



(51) International Patent Classification:

C40B 20/08 (2006.01) C40B 40/10 (2006.01)
C40B 30/10 (2006.01) C12N 9/00 (2006.01)

(21) International Application Number:

PCT/US2013/077583

(22) International Filing Date:

23 December 2013 (23.12.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/745,157 21 December 2012 (21.12.2012) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: ACTIN MOLECULE ORGANIZATION AND USES THEREOF

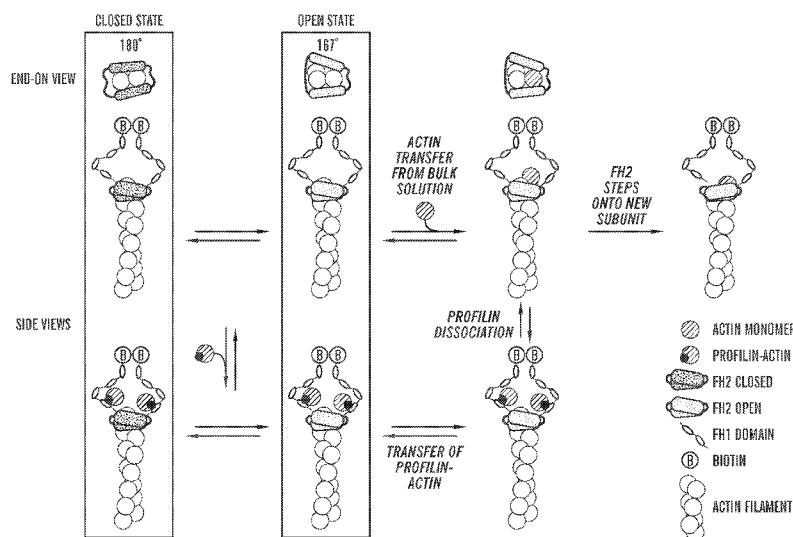


FIG. 1A

(57) Abstract: The invention is related to actin arrays and methods of using actin arrays.

ACTIN MOLECULE ORGANIZATION AND USES THEREOF

[0001] This application claims priority to U.S. Application Ser. No. 61/745,157, filed on December 21, 2012, the contents of which is hereby incorporated by reference in its entirety.

[0002] All patents, patent applications and publications cited herein are hereby incorporated by reference in their entirety. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

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GOVERNMENT INTERESTS

[0004] The work described herein was supported in whole, or in part, by National Institute of Health Grant No. GM074739. The United States Government has certain rights to the invention.

BACKGROUND OF THE INVENTION

[0005] Formins promote processive elongation of actin filaments for cytokinetic contractile rings and other cellular structures. *In vivo* these structures are exposed to tension, but the effect of tension on these processes remains unknown.

SUMMARY OF THE INVENTION

[0006] Here single-molecule imaging was used to investigate the effects of tension on formin-mediated actin filament polymerization. The experiments demonstrate that sub-picoNewton forces on the filaments dramatically slow formin-mediated polymerization in the absence of profilin, but result in faster polymerization in the presence of profilin. It is proposed that force shifts the conformational equilibrium of the end of a filament associated with formin FH2 domains toward the closed state that precludes polymerization, but that

profilin-actin associated with formin FH1 domains reverses this effect. Thus physical forces have an important influence on actin assembly.

[0007] In one aspect, the invention provides an array comprising: a) a solid support; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0008] In another aspect, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer. In one embodiment, the barrier comprises a mechanical barrier, a chemical barrier, a protein barrier, or a combination thereof.

[0009] In another aspect, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a protein barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0010] In one embodiment, the linkage is formed between neutravidin and biotin, or streptavidin and biotin. In one embodiment, the protein filament is aligned along the barrier through application of a hydrodynamic force, an electrophoretic force, or a combination thereof. In one embodiment, one end of the protein filament is attached by a linkage. In one embodiment, the protein filament is reversibly attached to the lipid bilayer along the contour of the lipid bilayer. In one embodiment, both ends of the protein filament are attached by a linkage. In one embodiment, the ends of the protein filament are each attached by different linkages. In one embodiment, the protein filament is an actin filament. In one embodiment, the protein filament is an intermediate filament. In one embodiment, the protein filament is a microtubule protofilament. In one embodiment, the intermediate filament comprises lamin, vimentin, neurofilament, or keratin. In one embodiment, the microtubule protofilament comprises alpha tubulin, beta tubulin, or a combination thereof. In one embodiment, the protein filament comprises at least 10 monomer units. In one embodiment, the filament binding protein comprises cross-linking proteins, motor proteins, branching proteins, severing

proteins, polymerization proteins, capping proteins, depolymerizing proteins, end tracking proteins, nucleators, monomer binding proteins, stabilizers, sidebinders or membrane anchors. In one embodiment, the filament binding protein comprises formin, or profilin. In one embodiment, the protein filament is coupled to a label. In one embodiment, the label is a fluorescent label. In one embodiment, the solid support comprises SiO₂. In one embodiment, the lipid bilayer comprises zwitterionic lipids.

[0011] In one embodiment, the protein filament binding protein comprises alpha-actinin, fascin, spectrin, transgelin, fimbrin, scribbin, villin, espin, myosins, Arp2/3, WASP/SCAR/WAVE, gelsolin, fragmin, capping protein (capZ), formin, tensin, tropomodulin, CapG, ADF/cofilin, AIP1, N-WASP, WASP, profilin, thymosins, twinfilin, Srv2/CAP, verprolin/WIP, adducin, caldesmon, calponin, nebulins, tropomyosin, IQGAP, Abp1, cortactin, coronin, drebrin, ENA/VASP, annexin II, alpha-catenin, BPAG, dystrophin, ERM proteins, plectin, spectrin, Sla2 (HIP1R), talin, tensin, utrophin, or vinculin.

[0012] In another aspect, the invention provides a microfluidic flowcell comprising an array comprising: a) a solid support; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer. In one embodiment, the protein filament is aligned along the barrier through application of a hydrodynamic force, an electrophoretic force, or a combination thereof. In one embodiment, the hydrodynamic force is tangential, perpendicular, or a combination thereof. In one embodiment, the electrophoretic force is tangential, perpendicular, or a combination thereof.

[0013] In another aspect, the invention provides a microfluidic flowcell comprising an array comprising: a) a solid support, wherein the solid support comprises a barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer. In one embodiment, the protein filament is aligned along the barrier through application of a hydrodynamic force, an electrophoretic force, or a combination thereof. In one embodiment, the hydrodynamic force is tangential, perpendicular, or a combination thereof. In one embodiment, the electrophoretic force is tangential, perpendicular, or a combination thereof.

[0014] In another aspect, the invention provides a microfluidic flowcell comprising an array comprising: a) a solid support, wherein the solid support comprises a protein barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer. In one embodiment, the protein filament is aligned along the barrier through application of a hydrodynamic force, an electrophoretic force, or a combination thereof. In one embodiment, the hydrodynamic force is tangential, perpendicular, or a combination thereof. In one embodiment, the electrophoretic force is tangential, perpendicular, or a combination thereof.

