodically arranged posts. This is in good agreement with the results of the convection-diffusion model as demonstrated in Figure 3b, in which positive DEP was assumed. Similarly to the case of IgG, concentration enhancement is observed for the positive DEP simulation in accordance with the observed streamline behavior. We thus conclude that BSA also demonstrated positive DEP behavior under our experimental conditions.

It is important to notice that the experimental streamline behavior is in excellent qualitative agreement with the performed numerical simulations as indicated by the match of streaming iDEP concentration enhancement. Quantitatively, however, we find a discrepancy in the absolute magnitude of the concentration factor. While we observe concentration up to a factor of $\sim 24\%$ in the case of IgG in the triangular post geometry, we find only a maximum value of $\sim 3\%$ from the simulation. This can be attributed to differences of the input parameters used for the numerical simulations in comparison to our detailed experimental conditions. Clearly, we are currently limited to the reported data on protein conductivity and thus chose to adapt our simulation input parameters to the data most relevant to IgG in solution as previously reported by Clarke et al. [54]. Additionally, other influences may arise from deviations of the actual electroosmotic mobility in the microchannels. Again, we adapted the simulation parameters to our previously reported data in coated PDMS channels [57]. In this study, we however used deviating buffer conductivity and pH, possibly giving rise to changes in the absolute value of μ_{eo} . As we have found in our simulation, slightly reduced μ_{eo} can increase the streaming concentration considerably (data not shown). Future optimization of the device geometry and convection influences might thus be readily performed with the here employed DEP model and numerical simulations.

Conclusion

In summary, we demonstrated successful iDEP-based manipulations of proteins with different geometries in a PDMS-based microfluidic system. Theoretical considerations predicted positive DEP behavior for proteins resulting in locally concentrated streamlines, which were accurately observed in experiments and were in excellent qualitative agreement with simulations. Moreover, this work demonstrates that protein aggregation readily occurs using standard phosphate buffers without the use of additives. These protein aggregates exhibit a different behavior (i.e. trapping) as opposed to individual proteins in presence of a surfactant. Our results indicate the potential for manipulation of biomolecules with

molecular dimension smaller than 10 nm thus highlighting the ability to manipulate proteins using DEP. Specifically, streaming DEP has demonstrated the ability to concentrate proteins within a microfluidic device thus providing an exciting new tool for protein analysis devices in microfluidic format. We anticipate the enhancement of this iDEP concentration by further optimization of the device geometry with the aid of the theoretical model presented herein and the future application of iDEP for analytical applications of proteins on microfluidic platforms.

Acknowledgments

Financial support from the National Institute of Health under grant 1R21RR025826-01A2 is gratefully acknowledged. We also thank Lin Gan, Arizona State University, for preparation of figures.

Abbreviations

DEP	Dielectrophoresis
iDEP	insulator-based dielectrophoresis

References

1. Reyes DR, Iossifidis D, Auroux PA, Manz A. *Anal.Chem.* 2002; 74:2623–2636. [PubMed: 12090653]
2. Huang LR, Tegenfeldt JO, Kraeft JJ, Sturm JC, Austin RH, Cox EC. *Nature Biotechnol.* 2002; 20:1048–1051.
3. Huang LR, Cox EC, Austin RH, Sturm JC. *Science.* 2004; 304:987–990. [PubMed: 15143275]
4. Kaji N, Tezuka Y, Takamura Y, Ueda M, Nishimoto T, Nakanishi H, Horiike Y, Baba Y. *Anal.Chem.* 2004; 76:15–22. [PubMed: 14697027]
5. Doyle PS, Bibette J, Bancaud A, Viovy JL. *Science.* 2002; 295:2237. [PubMed: 11910102]
6. Minc N, Fuetterer C, Dorfman KD, Bancaud A, Gosse C, Goubault C, Viovy JL. *Anal.Chem.* 2004; 76:3770–3776. [PubMed: 15228353]
7. Tabuchi M, Ueda M, Kaji N, Yamasaki Y, Nagasaki Y, Yoshikawa K, Kataoka K, Baba Y. *Nature Biotechnol.* 2004; 22:337–340.
8. Fu J, Yoo J, Han J. *Phys.Rev.Lett.* 2006; 97:018103. [PubMed: 16907412]
9. Han J, Craighead HG. *Anal.Chem.* 2002; 74:394–401. [PubMed: 11811414]
10. Han J, Craighead HG. *Science.* 2000; 288:1026–1029. [PubMed: 10807568]
11. Huang L, Cox E, Austin RH, Sturm JC. *Anal.Chem.* 2003; 75:6963–6967. [PubMed: 14670059]
12. Fu J, Schoch RB, Stevens AL, Tannenbaum SR, Han J. *Nature Nanotechnology.* 2007; 2:121–128.
13. Fu J, Mao P, Han J. *Appl.Phys.Lett.* 2005; 87:263902. [PubMed: 18846250]
14. Pohl, HA. *Dielectrophoresis.* Cambridge: Cambridge University Press; 1978.
15. Jones, TB. *Electromechanics of Particles.* New York: Cambridge University Press; 1995.
16. Chen KP, Pacheco JR, Hayes MA, Staton SJR. *Electrophoresis.* 2009; 30:1441–1448. [PubMed: 19425000]
17. Hughes MP. *Electrophoresis.* 2002; 23:2569–2582. [PubMed: 12210160]
18. Cummings EB, Singh AK. *Anal.Chem.* 2003; 75:4724–4731. [PubMed: 14674447]
19. Lapizco-Encinas BH, Simmons BA, Cummings EB, Fitschenko Y. *Anal.Chem.* 2004; 76:1571–1579. [PubMed: 15018553]
20. Chou CF, Tegenfeldt JO, Bakajin O, Chan SS, Cox EC, Dorton N, Duke T, Austin RH. *Biophys.J.* 2002; 83:2170–2179. [PubMed: 12324434]
21. Hawkins BG, Smith AE, Syed YA, Kirby BJ. *Anal.Chem.* 2007; 79:7291–7300. [PubMed: 17764153]
22. Pysher MD, Hayes MA. *Anal. Chem.* 2007; 79:4552–4557. [PubMed: 17487977]

