Fringe Dielectrophoresis Nanoaperture Optical Trapping with Order of Magnitude Speed-Up of Unmodified Proteins

Elham Babaei, Demelza Wright, and Reuven Gordon*

Department of Electrical and Computer Engineering, University of Victoria, Victoria, BC

E-mail: rgordon@uvic.ca

Phone: 250-472-5179

Abstract

Single molecule analysis of small proteins in an aqueous environment without modification (e.g., labels or tethers) elucidates their biophysics and interactions relevant to drug discovery. By combining fringe-field dielectrophoresis with nanoaperture optical tweezers we demonstrate an order of magnitude faster trapping of proteins when the reference electrode is outside of the solution. When the reference electrode is inside the solution (the more common configuration found in the literature), electrophoresis speeds up the trapping of polystyrene nanospheres, but this was not effective for proteins in general. Since time-to-trap is critical for high-thoughput analysis, these findings are a major advancement to the nanoaperture optical trapping technique for protein analysis.

Introduction

Single-molecule techniques can resolve heterogeneity in behavior and give access to kinetics without synchronization.[1] Ideally these methods would allow for seeing dynamics at a

rapid timescale without modifications to the protein, in a physiological environment and be able to study small and large biomolecules. While used extensively in free-solution studies, modifications like labelling and tethering disrupt the natural function of biomolecules with significant impact on properties such as diffusion, surface potential and binding kinetics.[2]

Various single molecule techniques allow for analyzing unmodified proteins in aqueous environments, including nanopores, iScat and nanoaperture optical tweezers (NOTs). NEOtrap is a nanopore technique that sizes proteins by monitoring changes in ionic current for a blocked nanopore,[3] and it has recently been used to achieve sizing of 14 kDa proteins.[4] Early works on iScat achieved a 40 kDa size limit of detection,[5] which can be further enhanced below 9 kDa by using sophisticated machine learning techniques.[6] NOTs have previously sized single proteins down to 6.5 kDa, even in heterogeneous unprocessed solutions.[7,8] More generally, various shaped apertures have been used to trap and analyze nanoparticles.[9–14,14–29]

Later works combined nanopores with NOTs, which confirms that single proteins were trapped in NOTs, as generally expected due to natural repulsion.[17,22,30] Even without nanopores, the normal single protein analysis of NOTs is clear from the uniform distinct steps observed with rare multiple-trapping events[31] and from the consistent single-particle mass sizing results.[7] While trapped, single protein dynamics have been observed, including conformational changes (giving information about protein shape) and enzyme action.[8,32]

Previous works have introduced electric fields with nanoapertures in metal films to manipulate nanoparticles. By applying an AC electric field for a reference electrode in solution, as well as a laser beam, a balance was achieved between electroosmotic and thermophoretic forces to trap fluorescently labelled proteins at a distance from an array of holes.[33] Dielectrophoresis has also been used with gold-coated nanopipettes to isolate individual fluorescently labelled DNA where the reference electrode was again placed in solution.[34] There are two main distinctions between these (and similar) past works and our present work: we are applying the use of electrodes to NOTs for the first time without the use of fluorescence, and we are experimenting with the placement of the reference electrode to create a fringe field

and thereby enhance performance.

Here we examine the use of a electrode for enhancing the trapping speed of a NOT for unlabelled proteins. When the reference electrode is placed on the dielectric substrate supporting the gold film (on the opposite side from the solution), so that a fringe field is created in the solution by the nanohole, an order of magnitude reduction in trapping time is achieved. This occurs for both negative and positive applied voltage (and AC voltage) demonstrating that the speedup comes from dielectrophoresis. When the reference electrode is placed in the solution, which is the more common practice, a positive voltage on the gold film with respect to the reference electrode speeds up the trapping of polystyrene nanospheres, but this was not generally effective for proteins.

Dielectrophoretic Speed Up of Time to Trap

Figure 1(a) shows a schematic of the optical tweezer system containing a gold film with double nanoholes, fabricated as described previously.[35,36] A reference electrode is placed on the opposite side of the glass substrate to the sensing volume (away from protein solution) to achieve a fringe field through the aperture, as shown schematically in Figures 1 (b) and (c). The fringe field can attract particles by dielectrophoresis.