[0015] In another aspect, the invention provides a method for analyzing an interaction between a protein filament and a polypeptide, the method comprising: a) providing an array, wherein the attached protein filament is coupled to a first fluorescent label that permits visualization of the protein filament; b) contacting a polypeptide to the attached protein filament, wherein the polypeptide is coupled to a second fluorescent label that permits visualization of the polypeptide; c) applying a hydrodynamic force or an electrophoretic force tangential to the surface of the support to align the attached protein filament s in a desired orientation; d) visualizing the protein filament and the polypeptide; and e) determining whether the protein filament interacts with the polypeptide, wherein localization of the polypeptide anywhere along the length of the protein filament is indicative of interaction. In one embodiment, the determining comprises observing a static localization or dynamic localization of the polypeptide along the protein filament. In one embodiment, the static localization of the polypeptide along the protein filament indicates binding between the protein filament and the polypeptide. In one embodiment, the dynamic localization of the polypeptide along the protein filament indicates binding and movement of the polypeptide along the protein filament.

[0016] In one embodiment, the invention provides an array comprising: a) a solid support; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0017] In one embodiment, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the

protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0018] In one embodiment, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a protein barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0019] In one embodiment, the steps are automated. In one embodiment, the method optionally comprises applying continuously the hydrodynamic or electrophoretic force tangential to the surface of the support.

[0020] In another aspect, the invention provides a method for identifying a polypeptide sequence or compound that disrupts an interaction between a protein filament and a polypeptide, the method comprising: a) providing a first array, wherein the first array comprises a first population of identical protein filaments, and wherein the protein filaments are coupled to a first fluorescent label; b) providing a second array, wherein the second array comprises a second population of identical protein filaments, wherein the protein filaments are coupled to the first fluorescent label, and wherein the second population of protein filaments differ from the first population of protein filaments by at least one monomer unit; c) contacting a polypeptide sequence or compound to the arrays, wherein the polypeptide sequence or compound is coupled to a second fluorescent label that permits visualization of the polypeptide sequence or compound; and d) determining whether the first population of protein filaments and the second population of protein filaments interact with the polypeptide sequence or compound, wherein localization of the polypeptide sequence or compound anywhere along the length of the first population of protein filaments is indicative of an interaction between the first population and the polypeptide sequence or compound, and wherein an absence of localization of the polypeptide sequence or compound along the length of the second population of protein filaments is indicative that the second population comprises a polypeptide sequence or compound that disrupts the interaction between the first protein filament and the polypeptide sequence or compound.

[0021] In one embodiment, the invention provides an array comprising: a) a solid support; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament;

d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0022] In one embodiment, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0023] In one embodiment, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a protein barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0024] In one embodiment, the steps are automated. In one embodiment, the method optionally comprises applying continuously the hydrodynamic or electrophoretic force tangential to the surface of the support.

[0025] In another aspect, the invention provides a method for identifying a polypeptide sequence or compound that alters the structure of a protein filament, the method comprising: a) providing an array, wherein the protein filament is coupled to a first fluorescent label that permits visualization of the protein filament; b) applying a hydrodynamic force or an electrophoretic force tangential to the surface of the support to align the protein filament in a desired orientation; c) visualizing the length of the protein filament; d) contacting a polypeptide sequence or compound to the protein filament, wherein the polypeptide sequence or compound is optionally coupled to a second fluorescent label that permits visualization of the polypeptide sequence or compound; e) visualizing the length of the protein filament and, optionally, visualizing the polypeptide sequence or compound; and f) determining whether the protein filament changes length following the contacting step, wherein an increase or a decrease in the length of the protein filament is indicative of a polypeptide sequence or compound that alters the structure of the protein filament.

[0026] In one embodiment, the invention provides an array comprising: a) a solid support; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament;

d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0027] In one embodiment, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0028] In one embodiment, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a protein barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0029] In one embodiment, the steps are automated. In one embodiment, the method optionally comprises applying continuously the hydrodynamic or electrophoretic force tangential to the surface of the support.

[0030] In another aspect, the invention provides a method for identifying an agent that disrupts the interaction of a polypeptide and a protein filament, the method comprising: a) providing an array, wherein the protein filament is coupled to a first fluorescent label that permits visualization of the protein filament; b) contacting polypeptide to the protein filament, wherein the polypeptide is capable of interacting with the protein filament, and wherein the polypeptide is coupled to a second fluorescent label that permits visualization of the polypeptide; c) contacting an agent to the protein filament and the polypeptide; d) applying a hydrodynamic force or an electrophoretic force tangential to the surface of the support to align the attached protein filaments in a desired orientation; e) visualizing the protein filament and the polypeptide; and f) determining whether the agent disrupts the interaction between the protein filament and the polypeptide, wherein loss of localization of the polypeptide anywhere along the length of the protein filament is indicative of an agent that disrupts the interaction between the protein filament and the polypeptide.

[0031] In one embodiment, the invention provides an array comprising: a) a solid support; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament;

d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0032] In one embodiment, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0033] In one embodiment, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a protein barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0034] In one embodiment, the agents are from a library. In one embodiment, the steps are automated. In one embodiment, the method optionally comprises applying continuously the hydrodynamic or electrophoretic force tangential to the surface of the support.

[0035] In another aspect, the invention provides a method for identifying one or more agents that disrupt the interactions between one or more polypeptides and a protein filament, the method comprising: a) providing an array, wherein the array comprises a plurality of identical protein filaments, wherein the protein filaments are coupled to a first fluorescent label that permits visualization of the protein filaments; b) contacting one or more polypeptides to the protein filaments, wherein the one or more polypeptides are each capable of interacting with the protein filaments at different known locations, and wherein the one or more polypeptide are coupled to a second fluorescent label that permits visualization of the polypeptides; c) applying a hydrodynamic force or an electrophoretic force tangential to the surface of the support to align the attached protein filaments in a desired orientation and visualizing the protein filaments and the polypeptides; d) contacting a first agent to the array; e) visualizing the protein filaments and the polypeptides; f) determining whether the first agent disrupts the interaction between the protein filaments and one or more of the polypeptides, wherein loss of localization of one or more of the polypeptides along the length of the protein filaments is indicative of an agent that disrupts the interaction between the

protein filaments and the one or more polypeptides; and g) optionally contacting a second agent to the array and repeating steps e) and f).

[0036] In one embodiment, the invention provides an array comprising: a) a solid support; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0037] In one embodiment, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0038] In one embodiment, the invention provides an array comprising: a) a solid support, wherein the solid support comprises a protein barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

[0039] In one embodiment, the agents are from a library. In one embodiment, the steps are automated. In one embodiment, the method optionally comprises applying continuously the hydrodynamic or electrophoretic force tangential to the surface of the support.

BRIEF DESCRIPTION OF THE FIGURES

[0040] To conform to the requirements for PCT patent applications, many of the figures presented herein are black and white representations of images originally created in color. The original color versions of Figures 1-12 can be viewed in Courtemanche *et al.*, (2013) *Proc Natl Acad Sci U S A.* 110(24):9752-57 (including the accompanying Supplementary Information available in the on-line version of the manuscript available on the Proceedings of the National Academy of Sciences of the United States of America web site). For the purposes of the PCT, the contents of Courtemanche *et al.*, (2013) *Proc Natl Acad Sci U S A.* 110(24):9752-57, including the accompanying "Supplementary Information," are herein incorporated by reference in their entireties.