23. Thwar PK, Linderman JJ, Burns MA. Electrophoresis. 2007; 28:4572–4581. [PubMed: 18072223]
24. Cho YK, Kim S, Lee K, Park C, Lee JG, Ko C. Electrophoresis. 2009; 30:3153–3159. [PubMed: 19722215]
25. Iliescu C, Xu GL, Loe FC, Ong PL, Tay FEH. Electrophoresis. 2007; 28:1107–1114. [PubMed: 17330223]
26. Kang YJ, Cetin B, Wu ZM, Li DQ. Electrochim. Acta. 2009; 54:1715–1720.
27. Kang YJ, Li DQ, Kalams SA, Eid JE. Biomed. Microdev. 2008; 10:243–249.
28. Srivastava SK, Baylon-Cardiel JL, Lapizco-Encinas BH, Minerick AR. Journal of Chromatography A. 1218:1780–1789.
29. Voldman, J.; Bashir, R.; Wereley, S.; Ferrari, M. BioMEMS Biomed. Nanotech. Kluwer; 2006. p. 159-186.
30. Fiedler S, Shirley SG, Schnelle T, Fuhr G. Anal.Chem. 1998; 70:1909–1915. [PubMed: 9599586]
31. Huang Y, Joo S, Duhon M, Heller M, Wallace B, Xu X. Anal.Chem. 2002; 74:3362–3371. [PubMed: 12139041]
32. Morgan H, Hughes MP, Green NG. Biophys.J. 1999; 77:516–525. [PubMed: 10388776]
33. Voldman J, Toner M, Gray ML, Schmidt MA. Anal.Chem. 2002; 74:3984–3990. [PubMed: 12199564]
34. Wang XB, Yang J, Huang Y, Vykoukal J, Becker FF, Gascoyne PRC. Anal.Chem. 2000; 72:832–839. [PubMed: 10701270]
35. Yang J, Huang Y, Wang XB, Becker FF, Gascoyne PRC. Biophys. J. 2000; 78:2680–2689. [PubMed: 10777764]
36. Yang J, Huang Y, Wang XJ, Wang XB, Becker FF, Gascoyne PRC. Biophys. J. 1999; 76:3307–3314. [PubMed: 10354456]
37. Pommer MS, Zhang Y, Keerthi N, Chen D, Thomson JA, Meinhart CD, Soh HT. Electrophoresis. 2008; 29:1213–1218. [PubMed: 18288670]
38. Li H, Bashir R. Sens. & Actuat. B. 2002; 86:215–221.
39. Gascoyne PRC, Vykoukal JV. Electrophoresis. 2002; 23:1973–1983. [PubMed: 12210248]
40. Regtmeier J, Bugonovic L, Hoefemann H, Anselmetti D, Eichhorn R, Ros A. Anal.Chem. 2010 *accepted*.
41. Regtmeier J, Eichhorn R, Bogunovic L, Ros A, Anselmetti D. Anal. Chem. 2010; 82:7141–7149. [PubMed: 20690609]
42. Hoelzel R. IET Nanobiotechnology. 2009; 3:28–45. [PubMed: 19485551]
43. Tuukkanen S, Kuzyk A, Toppari JJ, Hakkinen H, Hytonen VP, Niskanen E, Rinkio M, Torma P. Nanotechnology. 2007; 18:295204.
44. Kuzyk A, Yurke B, Toppari JJ, Linko V, Torma P. Small. 2008; 4:447–450. [PubMed: 18350556]
45. Washizu M, Suzuki S, Kurosawa O, Nishizaka T, Shinohara T. IEEE Trans.Ind.Appl. 1994; 30:835–843.
46. Lapizco-Encinas BH, Ozuna-Chacon S, Rito-Palomer M. J. Chromatogr. A. 2008; 1206:45–51. [PubMed: 18571183]
47. Lapizco-Encinas BH, Rito-Palomer M. Electrophoresis. 2007; 28:4521–4538. [PubMed: 18072220]
48. Clarke RW, White SS, Zhou D, Ying L, Klenerman D. Angew. Chemie - Intl. Ed. 2005:3747–3750.
49. Bakewell DJG, Hughes MP, Milner JJ, Morgan H. Proc.20th Ann.Intern.Conf.IEEE Medicine and Biology Soc. 1998; 20:1079–1082.
50. Hoelzel R, Calander N, Chiragwandi Z, Willander M, Bier FF. Phys.Rev.Lett. 2005; 95:128102. [PubMed: 16197115]
51. Maruyama H, Nakayama Y. Applied Physics Express. 2008; 1
52. Zheng L, Brody JP, Burke PJ. Biosens. & Bioelectr. 2004; 20:606–619.
53. Agastin S, King MR, Jones TB. Lab Chip. 2009; 9:2319–2325. [PubMed: 19636462]
54. Clarke RW, Piper JD, Ying LM, Klenerman D. Phys.Rev.Lett. 2007; 98:198102. [PubMed: 17677664]