Figure 2 shows a typical trapping event for the protein aprotinin (6.5 kDa). The laser is turned on at 2.5 seconds and then 42 seconds elapses before there is a rapid discontinuity in the signal (trapping) and a large increase in noise (protein undergoing Brownian motion in the trap). The elapsed time is called the time-to-trap, T.

We repeated trapping measurements for several different proteins without voltage applied, applying a DC voltage, and applying an AC voltage. Figure 3(a) summarizes the results. Applying a DC voltage reduced the time to trap substantially (between 3 and 11 times). The results we repeated between 3 and 10 times for each protein.

We also experimented with applying an AC voltage, with frequencies between 10 Hz and

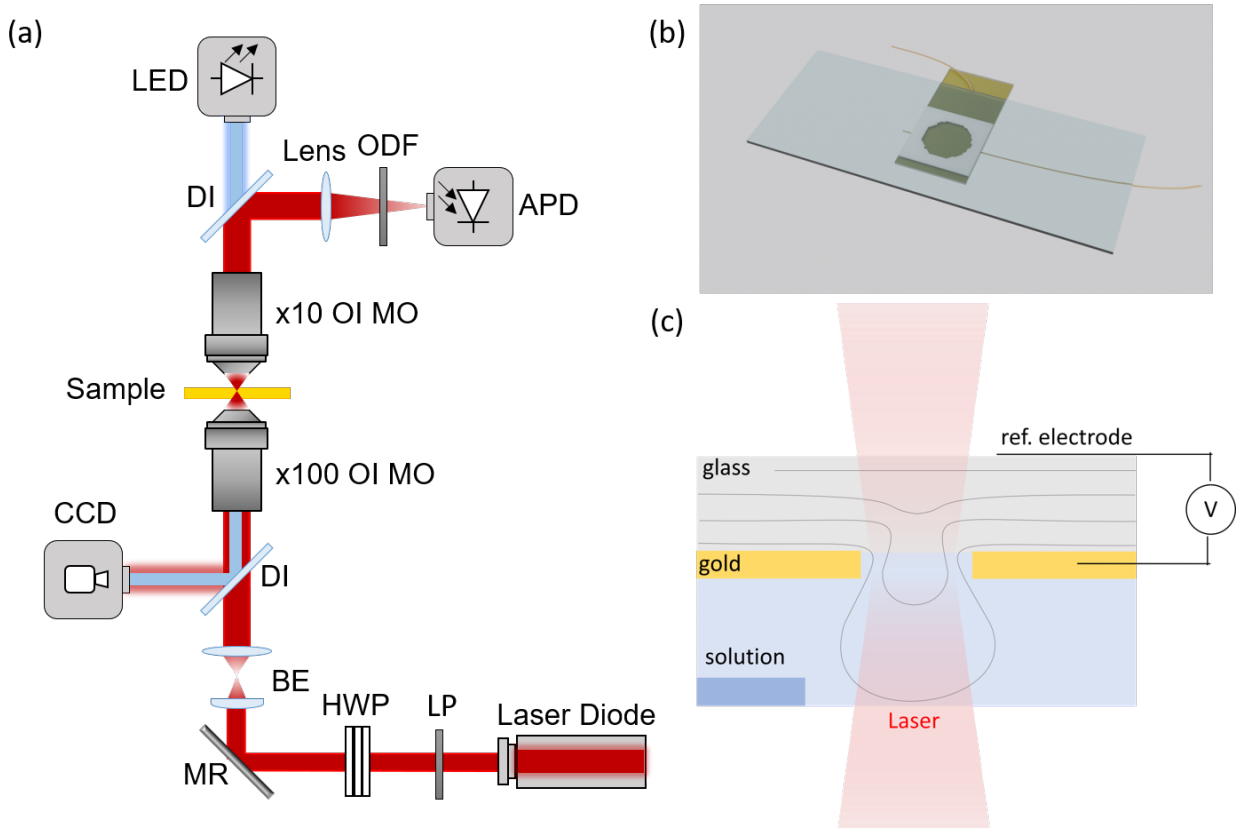

Figure 1: Dielectrophoretic double nanohole trapping. (a) Schematic of optical setup, with detail shown. (b) Electrode connections and equipotential lines of fringe electric fields around nanoaperture.