[0041] Figs. 1A-B. Formin-mediated actin filament polymerization in actin curtains. (A) Cartoon summary of the reaction pathways for actin from the bulk phase and profilin-actin associated with formin FH1 domains to add to the barbed end of an actin filament associated with a dimer of FH2 domains. FH2 dimers are shown in red for the closed conformation and green for the open conformation. End-on views of the filament illustrate the hypothesis that the closed conformation corresponds to a 180° pitch of the filament, while the open conformation has a 167° pitch. (B) Schematic of actin curtains. Biotinylated Bni1(FH1FH2)p (formin) is anchored via streptavidin to a lipid bilayer and polymerizes actin filaments that are aligned along nanofabricated barriers by solvent flow, allowing the filaments to be visualized by total internal reflection fluorescence microscopy.

[0042] Figs. 2A-D. Lipid-tethered formins polymerize actin filament curtains. Buffer conditions: 1.5 μ M actin (33% Oregon Green-actin) in microscopy buffer (10 mM imidazole (pH 7.0), 50 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 50 mM DTT, 0.2 mM ATP, 0.02 mM $CaCl_2$, 15 mM glucose, 0.02 mg/ml catalase, 0.1 mg/ml glucose oxidase). (A) Image of an actin curtain with flow on. The dotted box highlights an individual actin filament. “B” indicates the location of barriers. The buffer flow is from top to bottom. (B) Kymograph showing alternating examples of extension and diffusion of a single actin filament that result from applying and stopping buffer flow over the actin curtain shown in (A). (C) Kymograph showing a single formin-anchored actin filament growing over time. (D) Pulse-chase experiment demonstrating that polymer growth occurs only at the barbed end of the filament associated with the formin anchored at the barrier. Polymerization was initiated with fluorescent actin monomers, followed by a pulse of unlabeled actin monomers and finally chased with additional fluorescent actin monomers. The dark section in the middle of the actin filament at the right of the kymograph is due to the incorporation of the unlabeled actin monomers.

[0043] Figs. 3A-C. Force inhibits formin-mediated elongation of actin filaments in the absence of profiling. Buffer conditions: 1.5 μ M actin (33% Oregon Green-actin) in microscopy buffer with 0.25% methylcellulose (15 cP at 2%) to increase the viscosity to 1.88×10^{-3} Pa·s. (A) Time course of elongation of a single filament in the absence of profilin with a bulk flow of 0.1 mL/min. The black line is the length. The red (grey in black and white image) and blue (dark grey in black and white image) dashed lines are tangents to the beginning and the end of the elongation curve to emphasize the decline in the rate of

elongation as the filament grew longer. The hydrodynamic force applied to the filament (right axis) is proportional to the length of the filament. **(B)** Dependence of polymerization rate of filaments growing with bulk flow rates of 0.05 (red circles (grey in black and white image)), 0.075 (orange circles (light grey in black and white image)), 0.1 (blue circles (dark grey in black and white image)), 0.15 (green circles (grey in black and white image)), 0.2 (black circles) and 0.5 (purple circles (light grey in black and white image)) mL/min, which exert a drag force on each filament. Data were collected using 10 nm barriers (closed circles) or 25 nm barriers (open circles). Polymerization rates and drag forces (calculated according to equation 3) were measured at intervals over the course of the elongation experiment. The solid line represents data simulated using a thermodynamic model of formin-mediated polymerization with a force-sensitive gating factor(7). **(C)** Cartoon depiction of the effect of force on the equilibrium between the open (green (light grey in black and white image)) and closed (red (grey in black and white image)) conformations of the filament (gray)-bound formin in the absence of profilin. The FHI domains are depicted in black and magenta ovals and the streptavidin-bound biotin labels are shown in cyan circles.

[0044] Figs. 4A-D. Force increases the rate of formin-mediated polymerization in the presence of profilin. Buffer conditions as in Figure 3 except for the presence of profilin. **(A)** Dependence of polymerization rates on the concentration of profilin and the flow rate: open cyan square, no flow; red circles, 0.05 mL/min (grey in black and white image); orange circles, 0.075 mL/min (light grey in black and white image); blue circles, 0.1 mL/min (dark grey in black and white image); green circles, 0.15 mL/min (grey in black and white image); black circles, 0.2 mL/min; purple circle, 0.5 mL/min (grey in black and white image). Error bars are standard errors of the mean. **(B)** Elongation profiles of 10 filaments in the presence of 2.5 μ M profilin and 0.2 mL/min bulk flow, aligned by length. Each filament is represented by different color, and every third data point is shown. The red and blue dashed lines are tangents to the extension curves of the shortest and longest filaments. **(C)** Dependence of polymerization rates of filaments growing in the presence of 2.5 μ M profilin with bulk flow rates of 0.05 (red circles (grey in black and white image)), 0.075 (orange circles (light grey in black and white image)), 0.1 (blue circles (dark grey in black and white image)), 0.15 (green circles (grey in black and white image)), 0.2 (black circles) and 0.5 (purple circles (grey in black and white image)) mL/min to produce drag force on each filament. Data were collected using 10 nm barriers (closed circles) and 25 nm barriers (open circles). Polymerization rates and drag forces (Calculated according to equation 3) were measured at various intervals over

the course of the elongation experiment. The solid line represents data simulated using a thermodynamic model of formin-mediated polymerization in the presence of 2.5 μM profilin with the same force-sensitive gating factor as in **Figure 3B**. **(D)** Cartoon depiction of the effect of force on the equilibrium between the open (green (light grey in black and white image)) and closed (red (grey in black and white image)) conformations of the filament (gray)-bound formin in the presence of profilin (dark blue). The FH1 domains are depicted in black and magenta ovals, the streptavidin-bound biotin labels are shown in cyan outlined-circles, and the incoming actin subunit is shown in orange (grey in black and white image).

[0045] **Fig. 5.** Dependence of force per μm of filament on the bulk flow rate in the observation chamber.

[0046] **Figs. 6A-B. Effect of drag force on formin-mediated polymerization in the presence of 5 μM profilin.** Buffer conditions: 1.5 μM actin (33% Oregon Green-actin) with 5 μM profilin in microscopy buffer with 0.25% methylcellulose (15 cP at 2%). Barrier height was 25 nm. **(A)** Collection of elongation trajectories of filaments in the presence of 0.2 mL/min bulk flow, aligned by length. Each filament is represented by different color and, for clarity, every third data point is shown. Red (grey in black and white image) and blue (dark grey in black and white image) lines are tangents to the elongation profiles of the shortest and longest filaments. **(B)** Dependence of polymerization rates on the drag force exerted on filaments at 0.05 (red circles (light grey in black and white image)), 0.1 (blue circles (grey in black and white image)) and 0.2 (black circles) mL/min bulk flow using 25 nm barriers. Polymerization rates and drag forces (Calculated according to equation 3) were measured at various intervals over the course of the elongation experiment.

