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PROVISIONAL PATENT APPLICATION

METHODS AND SYSTEMS FOR CONTROLLING THE

SHEAR MODULUS OF GENOMIC LENGTH DSDNA MOLECULES

BACKGROUND

[0001] The relative statistical length scale of double-stranded DNA molecules (dsDNA, the terms “DNA” and “dsDNA” are used interchangeably throughout the present disclosure) is set by the number of statistical segments \( N = L/\kappa \) in the polymer, where \( L \) is the total length of the polymer and \( \kappa \) is the Kuhn length, described below. If \( N >> 1 \), the dsDNA is said to be very long.

[0002] There are generally two ways to sort extremely long dsDNA as a function of \( L \): gel electrophoresis at very low fields (and corresponding multi-day run times), to avoid elongation of the spherical random-coil polymer [6], or by full elongation in crossed fields [1, 9] or by stretching in microfabricated structures [22]. While stretching of a dsDNA polymer in post arrays or in microstructures or nanochannels is attractive and popular [12], it lacks the scalable high throughput that continuous flow particle separation technologies can provide [18, 21], which are necessary for many preparative applications [3].

[0003] Techniques which do not stretch dsDNA rely on a conformation of the genomic dsDNA which is as close to spherical form as possible. Indeed, the first attempt to sort dsDNA in a microfabricated device [23] failed precisely because the dsDNA was so easily elongated in shear fields.

SUMMARY OF SOME OF THE EMBODIMENTS

[0004] To that end, in embodiments of the present disclosure, methods and systems are presented to control the conformation of DNA to a non-elongated configuration so that it can be easily handled, e.g., sorted, concentrated, and the like (using, for example, a deterministic
lateral displacement array for concentrating DNA).

[0005] Accordingly, in some embodiments, a method for manipulating DNA molecules for use in a microfluidic device are provided. In such embodiments, the method comprises providing a solution of a plurality of DNA molecules having an approximately spherical shape and a first radius of gyration under under a zero flow velocity, and maintaining the DNA molecules in an approximately spherical shape under a useful range of flow velocity in a microstructured environment. For some embodiments, a useful range of flow velocity is from about 1 micron/second to about 1 millimeter/second.

[0006] In some embodiments (similar to that noted above), a method for manipulating DNA molecules in a microfluidic device is provided, where the method comprises providing a solution of a plurality of DNA molecules having a first radius of gyration under under a zero flow velocity, and maintaining and/or decreasing the first radius of gyration of the DNA molecules under a useful range of flow velocity.

[0007] In some embodiments, a method for manipulating DNA molecules in a microfluidic device is provided, where the method comprises providing a plurality of DNA molecules in a solution comprising agents that increase the shear modulus of the DNA molecules. These embodiments are configured such that the DNA molecules are able to maintain an approximately spherical conformation under a useful range of flow velocity..

[0008] In some embodiments, maintaining an approximately spherical shape of the DNA molecules, or maintaining and/or decreasing the first radius of gyration of the DNA molecules, or increasing the shear modulus of the DNA molecules comprises adding an amount of DNA condensation agent to the solution. The DNA condensation agent may be selected from the group consisting of: polyethylene glycol polymers (PEG), polyvinylpyrrolidone, spermine, spermidine, cobalt hexamine, and cetyltrimethylammonium bromide (CTAB).

[0009] In some embodiments, the amount of DNA condensation agent is between:

- about 1% to about 40% wt/vol.;
- about 1% to about 20% wt/vol.;
- about 5% to about 20% wt/vol.; or
- about 10% to about 20% wt/vol.

[0010] In some embodiments, one and/or another of the disclosed methods include flowing the solution (i.e., DNA combined with the the DNA condensation agent) through a microfluidic
device, where the microfluidic device comprises at least one of: a deterministic lateral displacement (DLD) array, a Brownian ratchet array, a pinched flow fractionation device, a hydrodynamic filtration device, and an anisotropic nanofilter array. In some embodiments, the microfluidic device manipulates the DNA molecules, where manipulation may comprise at least one of: fractionation by size, purification, chemical modification, and enzymatic modification.