1 kHz. Based on these initial studies, we selected 100 Hz for AC trapping; however, the speed-up was not as consistent with AC fields as for DC.

Figure 3(b) shows that the polarity of the field did not impact the time-to-trap, which distinguishes dielectrophoretic from electrophoretic effects. The observed trapping is dielectrophoretic in nature.

Electrophoretic-enhanced Trapping

We also placed the reference electrode inside the solution, as shown schematically in Figure 4(c). This is expected to have two effects: first, the fringe field should have a negative gradient towards the nanoaperture[37] (decreasing field strength when approaching the aper-

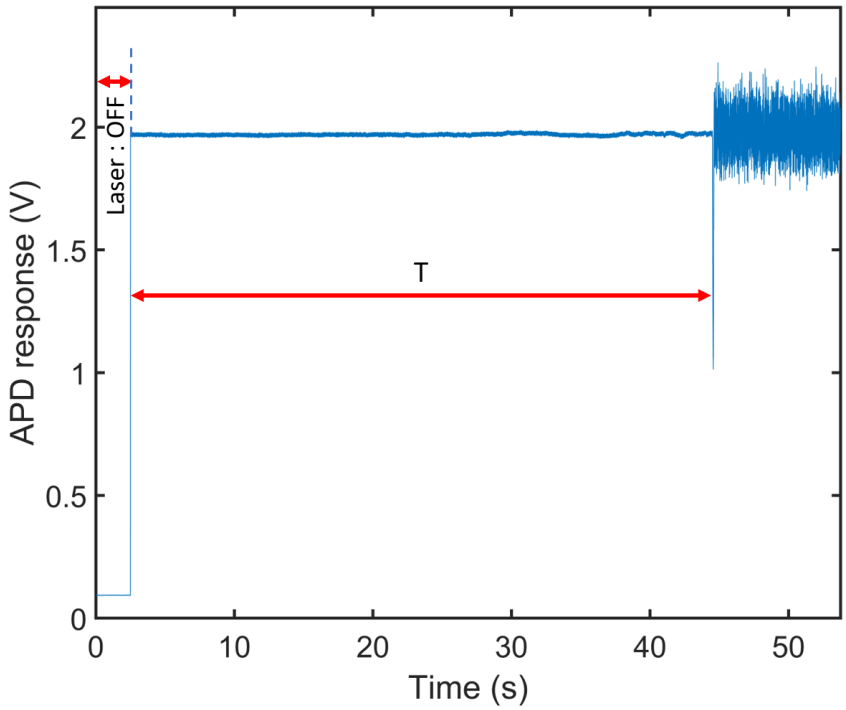

Figure 2: Time to trap (T) in a typical protein trapping event of aprotinin.

ture) and second, any surface-charge electrostatic interaction can be offset by the applied field. We found that having the reference electrode inside the solution did not speed up trapping for proteins in general (see Supporting Information); however, it did reduce the time-to-trap for 20 nm polystyrene spheres systematically. Furthermore, applying a greater positive potential to the gold film systematically reduced the time-to-trap, but a negative voltage did not allow for stable trapping, as shown in Figure 4(d). This suggests that an electrostatic interaction was attracting the negatively charged polystyrene particles to the surface to enhance trapping speed.

Discussion

Considering diffusion alone, we expect that the time to trap would be less than a millisecond, assuming diffusion coefficient of around 100 μ^2/s and average spacing of 0.1 μm (typical at micromolar concentrations). But this is not what is seen in NOTs: in the best case, trapping