[0047] **Fig. 7. Comparison of experimental and simulated polymerization rates in the presence of 2.5 μM profilin.** *Experimental data:* The points show the dependence of measured polymerization rates on the drag force exerted on each filament at bulk flow rates of 0.05 (red circles (grey in black and white image)), 0.075 (orange circles (light grey in black and white image)) 0.1 (blue circles (dark grey in black and white image)), 0.15 (green circles (grey in black and white image)), 0.2 (black circles) and 0.5 (purple circles (grey in black and white image)) mL/min. Data were collected using 10 nm barriers (closed circles) and 25 nm barriers (open circles). *Simulations:* Lines show the dependence of simulated polymerization rates on drag force using a gating factor that declines linearly from 0.75 without force to 0 at forces >0.2 pN. **Table 3** lists rate constants for the formation of

intermediate species (FH1-profilin, FH1-profilin-actin, FH1-profilin-actin-barbed end, barbed end-profilin and filamentous actin). The simulations used with three different FH1 loop closure rates: (solid line) 5000 s^{-1} that reproduce the polymerization rates of filaments associated with wild-type Bni1(FH1FH2)p in the absence of force; (long dashes) 25 times faster than wild type; (short dashes) 125 times faster; and (dotted line) 625 times faster.

[0048] **Figs. 8A-B. Height profiles of chromium barriers on fused silica slides.** (A) 10 nm and (B) 25 nm barriers were scanned by atomic force microscopy in tapping mode at a 1 Hz scanning rate.

[0049] **Figs. 9A-B** show graphs depicting the impact of surface proximity on surface-tethered filaments. (9A) Force estimates for a tethered actin filament. Black diamonds: estimated force calculated from the Batchelor formula with no correction for filament height from the surface (P1). Red circles (grey in black and white image): estimated force-corrected effects due to surface proximity over a range of distances from the surface. (Inset) List of the relevant parameters (bulk flow rate, filament radius, and solution viscosity). Representative calculations are shown for a 10- μm actin filament, approximately the average length of the filaments observed in this study. These calculations illustrate that proximity to the surface has a substantial effect on the force on filaments, but this effect diminishes rapidly with distance from the surface and is less than threefold for filaments located $>50\text{ nm}$ from the surface. (9B) Flow velocity profiles for (Upper) the entire cross-section of the chamber and (Lower) the region close to the surface. Colored lines indicate the calculated flow velocity profile as a function of distance from the surface at different bulk flow rates, as indicated (Inset). The linear flow velocities in the imaging plane were measured by tracking filaments broken from anchored filaments (Table 2A), and these data were superimposed on the flow velocity profiles to estimate the positions of the filaments relative to the surface. The open circles indicate mean velocities of broken filaments plotted on the flow velocity profiles for each different flow rate; vertical and horizontal error bars correspond to SEM on the flow measurements and corresponding height calculations, respectively, based on the experimentally measured linear flow velocities. At all bulk flow rates, the velocities of broken filaments correspond to the flow rate at $\sim 100\text{--}130\text{ nm}$ from the surface.

[0050] **Figs. 10A-B** show graphs of filament elongation before and after spontaneous filament breakage. Buffer conditions: 1.5 μM actin (33% Oregon Green-actin) in microscopy buffer with 0.25% methylcellulose (15 centipoise at 2%). Barrier height was 25 nm. (10A)

Evolution of the length of a single filament in the absence of profilin with a bulk flow of 0.05 mL/min. The red line (grey in black and white image) corresponds to elongation that occurs before a spontaneous breakage event and the blue line corresponds to elongation that occurs following the breakage event. The estimated hydrodynamic force applied to the filament is shown on the right axis and is proportional to the length of the filament. **(10B)** Filament growth before (red line (grey in black and white image)) and after (blue line (dark grey in black and white image)) the filament breakage event depicted in **10A**. The filament break instantaneously decreases the drag force (seen in **10A**) and produces an increase in the polymerization rate.

[0051] Figs. **11A-B** are graphs showing the effect of tension on formin-mediated polymerization in the presence of 5 μ M profilin. Buffer conditions: 1.5 μ M actin (33% Oregon Green-actin) with 5 μ M profilin in microscopy buffer with 0.25% methylcellulose (15 cP at 2%). Barrier height was 25 nm. **(11A)** Collection of elongation trajectories of filaments in the presence of 0.2 mL/min bulk flow, aligned by length. Each filament is represented by a different color and, for clarity, every third data point is shown. **(11B)** Dependence of polymerization rates on the drag force exerted on filaments at 0.05 (red circles (light grey in black and white image)), 0.1 (blue circles (dark grey in black and white image)), and 0.2 (black circles) mL/min bulk flow. Polymerization rates and drag forces (calculated according to Eq. 3) were measured at various intervals over the course of the elongation experiment.

[0052] Fig. **12** is a frame from a movie available at <http://www.pnas.org/content/110/24/9752/suppl/DCSupplemental>. Lipid-tethered, formin-bound actin filaments extend upon introduction of buffer flow. Buffer conditions: 1.5 μ M actin (33% Oregon Green-actin) in microscopy buffer (10 mM imidazole, pH 7.0, 50 mM KCl, 1 mM MgCl₂, 1 mM EGTA, 50 mM DTT, 0.2 mM ATP, 0.02 mM CaCl₂, 15 mM glucose, 0.02 mg/mL catalase, 0.1 mg/mL glucose oxidase). Movie of formin-bound actin curtains assembled at four barriers on the lipid-coated surface of a microfluidic chamber. Flow is introduced into the chamber and transiently paused. (Scale bar, 20 μ m.) The elapsed time is indicated.

[0053] Fig. **13** is a schematic of an overview of a Total Internal Reflection Fluorescence Microscope (TIRFM).

[0054] Figs. 14A-B are designs for flowcells.

DETAILED DESCRIPTION OF THE INVENTION

[0055] *In vivo*, actin filaments are subject to tension, yet the influence of force on formin-mediated polymerization is unknown, and existing theories predict different outcomes in the absence and presence of profilin. Kozlov & colleagues proposed that the elasticity of the FH2 domain and the formin-barbed end binding energy govern the polymerization rate (8, 9), and their simulations suggested that tension would increase the rate of polymerization by both lowering the activation barrier for actin subunit addition and by energetically favoring FH2 domain stepping onto the incoming actin subunit (8). In contrast, Vavylonis *et al.* (10) predicted that force-induced stretching of the FH1 domains would reduce the rate of profilin-actin transfer to the growing filament, thereby reducing the rate of polymerization for actin filaments under tension in the presence of profilin

[0056] Two Formin Homology 2 (FH2) domains form a donut-shaped head-to-tail dimer that encircles the fast growing barbed end of actin filaments and promotes nucleation and polymerization (1, 2). When an actin monomer binds to the barbed end of a filament, the formin FH2 domain steps onto the new subunit, allowing the formin to remain attached to the filament through thousands of cycles of subunit addition (Figure 1A) (3, 4). FH2 domains slow barbed end elongation, a phenomenon termed “gating”, which reflects an equilibrium between two conformations of a filament end associated with an FH2 dimer: a “closed” conformation that cannot bind actin monomers, and an “open” conformation that can bind actin monomers (Figure 1A) (1, 5). In spite of gating, FH2 domains can promote rapid filament elongation when coupled to Formin Homology 1 (FH1) domains (6), which are located N-terminal to the FH2 domain (3). Multiple polyproline tracks in FH1 domains bind complexes of the protein profilin with actin monomers. Profilin-actin transfers rapidly from the FH1 domains to the FH2-bound barbed end of the growing actin filament, presumably via diffusion of the FH1 domain (Figure 1A) (7).