[0011] In some embodiments, a system for concentrating DNA in a microfluidic environment is provided, and may comprise a deterministic lateral displacement (DLD) array as described in references [5, 10, 11, 14, 15, 16], hereby incorporated by reference. The DLD array may be configured to cause concentration of DNA molecules having a radius of gyration greater than a first (critical) radius from a solution of DNA molecules comprising an amount of DNA condensation agent. The system may also include a source of hydrodynamic pressure configured to cause the solution to flow in the microfluidic channels at a fluid flow velocity. The fluid flow velocity and amount of DNA condensation agent are configured so as to cause the DNA molecules to remain in an approximately spherical conformation.

[0012] In some embodiments, a system for concentrating DNA in a microfluidic environment is provided, and may comprise a deterministic lateral displacement (DLD) array. The DLD array may be configured to cause concentration of DNA molecules having a radius of gyration greater than a first (critical) radius from a solution of DNA molecules comprising an amount of DNA condensation agent. The embodiment may use electric fields to move DNA molecules through the DLD arrays at an electrophoretic mobility. Electrophoretic fields may be continuous [26], or alternating in direction and duration as in pulsed field electrophoresis [9]. The electrophoretic mobility and amount of DNA condensation agent are configured so as to cause the DNA molecules to remain in an approximately spherical conformation.

[0013] In some embodiments, a system for concentrating DNA in a microfluidic environment is provided, and may comprise a deterministic lateral displacement (DLD) array having a critical diameter for bumping within the range of about 0.2 to about 3 microns (for example).

[0014] In some embodiments, the fluid flow velocity ranges from about 1 \( \mu \text{m/s} \) to about 1000 \( \mu \text{m/s} \).

[0015] As noted above, in some embodiments, a DNA condensation agent is added to a solution of DNA molecules, which may be (e.g.) polyethylene glycol polymers (PEG). The presence of PEG molecules reduce the intrinsic radius of gyration of the DNA molecules
resulting in segments of DNA overlapping at an intersection to reduce the total volume of depletion zones (an excluded volume between DNA and PEG), which the PEG molecule cannot occupy due to the exclusion interaction between DNA and PEG molecule. This results in an increase of the shear modulus of DNA in microfluidic structures (e.g., in some embodiments, the increase may be up to about $10^4$).

[0016] Using such methodology to control DNA conformation, DNA can then be flowed through a microfluidic device for certain purposes (e.g., sorting, concentrating). For example, the microfluidic device may comprise a deterministic lateral displacement (DLD) array, which enables the DNA to be concentrated and isolated.

**DETAILED DESCRIPTION OF THE INVENTION**

[0017] Polymer dynamics studies begin by assuming a simple random walk in 3-dimensional space with Kuhn step size $\kappa$ equal to twice the persistence length $L_p$, and also include with respect to embodiments of the present disclosure (e.g., for statistical analysis) two physical constraints: (1) the (sequence dependent [8]) molecular Young’s modulus $Y_i$ contribution to the persistence length; and (2) the influence of self-avoidance which must be included in the statistical analysis. These physical constraints make the problem of truly understanding the dynamics of the polymer under shear a formidable problem.

[0018] Accordingly, the connection between the $L_p$ and the intrinsic (molecular) Young’s modulus $Y_i$ is given by:

$$L_p = \frac{Y_i L}{k_BT}$$

where $L$ is the surface moment of inertia of the polymer. That is, the intrinsic bending rigidity $Y_i$ of the polymer is folded into the thermodynamic parameter $\kappa = 2L_p$ which is not changed in the process of dsDNA compaction to first order in the distortion of the dsDNA molecular structure for the deformations discussed here [24].

[0019] Self-avoidance appears as a modification of the simple expected dependence $L^{1/2}$ of the radius of gyration $R_g$ of the dsDNA, where $L$ is the total length of the polymer. Self-avoidance can be viewed in a mean-field approach as due to a repulsive interaction between segments of length $\kappa$ caused by the interaction of irreducible excluded volume $v_{ex}$ of each segment. In a mean field approximation [20] the local density of links $\rho$ in a space of
dimension $d$ for a polymer of radius of gyration $R_g$ is:

$$\rho \approx \frac{N}{R_g^d} \quad (2)$$

[0020] Since the excluded volume interaction energy $E_{ex} \approx k_BT v_{ex} N \rho$ and the entropic energy due to expansion $E_{en} \approx k_BT R_g^2 / N \kappa^2$, the total free energy $G_f = E_{ex} + E_{en}$ is:

$$G_f \approx k_BT (v_{ex} \frac{N^2}{R_g^d} + \frac{R_g^2}{N \kappa^2}) \quad (3)$$