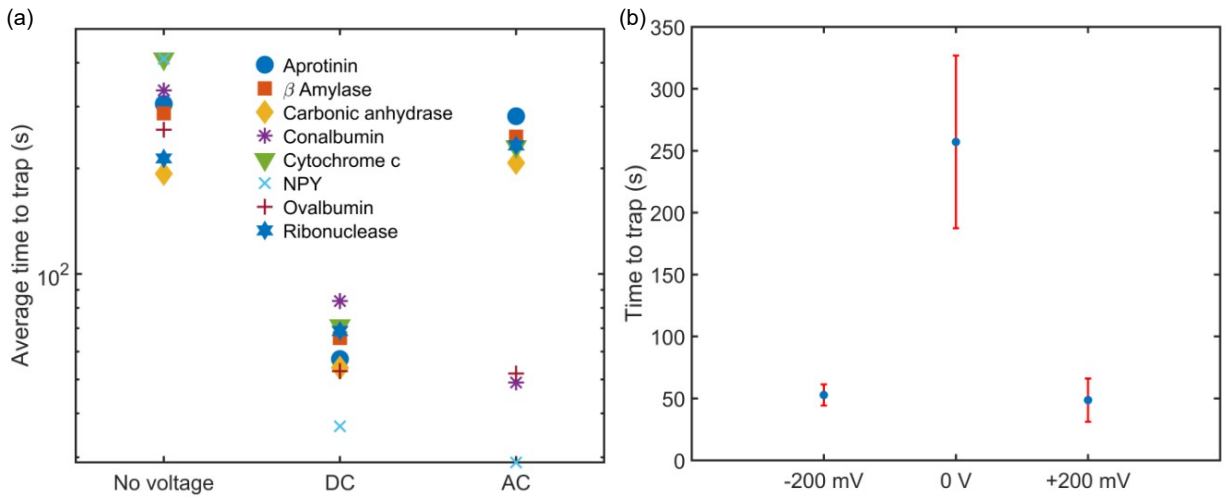

Figure 3: (a) Average time to trap vs applied DC and AC voltages for different proteins. (b) Trapping time for ovalbumin trapped applied DC voltage with DEP method

usually takes minutes, and in some extreme cases, tens of minutes.[38] In some cases, it can be faster (10s of seconds for 20 nm polystyrene nanospheres).[32] This can also be sped up by using surfactants to modify the Soret coefficient and then attract particles to the trap.[23] However, surfactants can have a damaging role on proteins.[39]

Trapping is slow because of competing effects that can keep the nanoparticles away from the trapping site. These effects include electrostatic repulsion and thermophobicity (when the trapping site at higher temperature due to the laser). Nanopores have been used to direct biomolecules to the trapping site with electrophoresis.[22,30] While this allows for rapid trapping, it requires: fabrication of a nanopore in addition to the trapping nanoaperture in a gold film (more nanofabrication time/cost); using a fragile thin membrane for the nanopore (more delicate); and, using two chambers (more complicated). Considering these challenges, the present approach does not add nanofabrication steps, uses a robust substrate and uses a simple single chamber.

This study shows that the location of the reference electrode plays an important role. In most works, the reference electrode is placed within the solution, and this can lead to repulsive electro-osmotic flow.[33] If we aim to direct the particles to the trapping site, repul-