[0057] The present invention is based in part on the discovery that protein filaments can be disposed on a substrate and positionally aligned to allow analysis of individual protein filaments. In particular, the methods and compositions described herein include a substrate, coating material, e.g., a lipid bilayer, and protein filaments coupled to filament binding

proteins that are attached directly to the substrate, attached to the substrate via a linkage, or attached to the lipid layer via a linkage.

[0058] Preparation of Substrate

[0059] Essentially, any conceivable substrate may be employed in the compositions and methods described herein. The substrate may be biological, nonbiological, organic, inorganic, or a combination of any of these, existing, e.g., as particles, strands, precipitates, gels, sheets, tubing, spheres, containers, capillaries, pads, slices, films, plates, or slides. The substrate may have any convenient shape, such as, e.g., a disc, square, sphere or circle. The substrate and its surface can form a rigid support on which to carry out the reactions described herein. The substrate can be, e.g., a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO₂, SiN₄, modified silicon, or any one of a wide variety of gels or polymers such as (poly)tetrafluoroethylene, (poly)vinylidenedifluoride, polystyrene, polycarbonate, or combinations thereof. Other substrate materials will be readily apparent to those of skill in the art upon review of this disclosure. In some embodiments, the substrate is made of SiO₂ and is flat.

[0060] In some embodiments, the substrate is coated with a linker to which the filament binding protein or the protein filament attaches. Such linkers can be, e.g., chemical or protein linkers. For example, the substrate can be coated with a protein such as neutravidin or an antibody. In one embodiment, proteins used to coat the substrate include, but are not limited to, avidin, streptavidin or neutravidin.

[0061] In some embodiments, the substrate includes a diffusion barrier, e.g., a mechanical, chemical or protein barrier. Diffusion barriers can be prepared by applying barrier materials onto the substrate prior to deposition of the lipid bilayer; the bilayer then forms around the barriers. A mechanical barrier can be, e.g., a scratch or etch on the substrate, which physically prevents lipid diffusion.

[0062] In the case of a chemical barrier, the chemical nature of the barrier, and not its surface topography, is the primary factor in preventing lipid diffusion [Q13]. Barrier materials can be made that are similar to the thickness of the bilayer itself (e.g., 6-8 nm), or thinner than the bilayer. Protein barriers can be deposited onto substrates, e.g., SiO₂ substrates, by a variety of methods. For example, protein barriers can be deposited in well-defined patterns by a process called microcontact printing [Q11, Q14]. Microcontact printing

uses a PDMS (poly[dimethylsiloxane]) template as a stamp for generating specific patterns on substrates. PDMS stamps can transfer proteins to a SiO₂ substrate in patterns with features as small as 1 μm, and thicknesses on the order of 5-10 nm [Q11, Q14]. The PDMS stamps used for microcontact printing can be made, e.g., by soft-lithography as described in [reference 14]. Once made, the PDMS can be incubated with a solution of protein, dried, and then placed into contact with the substrate, e.g., SiO₂, resulting in transfer of the protein "ink" from the PDMS stamp to the substrate and yielding a pattern defined by the stamp design. For example, protein barriers can be made from fibronectin.

[0063] To the substrate is then attached a layer of a material. In one embodiment, the material is one that renders the substrate inert. For example, the material can be lipids, forming, e.g., a lipid bilayer. In another embodiment, the layer is made of zwitterionic lipids. A lipid bilayer can be deposited onto the substrate by applying liposomes to the substrate. Liposomes can be produced by known methods from, e.g., 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) or 0.5% biotin-phosphatidylethanolamine (biotin-PE) plus 99.5% DOPC (Avanti Polar Lipids, Alabaster, Ala.). In some embodiments, the lipid bilayer can include polyethylene glycol (PEG). For example, in embodiments where quantum dots are used to label polypeptides, PEG can be included in the lipid bilayer. PEG can also be included to make the surface of the bilayer inert to reagents added to the array.

[0064] **Protein Filaments**

[0065] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms can apply to amino acid polymers in which one or more amino acid residues is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. A polypeptide can include a filament binding protein.

[0066] The term "protein filament" includes, but is not limited to, actin filaments, intermediate filaments, or microtubule protofilaments.

[0067] Actin filaments (F-actin) are linear polymers of the actin polypeptide (G-actin) which is the monomeric subunit of actin filaments. Each microfilament is made up of two helical interlaced strands of subunits. Microfilaments are polarized, with a barbed end and a pointed end. ATP-bound G-actin is the normal substrate for filament formation and can polymerize at the barbed end to form ATP-containing F-actin. F-actin is a slow ATPase and

ATP containing F-actin is hydrolyzed to ADP-F-actin. ADP-F-actin is less stable than ADP-G-actin enabling actin filaments to be disassembled as part of the actin recycling process in cells. Actin filaments can be microfilaments or thin filaments. Actin filaments can further assemble into bundles or networks, which can be polarized (actin filament barbed ends all pointing to one end of the bundle) or non-polarized (actin filament barbed ends pointing towards both ends of the bundle). In one embodiment, the actin filament comprises at least 10 monomer units. In another embodiment, the actin filament comprises at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 250, at least 500, at least 750 or at least 1000 monomer units.

Properties of actin filaments will be known to one of skill in the art and are described in Kries, T. and Vale, R., 1999, Guidebook to the Cytoskeletal and Motor Proteins, 2nd Edition, Oxford University Press, Oxford, U.K. and Dominguez, R. and Holmes, K.C., 2011, Actin Structure and Function, Annu. Rev. Biophys. 40: 169-186.

[0068] For example, the polypeptide sequence of chicken skeletal muscle actin is depicted in SEQ ID NO: 1. The nucleotide sequence of chicken skeletal muscle actin is shown in SEQ ID NO: 2. Sequence information related to ACTA1, chicken skeletal muscle actin is accessible in public databases by GenBank Accession numbers NP_001026234.1 (protein) and NM_001031063.1 (nucleic acid).