55. Sandin S, Ofverstedt LG, Wikstrom AC, Wrangé O, Skoglund U. *Structure*. 2004; 12:409–415. [PubMed: 15016357]
56. Morgan H, Green NG. *J. Electrostatics*. 1997; 42:279–293.
57. Hellmich W, Regtmeier J, Duong T, Ros R, Anselmetti D, Ros A. *Langmuir*. 2005; 21:7551–7557. [PubMed: 16042494]
58. Probstein, R. *Physicochemical Hydrodynamics*. Hoboken, New Jersey: John Wiley & Sons; 2003.
59. Cummings EB. *IEEE Engineering in Medicine and Biology Magazine*. 2003; 22:75–84. [PubMed: 15007994]
60. Duong T, Kim G, Ros R, Streek M, Schmid F, Brugger J, Ros A, Anselmetti D. *Microelectronic Engineering*. 2003; 67–68:905–912.
61. Gencoglu A, Camacho-Alanis F, Nguyen VT, Nakano A, Ros A, Minerick AR. *Electrophoresis*. 2011 in press.
62. Hjelmeland LM, Nebert DW, Osborne JC. *Anal. Biochem*. 1983; 130:72–82. [PubMed: 6869811]
63. Rosenqvist E, Jossang T, Feder J, Harbitz O. *J. Protein Chem*. 1986; 5:323–333.

Figure 1. Schematic representation of post arrays in the microfluidic device for iDEP protein manipulation. The device contains periodic arrays of insulating posts in varying geometry that distort the electric field, thus creating local gradients. The inset shows electric field lines around circular posts as an example.

Figure 2. Simulation results for IgG molecules in a 20 μm triangular post array at 3000 V/cm: a) E , b) ∇E^2 , c) Concentration profile considering convection due to electroosmosis, diffusion and DEP in the case of positive DEP. Streaming DEP is observed along the posts indicating protein concentration. d) Concentration profile considering negative DEP. In this case, streaming DEP indicates concentration depletion. The scale bar represents 15 μm . The color scale in a) is V/m, in b) V^2/m^3 and in c) and d) relative concentration. The simulated iDEP streamline flow is from top to bottom.

Figure 3. Simulation results for IgG molecules in an elliptical post array at 3000 V/cm: a) E , b) ∇E^2 . c) Concentration profile considering convection due to electroosmosis, diffusion and DEP in the case of positive DEP. Streaming DEP is observed along the posts indicating protein concentration. d) Concentration profile considering negative DEP. In this case, streaming DEP indicates concentration depletion. The scale bar represents 10 μm . The color scale in a) is V/m, in b) V^2/m^3 and in c) and d) relative concentration. The simulated iDEP streamline flow is from top to bottom.

Figure 4.

Fluorescence microscopy images of fluorescently labeled IgG in an array of triangular posts. The flow direction is from top to bottom. a) IgG aggregate trapping is observed as indicated by the arrow. (b–e) Streaming behavior of IgG at an applied voltage of 3000 V/cm in the presence of 1.7mM CHAPS. (b) $\sigma_m = 0.01$ S/m, (c) $\sigma_m = 0.02$ S/m, (d) $\sigma_m = 0.03$ S/m and (e) $\sigma_m = 0.04$ S/m. Positive DEP streaming is observed and is reduced from b) to e) while increasing the buffer conductivity. (Scale bar is 20 μm for a–e.)

Figure 5.
Normalized concentration factor as a function of buffer conductivities.

Figure 6.

Fluorescence images of fluorescently labeled BSA subject to DEP in an array of elliptical posts. a) Aggregate trapping of BSA. b) Streamline DEP of BSA in presence of CHAPS at an applied electric field of 1900 V/cm. The flow direction is from top to bottom.

Table 1

Input values used for numerical simulations

σ_p [S/m]	σ_m [S/m]	f_{MCM}^a	DEP type	μ_{DEP}^b [m ⁴ /V ² s]	D^c [m ² /s]
24.6	0.01	5.75	positive	8.60×10^{-24}	3.89×10^{-11}
0	0.01	-1.21	negative	-7.14×10^{-24}	3.89×10^{-11}

^aCalculated from equation (4)

^bCalculated from equation (9) and using values corresponding to IgG.

^cFrom reference [63].