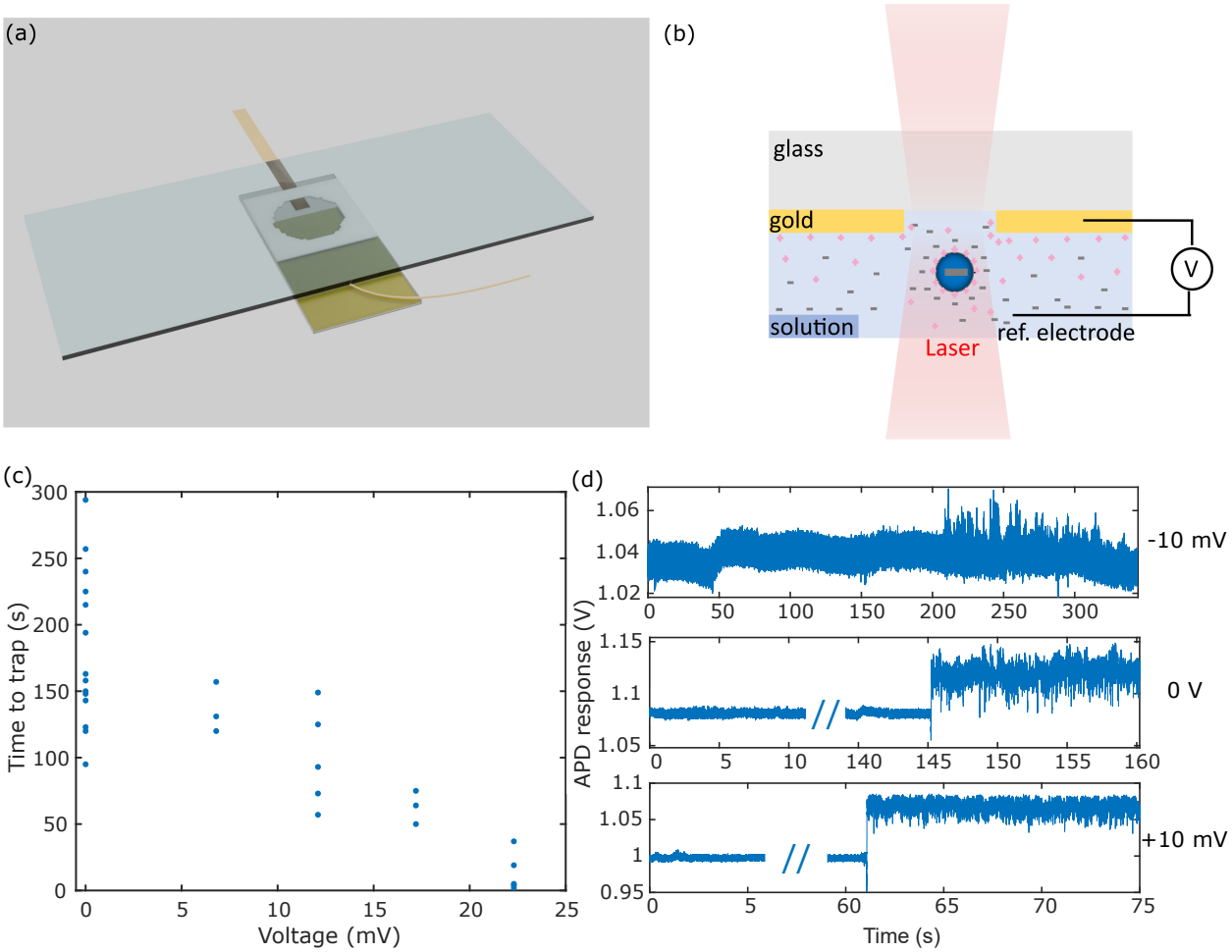

Figure 4: (a),(b) Method of sample preparation and electrode connections for applying voltage in electrophoresis technique. (c) Time to trap versus applied DC voltage for 20 nm polystyrene. (d) Trapping event of 20 nm polystyrene sphere at different DC voltages.

sion is not desirable. Dielectrophoretic effects are sensitive to the location of the reference electrode. The field gradient changes when moving the reference electrode from the solution to the opposite side of the film. If the reference electrode is placed in solution, the aperture in the metal plate sets up an opposing dipole to the applied normal electric field and therefore, the field decreases towards the aperture (negative gradient).[37] This causes a repulsive dielectrophoretic force (assuming the Claussius Mossitti factor is positive). On the other hand, placing the reference electrode on the opposite side of the metal film creates a positive field gradient that attracts particles by dielectrophoresis, which would lead to faster trapping as seen in the experiments for this configuration.

We believe that part of the reason dielectrophoresis works so well is because the proteins are highly polarizable in part because of their surface water interactions; however, this is a topic of ongoing investigation.[40] We confirmed both positive and negative voltages worked, proving that this was dielectrophoresis and not electrophoresis.

We have also used this technique to analyze various proteins, as done in the past, making use of noise amplitude and time constant to get information about the protein size (both from a hydrodynamic and optical scattering point of view, which are in general different quantities). The results are presented in the Supporting Information. Generally, the proteins showed linear increase in the noise amplitude with particle size, and a -2/3 exponent dependence with time constant (which was found by fitting the power spectral density of the thermally driven fluctuations). An exception was Neuropeptide Y, which showed significantly higher than expected noise amplitude but fit the time constant curve well. The larger fluctuations are possibly because of its rod-like shape and flexible tail end.[41]

Conclusions

A simple modification of placing an external reference electrode to the double-nanohole containing metal film allows for an order of magnitude faster trapping of unmodified proteins in solution. The speed-up was observed for both negative and positive potentials, showing that this is a dielectrophoretic effect. The approach was most effective at DC applied voltage. By contrast, putting the reference electrode in solution did not generally speed up trapping of proteins; it did however lead to voltage dependent faster trapping of polystyrene spheres, with increasing applied voltage, which we attribute to changes in the repulsive surface potential since it only worked for positive applied voltages. Since rapid trapping is required for high-throughput analysis of proteins and their interactions, this is a promising approach for the application to single molecule biosensors based on NOTs.