[0069] SEQ ID NO: 1 is the amino acid sequence corresponding to chicken skeletal muscle actin (residues 1-84):

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1 mcdedettal vcdngsglvk agfagddapr avfpsivgrp rhqgvvmvngm qkdsyvgdea
61 qskrgiltlk ypiehgiitn wddmekiwhh tfynelrvap eehptlltea plnpkanrek
121 mtqimfetfn vpamyvaiqa vlslyasgrt tgivldsgdg vthnvpieg yalphaimrl
181 dlagrdltdy lmkiltergy sfvttaerei vrdikeklcy valdfenema taasssslek
241 syelpdgqvi tignerfrcp etlfqpsfig mesagihett ynsimkcdid irkdlyannv
301 msggttmypg iadrmgkeit alapstmkik iiapperkys vwiggsilas lstfqgmwit
361 kqeydeagps ivhrkof

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[0070] SEQ ID NO: 2 is the nucleotide sequence corresponding to chicken skeletal muscle actin (residues 1-1134):

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1 atgtgtgacg aggacgagac caccgcgctc gtgtgcgaca acggctccgg cctagtgaag
61 gctggcttcg ccggggatga cggcccccagg gccgtgttcc cttccatcgt gggccggccc
121 cgccaccaag gtgtcatggt gggtatgggt cagaaggact cctacgtagg agatgaagct
181 cagagcaaga gaggtatcct caccctgaag taccocattg aacacggcat catcacaac
241 tgggacgaca tggagaagat ctggcaccac accttctaca atgagctgcg tgtggccct
301 gaggagcacc ccaccctgct cactgaggcc ccccttaatc ccaaagctaa ccgtgaaaag

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361 atgacccaaa tcatgtttga gacctttaac gtgcccgcga tgtacgtggc catccaagct
421 gtgctgtccc tgtatgectc tggccgtacc accggtattg tgctggactc cggcgatggg
481 gtgacacaca acgtcccat ctatgaaggg tatgccctgc cccatgccat catgcgcttg
541 gatctggctg gccgcgacct cactgactac ctgatgaaga tectgactga gcgtggctat
601 tcctttgtca ccacagctga acgtgagatt gtccgtgaca tcaaggagaa gctgtgctat
661 gtggctctgg actttgagaa cgagatggcc accgctgcct cctcctctc cttggagaag
721 agctatgagc tgctgatgg gcaggtcatc accatcgcca atgagcgttt ccgctgccc
781 gaaacctctc tccagccttc cttcattggg atggagtcg ctgggatcca tgagacaact
841 tacaacagca tcatgaaatg cgacattgac atcaggaagg acctgtatgc caacaacgct
901 atgtctgggg gtaccacat gtaccaggt attgctgacc gcatgcaaaa ggagatcaca
961 gccctggccc ccagcacaat gaagatcaag atcattgcc cactgagcg caagtactct
1021 gtctggatcg gtggctccat cttggcctcc ctgtccacct tccagcagat gtggatcaca
1081 aagcaggagt atgatgaagc cggcccatcc attgtccacc gtaaagtctt ctaa

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[0071] An actin gene can also encompass ortholog genes, which are genes conserved among different biological species such as humans, dogs, cats, mice, and rats, that encode proteins (for example, homologs (including splice variants), mutants, and derivatives) having biologically equivalent functions as the chicken-derived protein. Orthologs of actin include any mammalian ortholog inclusive of the ortholog in humans and other primates, experimental mammals (such as mice, rats, hamsters and guinea pigs), mammals of commercial significance (such as horses, cows, camels, pigs and sheep), and also companion mammals (such as domestic animals, e.g., rabbits, ferrets, dogs, and cats), or any other eukaryote (such as birds, *S.cerevisiae*, *D.melanogaster*, *C.elegans*, and *D.discoideum*). Actin can comprise a protein encoded by a nucleic acid sequence homologous to the chicken nucleic acid, wherein the nucleic acid is found in a different species and wherein that homolog encodes a protein similar to an actin protein. For example, sequence information related to human ACTA1, (human skeletal muscle actin) is accessible in public databases by GenBank Accession numbers NP_001091.1 (protein) and NM_001100.3 (nucleic acid).

[0072] Intermediate filaments are formed from two polypeptide monomers which interact to form a coiled-coil dimer. The dimers can then associate in a staggered antiparallel arrangement to form tetramers which assemble end to end into protofilaments. Around eight protofilaments wind around each other to form an intermediate filament. Dimers can be homodimers or heterodimers of intermediate filaments polypeptide monomers that, include, but is not limited to keratin, desmin, glial fibrillary acidic protein (GFAP), peripherin, vimentin, α -internexin, neurofilament, synemin, syncolin, lamin, nestin, filensin and phakinin. In one embodiment, the intermediate filament comprises at least 10 monomer units. In another embodiment, the intermediate filament comprises at least 5, at least 10, at least 20,

at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 250, at least 500, at least 750 or at least 1000 monomer units. Properties of intermediate filaments will be known to one of skill in the art and are described in Kries, T. and Vale, R., 1999, *Guidebook to the Cytoskeletal and Motor Proteins*, 2nd Edition, Oxford University Press, Oxford, U.K. and Cooper, G.M., 2000, *The Cell: A Molecular Approach*, 2nd Edition, Sinauer Associates, Sunderland, MA.

[0073] Microtubule protofilaments are hollow cylinders made up of polymerized dimers of alpha tubulin and beta tubulin. Tubulin dimers polymerize end to end into protofilaments. About thirteen protofilaments associate parallel to one another to form a single microtubule. Microtubules are polarized and tubulin polymerizes end to end, with the alpha subunits of one tubulin dimer contacting the beta subunits of the next. In a protofilament, the end with the alpha subunit exposed is known as the minus end, and the end with the beta subunit exposed is known as the plus end. GTP-bound beta tubulin is hydrolyzed to GDP-beta-tubulin shortly after polymerization. GDP-beta tubulin has a lower binding affinity for the adjacent tubulin molecules and favors depolymerization resulting in the dynamic behavior of microtubules. Properties of microtubules will be known to one of skill in the art and are described in Kries, T. and Vale, R., 1999, *Guidebook to the Cytoskeletal and Motor Proteins*, 2nd Edition, Oxford University Press, Oxford, U.K. and Cooper, G.M., 2000, *The Cell: A Molecular Approach*, 2nd Edition, Sinauer Associates, Sunderland, MA.

[0074] The term “filament binding protein” refers to any polypeptide that can bind to a protein filament. Examples of actin filament binding proteins include, but are not limited to, cross-linking proteins (e.g., alpha-actinin, fascin, spectrin, transgelin, fimbrin, scruin, villin, espin), motor proteins (e.g., myosins), branching proteins (e.g., Arp2/3, WASP/SCAR/WAVE), severing proteins (e.g., gelsolin, fragmin, villin), polymerization proteins, capping proteins (e.g., capping protein (capZ), formins, tensin, tropomodulin, CapG), depolymerizing proteins (e.g., ADF/cofilin, AIP1), end tracking proteins (e.g. formins, VASP, N-WASP), nucleators (e.g., Arp2/3, WASP, formin), monomer binding proteins (e.g., profilin, thymosins, twinfilin, Srv2/CAP, WASP, verprolin/WIP) stabilizers (e.g. adducing, caldesmon, calponin, nebulins, tropomyosin), sidebinders (e.g. IQGAP, Abp1, cortactin, coronin, drebrin, ENA/VASP) and membrane anchors (e.g., alpha-actinin, annexin II, alpha-catenin, BPAG, dystrophin, ERM proteins, plectin, spectrin, Sla2 (HIP1R), talin, tensin, utrophin, vinculin). Actin binding proteins will be known to one of skill in the art and

are described in Dos Remedios, C.G. et al., 2003, Actin Binding Proteins: Regulation of Cytoskeletal Microfilaments. *Physiol. Rev.* 83: 433-473 and Winder S.J. and Ayscough, K.R. 2005, Actin-Binding Proteins, *J. Cell Sci.* 118: 651-654. Examples of intermediate filament binding proteins include, but are not limited to, BPAG1, IFAP 300, lamin-associated proteins (e.g., LAP1, LAP2, LBR), filaggrin, plectin, plakoglobin, desmoplakin, desmogleins, and desmocollins. Examples of microtubule binding proteins include, but are not limited to, tau proteins, microtubule-associated proteins (e.g., MAP-1A, MAP-1B, MAP-1C, MAP-2, MAP-3, MAP-4, E-MAP-115), STOP, APC, mapmodulin, MARK protein kinases, MARPs, Op18/stathmin, radial spoke proteins, syncolin, Tea1, tektins, XMAPs, ensconsin, plus-end tracking proteins (e.g., EB1, EB2, EB3, p150Glued, Dynamitin, Lis1, CLIP170, CLIP115, CLASP1, CLASP2), motor proteins (e.g., kinesin, dynein) and centrosomal proteins (e.g. gamma-tubulin, centrosomin, CP190/CP60, Katanin, NuMA, pericentrin, yeast spindle pole body proteins).