Minimization of Eq. 3 with respect to $R_g$ then yields that within the confines of Flory theory for self-avoidance in $d=3$ dimensions the radius of gyration ($R_g$) is:

$$R_g \approx \frac{1}{v_{ex} N \kappa^2} \quad (4)$$

[0021] As discussed below, a 166 kbp T4 dsDNA molecule of length $L \approx 56 \mu m$ includes a persistence length $L_p$ of about 50 nm [24], and the measured average radius of gyration $R_g$ (also discussed below) is about 1.4 $\mu m$. From Eq. 4, this yields $v_{ex} \approx 3.1 \times 10^3$ nm$^3$.

[0022] Depletion forces occur when the DNA solution also contains solutes that are much larger than the solvent molecules. To that end, in some embodiments of the present disclosure, a small flexible polymer (in some embodiments, PEG) is added to a solution containing dsDNA in order to reduce the radius of gyration of the DNA molecule. The radius of gyration is a measure for the characterization of the time-averaged configuration of a polymer, which measures the root-mean-square distance of the collection of segments from their common center of mass. In such embodiments, the volume around each DNA molecule includes additional regions of excluded volume where the DNA molecule and PEG molecules are in close proximity, which may be referred to as a depletion zones. Other PEG molecules cannot occupy these zones due to the exclusion interaction between DNA and the closely apposed PEG molecules. The overlap of two segments of DNA (at an intersection) reduces the total volume of the depletion zones around the DNA molecule, thereby increasing the volume accessible to PEG molecule (see Fig. 1). This results in a network of smaller dsDNA chains, and also increases the entropy of the entire DNA + PEG system. One of the unexpected results of some of the embodiments of the present disclosure is that the reduction of the intrinsic radius of gyration of the DNA molecule caused by DNA condensation agents is accompanied by enhanced resistance of the DNA molecule to shear deformation in flow. This enhanced resistance makes it possible for the DNA molecules to maintain a spherical conformation,
which in turn allows manipulation of DNA molecules in a variety of rapid, scalable, continuous flow separation methods, which cannot be used in the absence of condensation agents, as outlined below.

[0023] Although the “radius of gyration” is a convenient term when defining the principles of some of the embodiments of the present disclosure, the “effective” size that the DNA exhibits in microfluidic devices is only approximated by the calculated radius of gyration. In some embodiments, the exact relationship between the size of the DNA and radius of gyration is estimated using the approximation that the DNA molecules are hard spherical particles. Nevertheless, as shown below, under some conditions the two values appear to scale together, and therefore, the concept of radius of gyration is useful for describing the principles of the invention.

[0024] Fig. 1 illustrates an example of depletion force induced by PEG molecule crowding.

[0025] There is a minimum radius of gyration \( R_g \) which can be reached by depletion forces, since the persistence length \( L_p \) is not changed by the compaction process caused by the depletion forces. The total excluded \( V_{ex} \) volume is then:

\[
V_{ex} = N \times v_{ex} \tag{5}
\]

It can be assumed if this volume represents the smallest possible volume that the polymer can have at temperature \( T \) even at very high concentrations of PEG, then a minimum radius of gyration \( R_{g,m} \) is:

\[
R_{g,m} \approx \left( \frac{3V_{ex}}{4\pi} \right)^{1/3} \tag{6}
\]

[0026] From the value for \( v_{ex} \) from Eq. 4, the minimum radius of gyration \( R_{g,m} \) can be computed from Eq. 6. For the T4 dsDNA molecule, the expected \( R_{g,m} \approx 75 \) nm, a factor of approximately 20 from the aqueous value and about twice the radius of the T4 capsid head [17].