Methods

Sample preparation

Samples were prepared as in past work.[36] The dimensions were 20 nm for the cusp size and 330 nm for the diameter. A typical electron micrograph of the sample is shown in the Supporting Information. The sample was cleaned with acetone between runs. After a few runs, samples were cleaned with KOH and H_2O_2 solution.

Protein samples

Cytochrome c, bovine serum albumin, β amylase are from the GE LMW filtration calibration kit (GE28-4038-41, Sigma Aldrich),and ovalbumin, conalbumin, aprotinin, carbonic anhydrase, ribonuclease are from Cytovia. The concentration of 0.1 percent weight per volume was used for all the proteins in 0.01 M phosphate buffer.

NOT

An optical tweezer setup was modified from Thorlabs OTKB by replacing the 980 nm laser diode with an 850 nm laser diode. The laser power was maintained at 16 mW.

Acknowledgement

The authors acknowledge funding from the NSERC (Canada) Discovery Grant RGPIN-2017-03830.

Declarations

The authors declare the submission of a patent application relating to this technique.

Contributions

E. B. performed all nanofabrication, measurements and analysis and wrote the manuscript. D. W. helped with data analysis and wrote the manuscript. R. G. conceived of experiments, helped with data analysis and wrote the manuscript.

Supporting Information Available

Scanning electron microscope image, trapping time dependent data for neuropeptide Y, constant and noise amplitude analysis, power spectral density plots, probability density function histogram, additional time to trap for carbonic anhydrase and bovine serum albumin.

References

(1) Cornish, P. V.; Ha, T. A survey of single-molecule techniques in chemical biology. *ACS Chemical Biology* **2007**, *2*, 53–61.

(2) Liang, F.; Guo, Y.; Hou, S.; Quan, Q. Photonic-plasmonic hybrid single-molecule nanosensor measures the effect of fluorescent labels on DNA-protein dynamics. *Science Advances* **2017**, *3*, e1602991.

(3) Schmid, S.; Stömmer, P.; Dietz, H.; Dekker, C. Nanopore electro-osmotic trap for the label-free study of single proteins and their conformations. *Nature Nanotechnology* **2021**, *16*, 1244–1250.

(4) Wen, C.; Bertosin, E.; Shi, X.; Dekker, C.; Schmid, S. Orientation-Locked DNA Origami for Stable Trapping of Small Proteins in the Nanopore Electro-Osmotic Trap. *Nano Letters* **2022**,

(5) Priest, L.; Peters, J. S.; Kukura, P. Scattering-based light microscopy: From metal nanoparticles to single proteins. *Chemical Reviews* **2021**, *121*, 11937–11970.

(6) Dahmardeh, M.; Dastjerdi, H. M.; Mazal, H.; Köstler, H.; Sandoghdar, V. Self-supervised machine learning pushes the sensitivity limit in label-free detection of single proteins below 10 kDa. **2022**,

(7) Wheaton, S.; Gordon, R. Molecular weight characterization of single globular proteins using optical nanotweezers. *Analyst* **2015**, *140*, 4799–4803.

(8) Ying, C.; Karakaci, E.; Bermudez-Urena, E.; Ianiro, A.; Foster, C.; Awasthi, S.; Guha, A.; Bryan, L.; List, J.; Balog, S.; Acuna, G. P.; Gordon, R.; Mayer, M. Watching single unmodified enzymes at work. *arXiv:2107.06407* **2021**,

(9) Kwak, E.-S.; Onuta, T.-D.; Amarie, D.; Potyrailo, R.; Stein, B.; Jacobson, S. C.; Schaich, W.; Dragnea, B. Optical trapping with integrated near-field apertures. *Journal of Physical Chemistry B* **2004**, *108*, 13607–13612.

