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Manipulation and capture of A β amyloid fibrils and monomers by DC insulator gradient dielectrophoresis (DC-iGDEP)[†]

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Here we report a novel method for the manipulation and concentration of A β amyloid fibrils, implicated in Alzheimer's disease, using DC insulating gradient dielectrophoresis (DC-iGDEP). Fibril enrichment was found to be ~400%. Simulations suggest that capture of the full range of amyloid protein aggregates is possible with optimized device design.

Protein aggregates have been associated with more than 25 human diseases, including Alzheimer's disease, Parkinson's disease and type 2 diabetes.¹ As a result, researchers in medical, biochemical and analytical chemistry fields are striving to understand the mechanisms of formation of amyloid aggregates and to identify toxic species. Protofibrils, transient metastable oligomeric aggregates that develop prior to the formation of amyloid fibrils, have been implicated in disease pathogenesis and are considered to be cytotoxic. Amyloid aggregation has been shown to form a heterogeneous mixture of oligomeric species *in vitro*, but the specific structure or structures of the cytotoxic species, their relationship to amyloid fibril formation and the mechanism of toxicity are unknown. Techniques capable of manipulating and concentrating various aggregate structures are of paramount importance^{2–7} because specific knowledge of all constituent aggregate species is needed to effectively develop therapeutics based on disrupting or altering the aggregation process. Each of the different aggregate species from monomer to mature fibrils can have unique chemical and physical properties providing a basis for differential toxicity.

Traditional means of isolating various oligomers and fibrils of A β amyloid and other misfolded proteins have relied heavily on techniques such as sedimentation, size-exclusion chromatography (SEC) and capillary electrophoresis (CE).^{8–17} Sedimentation typically uses large volume preparations and cannot effectively separate

oligomers into subpopulations, given their structural similarities and the typical small volumes of aggregate samples. While CE uses small volume samples and provides greater resolution compared to other separation techniques, target populations generally are diluted rather than concentrated during separation. Similarly, SEC has proven effective for isolating relatively stable protofibril species, but as with CE, samples are generally diluted during separation, and it has been shown to disrupt some protein aggregates.¹⁸ Several other methods used to isolate and characterize A β aggregates include HPLC, gel electrophoresis, and transmission electron microscopy (TEM); however, these methods do not provide a comprehensive account of the coexisting oligomeric populations during amyloid fibril formation.¹⁷ TEM measurements are made after extensive sample preparation and generally only capture stable end-point species. Considering amyloid fibril formation as a series of reversible reactions suggests that all separation schemes can alter the populations of aggregate species present before separation. For instance, protofibrils can convert back to monomers. To provide the most realistic view of the population of aggregates in a sample, a separation-based technique should be as rapid and gentle as possible.

The technique described here, direct current based insulator gradient dielectrophoresis (DC-iGDEP), rapidly resolves particulates by balancing electrophoresis and dielectrophoresis within a single separation channel.¹⁹ Relative to a capillary electrophoretic separation, the addition of dielectrophoretic forces increases the analyte-specific separation vectors to include the permeability and conductivity of the particle as well as the same parameters for the surrounding medium, which can be tuned. Combining these forces in opposition translates into discrete collection points that also concentrate the target species. This achieves both the separation and concentration of A β amyloid structures in a short time frame for direct quantification or use in downstream research on the properties of those structures. The separation is not based on the interaction of the aggregates with a stationary phase, reducing the impact of the separation process on the aggregate structures.

Previously, it has been demonstrated that DC-iGDEP can separate and concentrate spherical synthetic polymer nano- and microparticles from 20 nm to 1 μm .^{19,20} It was not known if DC-iGDEP could be used successfully to manipulate species with the size, shape and chemical properties of amyloid aggregates. Here we present initial studies demonstrating that DC-iGDEP is able to rapidly and selectively concentrate A β amyloid aggregates, and has the potential to

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[†] Electronic supplementary information (ESI) available: Device fabrication, fibril and monomer preparation, video of fibril capture, and COMSOL modeling. See DOI: 10.1039/c2an35138b

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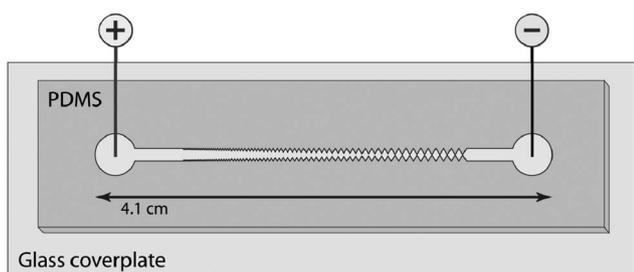


Fig. 1 Diagram of the direct current insulator gradient dielectrophoresis (DC-iGDEP) device. An insulated sawtooth pattern is integrated with a tapered microfluidic channel to generate localized non-uniform electric field gradients of increasing strength from left to right.

analyze populations of smaller protofibrillar aggregates with further tailoring of the channel design.