[0027] Considering the entropic elasticity of polymer chains, the collective shear modulus \( G_c \) of the dsDNA network is roughly estimated as [20]:

\[
G_c \approx \frac{n^* k_B T}{\frac{1}{2} R_g^3} \tag{7}
\]

where \( n^* \) (in the range of 1 to \( N \)) is the number of effective strands. As the PEG volume fraction increases, more overlap regions are generated (as well as the DNA strands in between these regions), which leads to an increase of \( n^* \).
[0028] At zero PEG concentration, no DNA network is formed, $n^* = 1$, which yields $G_{c,o} \approx 3.6 \times 10^{-4}$ Pa (confirmed for small displacement using optical tweezer [24]). In the case of maximum compaction, $n^* = N$, $G_{c,max} \approx k_BT/v_{ex}$ is independent of $L$ since it essentially represents an incompressible core of a compacted polymer, due to the physical constraints of self-avoidance and the elastic energy stored in the persistence length. For the T4 dsDNA molecules discussed below, the maximum value of the shear modulus $G_c$ may be about $1.3 \times 10^3$ Pa.

[0029] Some embodiments of the present disclosure which use depletion force to hold dsDNA molecules in a relatively non-deformable, spherical conformation enable the use of, for example, rapid, scalable continuous flow methods for DNA manipulation [18, 21]. According to some embodiments, a DNA concentrator is provided which utilizes one or more DLD arrays to concentrate genomic length dsDNA molecules (established according to some embodiments).

[0030] In such DLD arrays, particles smaller than a critical size $D_c$ follow a laminar flow direction, weaving through array in a “zigzag” trajectory, while particles larger than the critical size are displaced laterally in the array (i.e., by posts at each column), following a migration angle $\varepsilon$ in a bumping trajectory [5, 10, 11, 16, 14, 15]. Fig. 1(a) shows the schematic of a DLD apparatus according to some embodiments of the disclosure. In such DLD arrays, 6.3 $\mu$m diameter posts are arranged on a 8 $\mu$m grid, with the angle between the (two) grid axes at about $\varepsilon = 3.8^\circ$, giving a critical size $D_c$ of about 0.7 $\mu$m. To that end, for example, using such a DLD array according to some embodiments, a 166 kbp T4 dsDNA at 10% PEG volume fraction entering an input region of the array can be concentrated and collected along the center wall at a peak flow velocity $v_{f,max}$ in the middle of a gap of 30 $\mu$m/s (Fig. 1(b) and (c)). Accordingly, such embodiments (using the dsDNA spherical conformation produced according to some embodiments), yield increased separation rates and resolution, compared with the performance achievable with gel electrophoresis methods.

[0031] The spherical DNA conformation (produced according to some embodiments) are not deformed upon meeting the posts, instead, the polymer is pushed into adjacent stream lines. Otherwise, if the dsDNA is elongated, it follows the laminar flow direction in a zigzag trajectory (since its short axis length is below the critical size). Deformation of the polymer from the desired spherical shape may occur when the shear strain $\gamma \approx 1$. Thus, in useful embodiments, the shear stress $\tau$ should not exceed $G_c$ in the device. Since the hydrodynamic
shear stress $\tau = \mu \frac{d v_f}{d z}$ (where $\mu$ is the viscosity of solvent which depends on the PEG volume fraction [7]), the transverse velocity gradients $\frac{d v_f}{d z}$ that can be tolerated:

$$\frac{d v_f}{d z}_{\text{max}} \approx \frac{c_v}{\mu}$$

[0032] Fig. 2 illustrates the following:

- (a) The DNA concentrator schematic using a DLD array;

- (b) Micrograph composite of purified 166 kbp T4 dsDNA in a peak velocity 30 $\mu$m/s flow at 10% PEG volume fraction traveling through the microfluidic arrays;

- (c) The fluorescence intensity profile at the outputs indicates a good isolation of DNA molecules. The DNA was concentrated from a stream width of ~600 micrometers near the device input (left side of Fig. 2) to less than 50 micrometers near the output.

[0033] EXAMPLE: DNA sample preparation (according to some embodiments). Phage T4 dsDNA (strain GT7, Wako) was equilibrated with YOYO1 (Life Technologies) at 50 °C for 1 hour under the following conditions: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5 M NaCl, 1 ng/L T4 dsDNA, 0.15 M YOYO1, 10 mM DTT (the ratio of YOYO1/DNA bp = 1/10). Following equilibration, the YOYO1-labeled DNA was cooled to room temperature and diluted 100-fold with a buffer containing 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 10 mM DTT, and from 0 to 20% (w/v) PEG (Dow Carbowax Polyethylene Glycol 8000, average molecular weight 6000-9000) as indicated. The DNA solutions were stored at 4 °C in opaque plastic bottles until used. All solutions (except for those containing DNA) were filtered through 0.45 $\mu$m filters.