(10) Pang, Y.; Gordon, R. Optical trapping of 12 nm dielectric spheres using double-nanoholes in a gold film. *Nano Lett.* **2011**, *11*, 3763–3767.

(11) Juan, M. L.; Gordon, R.; Pang, Y.; Eftekhari, F.; Quidant, R. Self-induced back-action optical trapping of dielectric nanoparticles. *Nat. Phys.* **2009**, *5*, 915–919.

(12) Saleh, A. A.; Dionne, J. A. Toward efficient optical trapping of sub-10-nm particles with coaxial plasmonic apertures. *Nano Lett.* **2012**, *12*, 5581–5586.

(13) Berthelot, J.; Aćimović, S. S.; Juan, M. L.; Kreuzer, M. P.; Renger, J.; Quidant, R. Three-dimensional manipulation with scanning near-field optical nanotweezers. *Nat. Nanotechnol.* **2014**, *9*, 295–299.

(14) Kerman, S.; Chen, C.; Li, Y.; Van Roy, W.; Lagae, L.; Van Dorpe, P. Raman fingerprinting of single dielectric nanoparticles in plasmonic nanopores. *Nanoscale* **2015**, *7*, 18612–18618.

(15) Zhao, Y.; Saleh, A. A.; Dionne, J. A. Enantioselective optical trapping of chiral nanoparticles with plasmonic tweezers. *Acs Photonics* **2016**, *3*, 304–309.

(16) Zhao, Y.; Saleh, A. A.; Van De Haar, M. A.; Baum, B.; Briggs, J. A.; Lay, A.; Reyes-Becerra, O. A.; Dionne, J. A. Nanoscopic control and quantification of enantioselective optical forces. *Nature Nanotechnology* **2017**, *12*, 1055–1059.

(17) Peri, S. S. S.; Sabnani, M. K.; Raza, M. U.; Ghaffari, S.; Gimlin, S.; Wawro, D. D.; Lee, J. S.; Kim, M. J.; Weidanz, J.; Alexandrakis, G. Detection of specific antibody-ligand interactions with a self-induced back-action actuated nanopore electrophoresis sensor. *Nanotechnology* **2019**, *31*, 085502.

(18) Jensen, R. A.; Huang, I.-C.; Chen, O.; Choy, J. T.; Bischof, T. S.; Lončar, M.; Bawendi, M. G. Optical trapping and two-photon excitation of colloidal quantum dots using bowtie apertures. *ACS Photonics* **2016**, *3*, 423–427.

(19) Raza, M. U.; Peri, S. S. S.; Ma, L.-C.; Iqbal, S. M.; Alexandrakis, G. Self-induced back action actuated nanopore electrophoresis (SANE). *Nanotechnology* **2018**, *29*, 435501.

(20) Jiang, Q.; Rogez, B.; Claude, J.-B.; Baffou, G.; Wenger, J. Temperature measurement in plasmonic nanoapertures used for optical trapping. *ACS Photonics* **2019**, *6*, 1763–1773.

(21) Verschueren, D. V.; Pud, S.; Shi, X.; De Angelis, L.; Kuipers, L.; Dekker, C. Label-free optical detection of DNA translocations through plasmonic nanopores. *ACS Nano* **2018**, *13*, 61–70.

(22) Verschueren, D.; Shi, X.; Dekker, C. Nano-Optical Tweezing of Single Proteins in Plasmonic Nanopores. *Small Methods* **2019**, *3*, 1800465.

(23) Jiang, Q.; Rogez, B.; Claude, J.-B.; Baffou, G.; Wenger, J. Quantifying the role of the surfactant and the thermophoretic force in plasmonic nano-optical trapping. *Nano Lett.* **2020**, *20*, 8811–8817.

(24) Xu, Z.; Song, W.; Crozier, K. B. Direct particle tracking observation and Brownian dynamics simulations of a single nanoparticle optically trapped by a plasmonic nanoaperture. *ACS Photonics* **2018**, *5*, 2850–2859.