The design and fabrication of the DC-iGDEP device is detailed in Staton *et al.*¹⁹ Briefly, the DC-iGDEP microfluidic channel was fabricated in polydimethylsiloxane (PDMS) with a glass cover slide (Fig. 1), and 20 μL of A β sample was introduced into the reservoir at the broader end of the channel. The A β monomer and fibrils in PBS were prepared as described in detail previously.^{12,16,17,21} Additional information about the fabrication of the DC-iGDEP and A β sample preparation can be found in the ESI†. The monomer and fibril samples were tested separately by DC-iGDEP. The results reported here were consistent and representative of multiple experiments using three independent preparations of the monomer and fibrils and multiple devices. Experiments were performed by applying voltages between 400 and 1000 V (over the entire channel length) for 1 to 15 min for each experiment.

The DC-iGDEP experiments were monitored by fluorescence microscopy. When introduced into the DC-iGDEP channel, the monomer consistently created streaming patterns at all of the applied voltages (400 to 1000 V, Fig. 2), which were more apparent at higher voltages. The streamlines appearing along the centerline of the channel indicate significant influence of electrophoretic forces; however, none of the conditions tested resulted in the monomers being captured.

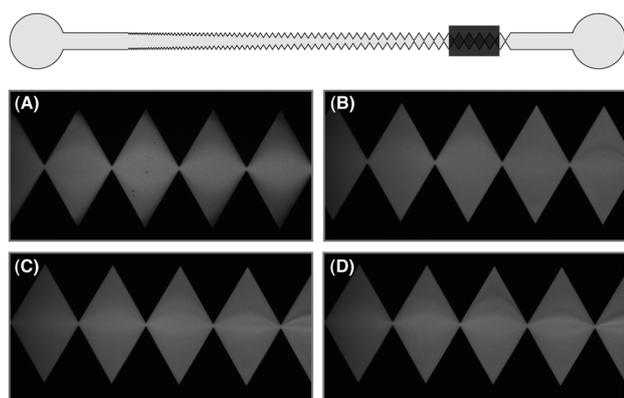


Fig. 2 Fluorescence images of the narrowest portion of the DC-iGDEP for A β monomer samples (30 μM) with (A) 400 V, (B) 600 V, (C) 800 V, and (D) 1000 V applied. The diffuse light areas show the fluorescently tagged A β monomer distributed throughout the channel. Streaming of monomer is more visually apparent in (C) and (D).

In contrast, when the A β amyloid fibril samples were tested using the DC-iGDEP channel under similar conditions, separation and concentration of the fibrils were observed (Fig. 3). A TEM of the fibril sample used in this experiment is presented in Fig. 4 and clearly shows the presence of mature amyloid fibrils. Thioflavin T (10 μM) fluorescence measurements also indicated that aggregation was complete after 7 days. Images of experiments below 400 V applied over the length of the channel (not shown) indicated movement of fibrils without capture. At 400–1000 V applied, the A β fibrils were captured and concentrated at the narrow points in the channel with similar local fluorescence intensity. Previous studies with synthetic nanospheres have demonstrated that particle capture typically does not obstruct the channels, and nanoparticles below the capture threshold size will pass through an intersection containing larger particles that have been concentrated and captured.¹⁹

In order to differentiate between nonspecific and DC-iGDEP-controlled capture, the applied voltage was removed after the capture and concentration of A β fibrils, and bright areas were monitored for dispersion away from the capture area. The bright fluorescence regions shown in Fig. 3 rapidly dissipated due to diffusion and slight convection of the A β fibrils away from the capture regions. Following

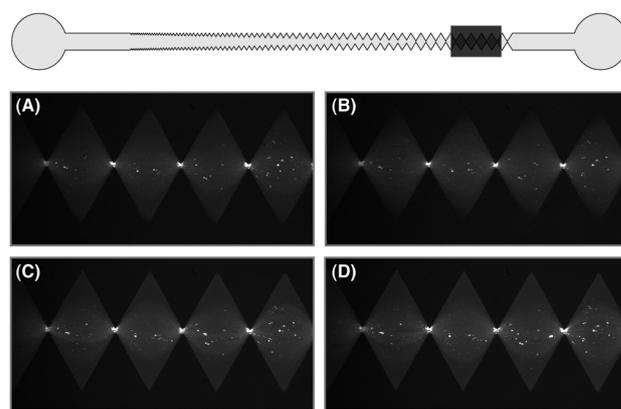


Fig. 3 Fluorescence images of the narrowest portion of the DC-iGDEP (same as in Fig. 2) showing capture of A β fibrils (30 μM) at (A) 400 V, (B) 600 V, (C) 800 V, and (D) 1000 V applied. The areas of high fluorescence localized between the tips of the sawtooth patterned insulator indicate small zones where the fibrils were captured and concentrated.