[0034] Figs. 3(a)-(d): Purified 166 kbp T4 dsDNA in microfluidic array at: (a) zero flow velocity, where the DNA coils up into a sphere, and , (b) 20 $\mu$m/s flow where the DNA is elongated and stretched under the shear flow, (c) 5% PEG volume fraction, where DNA is less stretched in PEG solution under the shear flow, and (d) 10% PEG volume fraction, where DNA maintains the spherical conformation and behaves like a solid particle. Accordingly, Fig. 3 shows fluorescent micrographs of purified 166 kbp T4 dsDNA under different conditions in
the DLD arrays according to some embodiments of the present disclosure. Accordingly, at zero fluid velocity, DNA includes a spherical conformation, with an observed radius of 1.4 \( \mu \text{m} \) (Fig. 3(a)). Upon a fluid flow is inputted in the structure, with an average flow in a direction 3.8° from an array axis (De for the DLD array was approximately 0.7 \( \mu \text{m} \), a velocity shear profile is then introduced as the fluid flows through the gaps (because of a boundary condition of zero velocity at the post walls). For a peak velocity \( v_{f,\text{max}} \) of 20 \( \mu \text{m/s} \), DNA is observed with an elongated conformation, with a length as long as 20 \( \mu \text{m} \). However, in accordance with the solution of dsDNA and PEG according to some embodiments of the present disclosure, under fluid flow, the length of dsDNA is shorter, as shown in Fig. 3(c) under a 5% PEG volume fraction, and has about a zero elongation as shown in Fig. 3(d) at 10% PEG volume fraction (both at a peak velocity of about 20 \( \mu \text{m/s} \)). This is due to the increase of collective shear modulus \( G_c \).

Further, the inventors of the embodiments of the present disclosure have checked the relationship between flow rate, PEG concentration, shear strain, and DNA conformation by analysis of video micrographs of T4 DNA molecules traversing DLD arrays, and classifying the conformation of the DNA molecules according to the following convention: if the length is larger than about four times the radius of gyration \( R_g \) (\( \approx 6 \mu \text{m} \), the shear strain \( \gamma >1 \)), DNA molecules are classified as elongated (open circles Fig. 4), otherwise they are classified as spherical (filled circles in Fig. 4). The results are shown in Fig. 4. As shown in figure, the induced depletion force has an effect on the spherical-elongated phase transition. To that end, assuming a parabolic flow profile in the gap [11], the maximum transverse velocity gradients \( \frac{dv_y}{dz} \) in the microfluidic arrays can be written as:

\[
\frac{dv_y}{dz_{\text{max}}} = \frac{4v_{f,\text{max}}}{d} \tag{9}
\]

where \( d = 1.7 \mu \text{m} \) is the minimum gap of this microfluidic structure. From Eq. 8, the collective shear modulus can be obtained and plotted in Fig. 4 with a black dash line. The shear modulus is increased by a factor of about 10^4 by introducing the depletion forces to the environment.

Using the data in Fig. 4, which illustrates a collection of data marking the “elongated” and “spherical” 166 kbp T4 dsDNA as a function of PEG volume fraction and flow velocity, Elongated DNA is marked with open circles while the spherical DNA is marked with filled circles. The black dash line is the collective shear modulus from Eq. 8., DNA’s behavior in DLD arrays can be predicted (i.e., the black dashed line). Below this line is the region of
conditions for DNA flows in bumping trajectory, while out of this region (i.e., above this line) DNA will follow a zig-zag trajectory. A collection of data marking bumping and zig-zag 166 kbp T4 dsDNA as a function of PEG volume fraction and flow velocity is also shown in Fig. 5. Accordingly, as the PEG volume fraction increases above 15%, DNA will undergo a coil-globule transition [19], and $R_g$ will fall below the critical size $D_c$, leading to a sharp drop of the prediction at high PEG volume fraction regime. This results in DNA being too compacted to be displaced in the single DLD array used for the experiments shown in Fig. 5 (although it still has a spherical conformation). In some embodiments, a DLD array having a smaller critical size for bumping is provided, and thus, a DNA with a 20% PEG may also be bumped. Also note that at low PEG volume fractions, DNA can be collected at a higher velocity flow than predicted. This may be because the short axis length of the DNA coil is still above the DLD array critical size even if DNA has a partially elongated conformation.