[0075] For example, the polypeptide sequence of *S.cerevisiae* formin is depicted in SEQ ID NO: 3. The nucleotide sequence of *S.cerevisiae* formin is shown in SEQ ID NO: 4. Sequence information related to Bni1p, *S.cerevisiae* formin is accessible in public databases by GenBank Accession numbers NP_014128.2 (protein) and NM_001183109.2 (nucleic acid).

[0076] SEQ ID NO: 3 is the amino acid sequence corresponding to *S.cerevisiae* formin (residues 1-1953):

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1  mlknsqskhs  nsleshsnss  sgifqnklrl  ansnatnsnt  gsptyasqqq  hspvgnevst
61  spassssfrk  inapsrstst  earpinkkst  lntqnlsqym  ngklsgdvpv  ssqharshsm
121  qskysyskrn  ssqasnkltr  qhtgqshsas  slisqgsltn  lskfttpdgg  iylempsdpy
181  evevlfedim  ykrnifqsls  edkqealmgy  siekkwliwk  qdlqlnelkm  rantsssta
241  srtsmasdhb  piltanssls  spksvlmtsa  ssptstvyss  slnhsttlls  vgtstskgkk
301  lvsgslkkqp  slnniyrqga  enntsastlp  gdrtnrppih  yvqriladkl  tsdemkdllw
361  tlrteqldwv  dafidhqghi  amanvlmsi  yktaprenlt  kellekensf  fkcfrvlsm
421  sqglyefsth  rlmtdtvaeg  lfstklatr  mateifvcml  ekknksrfea  vltsldkkfr
481  igqnlhmiqn  fkkmpqyfs  ltleshikii  qawlfaveqt  ldgrgkmgsl  vgasdefkng
541  ggenaileyc  qwtmvfinhl  cscsdninqr  mllrtklenc  gilrimnkik  lldydavidq
601  ielydnkkld  dfnvkleann  kafnvdlhdp  lsilknlwdi  ckgtenekl  vslvqhlfls
661  ssklieenqn  sskltkqikl  mdsivtnvsv  astsdeetnm  nmaiqrllyda  mqtdevarra
721  ilesraltkk  leeiqaerds  lseklskaeh  glvggledel  herdrilakn  qrvmqgleae
781  leelkkkhll  ekhqgevelr  kmtilnsrp  eesfnknegt  rgmnsslss  ekaniqkvlg
841  dqlsrakddy  kddskkfgmt  lqpnkrklml  rmqmeniene  arqlemtnfa  efekdrlepp
901  ihikkpkvkk  mknkdrkplv  kpqeadvnkl  ndlrralaei  qmesndiskf  nveervneif
961  nekkslalkr  ikeletkykg  fgidfnvdei  mdsppkntgd  veteedanya  sldpktyqkk
1021  ldeinritdq  lldiqttqte  eiqvveedgs  dlsssssdde  seeiyqdasp  tqelrshse
1081  lssgsgpgsf  ldalsqkygt  gqnvtsaaaf  gennngsgig  plhskvektf  mnrlrkstvs
1141  sapyleeltq  kvnkvepyeq  nedegldkks  lpenstasaa  safdkaekdm  rghvengkqg
1201  rrvnheedkt  adfsavskln  ntdgaedlst  qssvlssqpp  pppppppvp  aklfgeslek

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1261 ekkseddtvk qettgdspap pppppppppp pmalfgkpkg etppppplps vlssstdgvi
1321 ppappmmpas qiksavtsp1 lpqspslfek yprphkklkq lhweklidctd nsiwgtgkae
1381 kfaddlyekg vladlekafa areikslask rkedlqkitf lsrdissqqfg inlhmyssls
1441 vadlvkkiin cdrdflqtps vveflsksei ievsvnlarn yapystdweg vrnledakpp
1501 ekdpndlqra dqilyqlmvr lesywgsmr altvvtssyer eynellaklr kvdkavsalq
1561 esdnlnrvfn vilavgnfmn dtskqaggfk lstlqrltft kdttnsmtfl nyvekivrln
1621 ypsfndflse lepvl dvvkv sieqlvndck dfsqsi vve rsveignlsd sskfhpldkv
1681 liktlpvlpe arkkgdlled evkltimefe slmhtygeds gdkfakisff kkfadfiney
1741 kkaqaqlaa eeerlyikh kkiveeqqkr aqekekqken snspsegne edeaedravr
1801 mdklleqlkn agpaksdpss arkralvrkk ylsekdnapp lndldteeg silyspeamd
1861 ptadtvihae sptplatrgv mntsedlpsp sktsaledge eisdrarmll kelrgsdtpv
1921 kqnsildehl eklrarkers igeastgnrl sfk

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[0077] SEQ ID NO: 4 is the nucleotide sequence corresponding to *S.cerevisiae* formin
(residues 1-5862):