(25) Li, N.; Cadusch, J.; Liu, A.; Barlow, A. J.; Roberts, A.; Crozier, K. B. Algorithm-Designed Plasmonic Nanotweezers: Quantitative Comparison by Theory, Cathodoluminescence, and Nanoparticle Trapping. *Adv. Opt. Mater.* **2021**, *9*, 2100758.

(26) Yoon, S. J.; Song, D. I.; Lee, J.; Kim, M.-K.; Lee, Y.-H.; Kim, C.-K. Hopping of single nanoparticles trapped in a plasmonic double-well potential. *Nanophotonics* **2020**, *9*, 4729–4735.

(27) Kotnala, A.; Kollipara, P. S.; Li, J.; Zheng, Y. Overcoming diffusion-limited trapping in nanoaperture tweezers using opto-thermal-induced flow. *Nano Lett.* **2019**, *20*, 768–779.

(28) Yoo, D.; Gurunatha, K. L.; Choi, H.-K.; Mohr, D. A.; Ertsgaard, C. T.; Gordon, R.; Oh, S.-H. Low-power optical trapping of nanoparticles and proteins with resonant coaxial nanoaperture using 10 nm gap. *Nano Lett.* **2018**, *18*, 3637–3642.

(29) Kotsifaki, D. G.; Truong, V. G.; Chormaic, S. N. Fano-resonant, asymmetric, metamaterial-assisted tweezers for single nanoparticle trapping. *Nano Lett.* **2020**, *20*, 3388–3395.

(30) Peri, S. S. S.; Sabnani, M. K.; Raza, M. U.; Urquhart, E. L.; Ghaffari, S.; Lee, J. S.; Kim, M. J.; Weidanz, J.; Alexandrakis, G. Quantification of low affinity binding in-

teractions between natural killer cell inhibitory receptors and targeting ligands with a self-induced back-action actuated nanopore electrophoresis (SANE) sensor. *Nanotechnology* **2020**, *32*, 045501.

(31) Hacohen, N.; Ip, C. J.; Gordon, R. Analysis of egg white protein composition with double nanohole optical tweezers. *Acs Omega* **2018**, *3*, 5266–5272.

(32) Yang, W.; van Dijk, M.; Primavera, C.; Dekker, C. FIB-milled plasmonic nanoapertures allow for long trapping times of individual proteins. *iScience* **2021**, *24*, 103237.

(33) Hong, C.; Yang, S.; Ndukaife, J. C. Stand-off trapping and manipulation of sub-10 nm objects and biomolecules using opto-thermo-electrohydrodynamic tweezers. *Nat. Nanotechnol.* **2020**, *15*, 908–913.

(34) Freedman, K. J.; Otto, L. M.; Ivanov, A. P.; Barik, A.; Oh, S.-H.; Edel, J. B. Nanopore sensing at ultra-low concentrations using single-molecule dielectrophoretic trapping. *Nature Communications* **2016**, *7*, 1–9.

(35) Ravindranath, A. L.; Shariatdoust, M. S.; Mathew, S.; Gordon, R. Colloidal lithography double-nanohole optical trapping of nanoparticles and proteins. *Opt. Express* **2019**, *27*, 16184–16194.

(36) Hajisalem, G.; Babaei, E.; Dobinson, M.; Iwamoto, S.; Sharifi, Z.; Eby, J.; Synakewicz, M.; Itzhaki, L. S.; Gordon, R. Accessible high-performance double nanohole tweezers. *Opt. Express* **2022**, *30*, 3760–3769.

(37) Jackson, J. D. Classical Electrodynamics. 1999.

(38) Pang, Y.; Gordon, R. Optical trapping of a single protein. *Nano Lett.* **2012**, *12*, 402–406.

(39) Otzen, D. Protein–surfactant interactions: a tale of many states. *Biochim. Biophys. Acta, Proteins Proteomics* **2011**, *1814*, 562–591.

(40) Hayes, M. A. Dielectrophoresis of proteins: Experimental data and evolving theory. *Analytical and Bioanalytical Chemistry* **2020**, *412*, 3801–3811.

(41) Bader, R.; Bettio, A.; Beck-Sickinger, A. G.; Zerbe, O. Structure and dynamics of micelle-bound neuropeptide Y: comparison with unligated NPY and implications for receptor selection. *Journal of Molecular Biology* **2001**, *305*, 307–329.