[0037] It should be pointed out that, in some embodiments, the effective size of the DNA for determining its behavior in the DLD array (that is, whether it bumps or not) may scale as the radius of gyration. Accordingly, for some embodiments, an important principle is that in the presence of condensing agents such as PEG, the effective size of the DNA as it moves through the DLD array doesn’t decrease dramatically when the microfluidic flow velocity increases.

[0038] Thus, according to some embodiments of the present disclosure, dsDNA conformation can be controlled by molecular crowding induced depletion force via adjusting the shear modulus of DNA in microfluidic structures through the addition of PEG in determined amounts. This results in a simplified model and an increase in shear modulus of about $10^4$. In some embodiments, by controlling DNA conformation, DNA can be concentrated and isolated using DLD arrays in hydrodynamic fluid flow with higher separation rate and resolution than in current preparative electrophoresis methods.

[0039] Also, various inventive concepts may be embodied as one or more methods and systems of which examples have been provided herein. The acts performed as part of the method may be ordered in any suitable way. Accordingly, embodiments may be constructed in which acts are performed in an order different than illustrated, which may include performing some acts simultaneously, even though shown as sequential acts in illustrative embodiments.

[0040] Any and all references to publications or other documents, including but not limited to, patents, patent applications, articles, webpages, books, etc., presented anywhere in the present application, are herein incorporated by reference in their entirety.
[0041] Although a few variations have been described in detail above, other modifications are possible. For example, any logic flows depicted in the accompanying figures and/or described herein do not require the particular order shown, or sequential order, to achieve desirable results. Other implementations may be within the scope of at least some of the following exemplary claims.

[0042] As noted elsewhere, these embodiments have been described for illustrative purposes only and are not limiting. Other embodiments are possible and are covered by the disclosure, which will be apparent from the teachings contained herein. Thus, the breadth and scope of the disclosure should not be limited by any of the above-described embodiments but should be defined only in accordance with claims supported by the present disclosure and their equivalents. Moreover, embodiments of the subject disclosure may include methods, systems and apparatuses/devices which may further include any and all elements from any other disclosed methods, systems, and devices, including any and all elements corresponding to binding event determinative systems, devices and methods. In other words, elements from one or another disclosed embodiments may be interchangeable with elements from other disclosed embodiments. In addition, one or more features/elements of disclosed embodiments may be removed and still result in patentable subject matter (and thus, resulting in yet more embodiments of the subject disclosure). Also, some embodiments correspond to systems, devices and methods which specifically lack one and/or another element, structure, and/or steps (as applicable), as compared to teachings of the prior art, and therefore represent patentable subject matter and are distinguishable therefrom (i.e. claims directed to such embodiments may contain negative limitations to note the lack of one or more features prior art teachings).

[0043] All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

References


Exemplary Claims:

1. A method for manipulating DNA molecules for use in a microfluidic device, the method comprising:
   providing a solution of a plurality of DNA molecules having a set of first values for their radii of gyration, said first radii values varying in proportion to the molecular weight of each DNA molecule, under under a zero transport velocity; and
   substantially maintaining the DNA molecules in a spherical shape under a transport velocity.

2. A method for manipulating DNA molecules in a microfluidic device, the method comprising:
   providing a solution of a plurality of DNA molecules having a set of first values for their radii of gyration, said first radii values varying in proportion to the molecular weight of each DNA molecule, under under a zero transport velocity; and
   providing conditions wherein the DNA molecules:
   substantially maintain their first radii values under a transport velocity, and/or
   decrease their radii of gyration to a set of second values for their radii of gyration, said second radii of gyration varying in proportion to the molecular weight of each DNA under a transport velocity.

3. The method of claims 1 or 2, wherein maintaining the spherical shape of the DNA molecules, or maintaining and/or decreasing the set of first values for their radii of gyration of the DNA molecules comprises adding an amount of DNA condensation agent to the solution.

4. The method of claims 1 through 3, wherein the DNA molecules are transported through the microfluidic device by flow.
5. The method of claims 1 through 3 wherein the DNA molecules are transported through the microfluidic device by electric fields.

6. The method of claims 1 through 3 wherein the DNA molecules are transported through the microfluidic device by a combination of flow and electric fields.