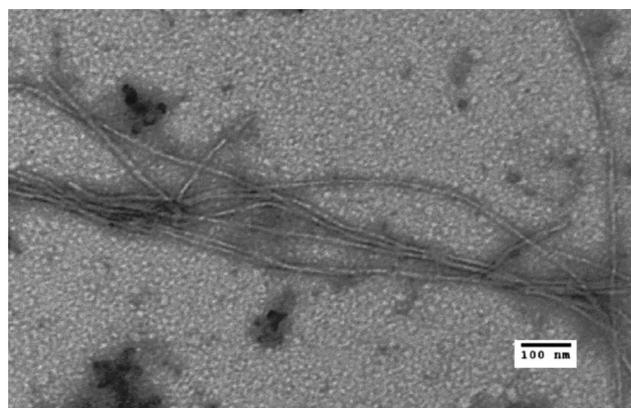


Fig. 4 TEM of the mature A β fibrils used in Fig. 3. The scale bar is 100 nm, and the fibril sample was in PBS before buffer exchange to Tris.

a short period of time, the voltage was reapplied to allow selective capture to occur again. This process was repeated several times to verify that the capture was the result of the DC-iGDEP as well as to demonstrate the ability of the technique to reproducibly manipulate the amyloid aggregates within a given experimental session. A video of this behavior is also included in the ESI†. The fibrils controllably collected near tips of the sawtooth-patterned insulating channel. The position of capture indicates that, under these experimental conditions, the fibrils exhibited properties consistent with positive dielectrophoresis, meaning that the collection points were where the electric field intensity is greatest.^{19,22,23} According to classic theory, particles that undergo positive dielectrophoretic capture are less permeable than the surrounding medium. Some evidence of nonspecific adsorption of the amyloid aggregates was observed along the channel surface. Without surface treatments or coatings nonspecific protein adsorption is common for PDMS channels.

The ability of the DC-iGDEP method to concentrate fibrils was examined semi-quantitatively by noting the fluorescence intensity at the collection points compared to background levels in the channel prior to collection and areas where no detectable capture had occurred. The resulting enrichment of the fibril material ranged from about 350% to over 500% depending on the applied voltage (see ESI†). However, under more ideal conditions with lower fibril loads, the potential capture efficiency could be as high as 600%, simply by reducing the amount of background fluorescence. The maximum observed enrichment was 520% at 600 V.

The overall goal of this work is to separate, capture and concentrate the full range of aggregate structures generated during mature amyloid fibril formation. The experimental results presented here demonstrate that mature A β fibrils, but not A β monomer, are captured and concentrated using the current device design and the described experimental conditions. COMSOL (finite element multiphysics modeling software) calculations allow for directed device design development by exploring alternative device design parameters not yet tested experimentally. Modeling with COSMOL confirmed that for the current DC-iGDEP device design, A β monomer should not be captured for any reasonable experimental conditions. Details of the models used to evaluate the particle capture potential of the device can be found in the ESI†. Modeling also predicts that by reducing the smallest sawtooth gap distance from 27 μ m in the current design to \sim 10 nm, the increased field gradient strength required to allow monomer capture along with all intermediate species could be generated. By modeling the amyloid aggregates, oligomers, and monomers based on their unique electrophoretic and dielectrophoretic properties, the speed of the device design evolution can be accelerated while also being tailored for capturing particular bioanalytes and positioning them along the length of the channel.

In conclusion, this study demonstrates that DC-iGDEP can be used to manipulate and selectively capture A β amyloid fibrils, while influencing but not capturing A β monomer. DC-iGDEP successfully combines high enrichment of fibrils (up to 520%) with short analysis times (1–15 min) on a cost effective platform. Production and operation of the DC-iGDEP microdevices using PDMS are very simple. This initial study and related simulations indicate that this technique

has the potential to rapidly isolate and concentrate various A β aggregate structures intermediate between monomer and mature fibrils. Development of new rapid and gentle separation techniques for isolating and characterizing amyloid aggregates is essential for understanding the role of protein aggregation in amyloid-linked diseases.

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