7. The method of claim 3, wherein the DNA condensation agent is selected from the group consisting of: polyethylene glycol polymers (PEG), Polyvinylpyrrolidone, Spermine, Spermidine, Cobalt hexamine, and Cetyltrimethylammonium bromide (CTAB).

8. The method of claim 3, wherein the amount of DNA condensation agent is between about 1% to about 40% wt/vol.

9. The method of claim 3, wherein the amount of DNA condensation agent is between about 1% to about 20% wt/vol.

10. The method of claim 3, wherein the amount of DNA condensation agent is between about 5% to about 20% wt/vol.

11. The method of claim 3, wherein the amount of DNA condensation agent is between about 10% to about 20% wt/vol.

12. The method of claims 1 or 2, further comprising flowing the solution through a microfluidic device.

13. The method of claim 12, wherein the microfluidic device is selected from the group consisting of: a deterministic lateral displacement (DLD) array, a Brownian ratchet array, a pinched flow fractionation device, a hydrodynamic filtration device, and a
anisotropic nanofilter array.

14. The method of claim 12, wherein the microfluidic device manipulates the DNA molecules, wherein manipulation comprises at least one of: fractionation by size, purification, and chemical modification.

15. The method of claim 14, wherein chemical modification comprises enzymatic modification.

16. A system for manipulating DNA in a microfluidic environment, comprising:

- a deterministic lateral displacement (DLD) array, wherein the dimensions of the array are configured to fractionate DNA molecules on the basis of size, such that DNA molecules greater than a critical size move in a first direction through the array, and DNA molecules less than a critical size move in a second direction through the array;
- a DNA condensation agent; and
- a transport means configured to cause the DNA molecules to move through in the microfluidic array at a transport velocity, wherein:
  - at least one of the transport velocity and amount of DNA condensation agent are configured such that the DNA molecules retain an approximately spherical conformation as they pass through the microfluidic array.

17. The system of claim 16, wherein the transport means comprises flow means.

18. The system of claim 16, wherein the transport means comprises at least one electric field.

19. The system of claim 16, wherein the transport means comprises a combination of flow means and at least one electric field.
20. The system of claim 16, wherein the DLD array is configured with a critical size for fractionation, expressed as a particle diameter of between 0.1 and 3 μm.

21. The system of claim 16, wherein the condensation agent causes the DNA molecules of the sample to adopt a more compact size when passing through the DLD array at the transport velocity.
ABSTRACT

In some embodiments, a method for manipulating DNA molecules for use in a microfluidic device is provided, where the method may comprise providing a solution of a plurality of DNA molecules having a first radius of gyration under under a zero flow velocity, and maintaining the DNA molecules in a spherical shape under a flow velocity.
Figure 1

Fig. 2
Fig. 3

Fig. 4
Fig. 5

A collection of data marking bumping and zig-zag behaviour of 166 kbp T4 dsDNA as a function of PEG volume fraction and flow velocity. The bumping DNA molecules are marked with open triangles while the zig-zag DNA are marked with filled triangles. The black dash line is the allowed flow velocity using the data in Fig. 4.
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<th>Application Data Sheet 37 CFR 1.76</th>
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<td>METHODS AND SYSTEMS FOR CONTROLLING THE SHEAR MODULUS OF GENOMIC LENGTH DSDNA MOLECULES</td>
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### Mailing Address of Inventor:

<table>
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<tr>
<th>Address 1</th>
<th>4 Colbert Road</th>
</tr>
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</table>

### Inventor 2

<table>
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<th>2</th>
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<th>Legal Name</th>
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<tbody>
<tr>
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<tr>
<td></td>
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<table>
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<tr>
<th>Address 1</th>
<th>243 Concord Road</th>
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</table>

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.

### Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).
<table>
<thead>
<tr>
<th>Application Data Sheet 37 CFR 1.76</th>
<th>Attorney Docket Number</th>
<th>SAGS-008/P01US 322059-XXX</th>
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<td>Title of Invention</td>
<td>METHODS AND SYSTEMS FOR CONTROLLING THE SHEAR MODULUS OF GENOMIC LENGTH DSDNA MOLECULES</td>
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☐ An Address is being provided for the correspondence Information of this application.

<table>
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<th>58249</th>
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<tbody>
<tr>
<td>Email Address</td>
<td><a href="mailto:zPATDCDocketing@cooley.com">zPATDCDocketing@cooley.com</a></td>
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Application Information:

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<td>Subject Matter</td>
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<td>(if any)</td>
<td>Suggested Figure for Publication (if any)</td>
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Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

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Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

<table>
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<th>☐ US Patent Practitioner</th>
<th>☐ Limited Recognition (37 CFR 11.9)</th>
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</tr>
</tbody>
</table>

### Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the application number blank.

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Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.

### Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Additional Foreign Priority Data may be generated within this form by selecting the Add button.

### Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.
Authorization to Permit Access:

- Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an assignee under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

- Assignee
- Legal Representative under 35 U.S.C. 117
- Joint Inventor

- Person to whom the inventor is obligated to assign.
- Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here.

Prefix | Given Name | Middle Name | Family Name | Suffix
--- | --- | --- | --- | ---
**Application Data Sheet 37 CFR 1.76**

| Title of Invention | METHODS AND SYSTEMS FOR CONTROLLING THE SHEAR MODULUS OF GENOMIC LENGTH DSDNA MOLECULES |

### Mailing Address Information:

| Address 1 |
| Address 2 |
| City | State/Province |
| Country | Postal Code |
| Phone Number | Fax Number |
| Email Address |

Additional Applicant Data may be generated within this form by selecting the Add button.

---

### Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

#### Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the “Assignee Information” section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. [ ]

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<th>Middle Name</th>
<th>Family Name</th>
<th>Suffix</th>
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### Mailing Address Information For Assignee including Non-Applicant Assignee:

| Address 1 |
| Address 2 |
| City | State/Province |
| Country | Postal Code |
| Phone Number | Fax Number |
| Email Address |

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.
<table>
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<tr>
<th>Application Data Sheet 37 CFR 1.76</th>
<th>Attorney Docket Number</th>
<th>SAGS-008/P01US 322059-XXX</th>
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**NOTE:** This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.

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<tr>
<td>/Brian P. Hopkins/</td>
<td>2014-10-16</td>
<td>Brian</td>
<td>Hopkins</td>
<td>42669</td>
</tr>
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</table>

Additional Signature may be generated within this form by selecting the Add button.

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.
Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency’s responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.
Provisional Application for Patent Cover Sheet
This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

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<td>S.</td>
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<td>Given Name</td>
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<tr>
<td>T. Christian</td>
<td>Boles</td>
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</tbody>
</table>

All Inventors Must Be Listed – Additional Inventor Information blocks may be generated within this form by selecting the Add button.

Title of Invention
METHODS AND SYSTEMS FOR CONTROLLING THE SHEAR MODULUS OF GENOMIC LENGTH DSDNA MOLECULES

Attorney Docket Number (if applicable)
SAGS-008/P01US 322059-XXX

Correspondence Address
Direct all correspondence to (select one):

- The address corresponding to Customer Number

Customer Number
58249

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- No.

- Yes, the invention was made by an agency of the United States Government. The U.S. Government agency name is:

- Yes, the invention was under a contract with an agency of the United States Government. The name of the U.S. Government agency and Government contract number are:
Entity Status
Applicant asserts small entity status under 37 CFR 1.27 or applicant certifies micro entity status under 37 CFR 1.29

- Applicant asserts small entity status under 37 CFR 1.27
- Applicant certifies micro entity status under 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
- No

Warning
Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Signature
Please see 37 CFR 1.4(d) for the form of the signature.

<table>
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<tr>
<th>Signature</th>
<th>/Brian P. Hopkins/</th>
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| Registration Number (If appropriate) | 42669

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. This form can only be used when in conjunction with EFS-Web. If this form is mailed to the USPTO, it may cause delays in handling the provisional application.
Privacy Act Statement

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3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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<td><strong>First Named Inventor/Applicant Name:</strong></td>
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**Payment information:**

- **Submitted with Payment:** yes
- **Payment Type:** Deposit Account
- **Payment was successfully received in RAM:** $130
- **RAM confirmation Number:** 4981
- **Deposit Account:** 501283
- **Authorized User:**

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

- Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)
- Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)
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**Multipart Description/PDF files in .zip description**

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**Total Files Size (in bytes):** 4296225
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Application Number: 62064890  
Document Date: 10/16/2014

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Form Revision Date: August 26, 2013