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1 atgttgaaga attcaggctc caaacattcg aactcaaagg aaagtcattc gaattcgagt
61 tctgggtatat tccaaaatth gaagcgtttg gctaactcaa atgcaacgaa tagcaatacg
121 ggtttctccga cctatgcac ccaacaacag cactctccag tgggaaacga agtgtccact
181 tcacctgcat cttcgtcat ttttagaaga ctgaatgcac cttctaggtc tacatctact
241 gaggcgaggc cgttgaataa aaaatcgaca ttaaacacac aaaactgtc tcaatatatg
301 aatggtaaat taagtggaga tgttccgta tctcacagc acgcaaggtc gcattcaatg
361 caatcgaaat attcgtatc caagaggaat tcttcacag cgtccaataa gctaacaagg
421 cagcataccg ggcaaagtca ctccgcatca agtcttctct ctcaagggtc gctaactaac
481 ttgagcaaat tcactacacc cgatggtaaa atttatttag aaatgcgctc ggaccatac
541 gaggtggaag ttttgtttga agatattatg tataaaagaa atatttttca gtctttatca
601 gaggacaaac aagaagctct gatgggctac agcattgaga agaaattggt gattgttaag
661 caggatttac aaaatgagct aaaaaaaatg cgagcaaca ctacatcttc ttcactgcc
721 tccagaactt cgatggcgtc ggaccatcat cctatcctta cggctaattc atctctttcg
781 tctcccaaat ctgttttgat gacaagcgcc tcttctccca cctcaactgt atacagcaac
841 agttttaaat attctactac tctttcatcg gtaggtacgt caacctcgaa ggggaaaaaa
901 ttagttagcg ggagtttgaa aaagcagcca tctctaaaca atattttatg aggtggggct
961 gagaacaata cgagtgcac tactcttcca ggagacagaa caaacagacc accgatacat
1021 tatgtgcaac gaatcctggc agacaaaact actagcgatg agatgaaaga tttatgggta
1081 atcttgaaga ctgaacagtt agactgggta gacgcttcta tagaccatca gggtcactat
1141 gctatggcta atgtactaat gaactctatt tataaagact caccocgaga aaacttgact
1201 aaagaattat tagagaaaga aaattcattt tttaaatgct tcagagtctt atcaatgctc
1261 tcacaagggt tgtatgagtt cagtacacat aggttaatga ctgatactgt tgcagaagg
1321 ttattctcta cgaactggc cacaaggaaa atggccactg aaatttttgt ttgtatgcta
1381 gaaaaaaaaa ataaaagcag attcgaagca gttcttacct cactagataa aaagtttagg
1441 atagggcaaa atcttcatat gattcaaaaac ttcaaaaaaa tgcccagta tttctctcat
1501 ttaaccttgg aaagtcattt gaaaattatt caagcctggg tatttgcgt tgaacaaact
1561 ttgatgggaa gaggaaaaat gggttcctta gtaggcgct cggatgaatt taaaaatggt
1621 gggggtgaaa atgcgattct agaatactgc caatggacaa tggtttttat caatcattta
1681 tgctcttgct ctgataacat aaatcaaaga atgctattaa ggacgaaact agaaaattgt
1741 ggaatttttac gaattatgaa caaaataaaa ctgctagatt acgacaaggt aattgaccac
1801 attgagttat atgacaataa taaacttgac gatttttaacg ttaaaattaga ggccaacaat
1861 aaggcctttta atgtagattt acacgatcca ctatcattat taaaaaacct ttgggatata
1921 tgtaaaggta ccgagaatga aaagctttta gtatctttag ttcaacatct tttctttct
1981 agttcaaagt taatagaaga aaacaaaaat tctctaaac ttactaagca attgaaactt
2041 atggattctt tggtagacta tgtagtggt gctcaactt ctgatgaaga aactaatatg
2101 aatatggcta tccagcggtt ttacgatgcc atgcagactg atgaagttgc acgtcgagct
2161 atattagaaa gtagagctct aacgaagaaa ttggaagaaa ttcaggcaga aagggtattct
2221 ttaagtgaag agttaagcaa ggcgagacac ggactcgttg gacagttaga agatgagttg
2281 catgagagag atcgtatttt agctaagaat cagagagttg tgcagcagct ggaagctgag
2341 ttagaagagt tgaaaaagaa gcatcttttg gaaaagcatc agcaagaggt agaattgaga
2401 aaaattgtga ctatattgaa ttcgaggcct gaagaaaagct tcaataagaa tgaaggcacc
2461 agagggtatga actcgagcct aaattcttca gagaaaagca atatccaaa ggtgttacag
2521 gacggattat caagagcaaa aaaggattac aaggatgatt caaaaaaatt cggcatgaca

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2581 cttcaacctta ataagagatt aaaaatgtta agaatgcaaa tggaaaatat tgaaaatgaa
2641 gccaggcaac tagagatgac aaattttgct gaatttgaga aagatcgtct tgaacctcca
2701 atacatatta agaagcccaa agtgaaagaag atgaaaaata aggataggaa accttttagtc
2761 aagcctcaag aggcggacgt aaataaacta aatgacctaa ggcgggcttt ggctgaaatc
2821 caaatggaaa gtaatgatat ttccaaatft aacgtcgaag aacgtgttaa tgagctatft
2881 aatgaaaaga aatctctggc tttaaagaga ctgaaaagaa tagaaaacaa atacaaagga
2941 tttgggtattg actttaatgt cgacgagatt atggacagtc caaagaaaaa tactggggac
3001 gtggaaaacgg aagaagacgc taactatgcc agtcttgacc ctaagacata tcagaaaaag
3061 ttagacgaga ttaatagaat aacggaccag ttgttagata ttcagactca aaccgaacac
3121 gaaatacaag tagaagaaga tggggaaaag gatctttcct cttcaagttc tgatgatgaa
3181 agtgaggaaa tataccaaga cgcctctcca actcaagaac taaggagcga acactcagag
3241 ctatcttcag gatcagggtcc tgggtctttt ctcgatgcct tatctcaaaa atatgggtact
3301 ggtcaaaaatg ttactgcctc ggctgctttt ggagagaaca ataacggttc ggggtattgga
3361 cctctacata gtaaagtfta aaagaccttt atgaacagac tcagaaaaatc caccgtgtct
3421 tctgtctccat atttagagga gttaacacag aaggtaaata aagttgaacc atatgaacaa
3481 aatgaagatg aaggtctaga taaaaaatct ttaccggaaa acagcacggc aagtgacagt
3541 tccgcctttt acaaagctga gaaagacatg agacaacatg tggaaaacgg aaagcaaggg
3601 cgtgtggtaa atcatgaaga agataaaaaca ggcgatttca gcgctgtgag taaactgaat
3661 aatacagatg ggcgagaaga tctctctact caatcatctg tactctctc acagccgcca
3721 ccgcctctct ctcctctctc tccagtctcc gccaaattgt tcgggtgaatc gttggagaaa
3781 gagaaaaagt cgaagacga cactgtcaag caagaaacta ctggggattc tctgtctct
3841 cccctctcac caccaccacc tctctctcca ccatggcat tgttcggcaa acccaaggga
3901 gaaacaccac caccgcgcgc gctaccatct gtattatctt catctactga tggcgtcatt
3961 ccgcgcgcct cactatgat gccgcgatog caaatcaaat cagctgtaac atcaccatta
4021 cttctcaaat ccgcctcatt atttgaagaag tatctctctc cacacaagaa attaaagcaa
4081 ctgcattggg aaaaattaga ttgtactgat aattctatft ggggtacagg taaagctgag
4141 aagttcgcag atgacttata cgaaaagggt gttcttgctg atttagaaaa ggcctttgcc
4201 gctagggaaa ttaaatcatt agcaagcaag cgcaaggaa atctgcagaa aattacattt
4261 ttatccaggg atattttctc acagtfttgg atcaatttgc atatgtattc atcactttcg
4321 gttgcgcgac tggtgaaaaa gattctaaac tgcgataggg attttttgca aacaccgagt
4381 gttgttgagt tttgtcaaa atctgaaata attgaagttt ctgttaattt agctaggaat
4441 tacgcgccgt attcgacaga ctgggagggc gtgaggaaac tcgaagatgc caagccctct
4501 gaaaaagatc caaacgattt acagagggtc gaccaaaatt atttgcaatt gatggttaat
4561 ctagaatcat actgggggtc acgtatgaga gctctcacag tggttacttc gtatgaaaga
4621 gaataataat agttattggc aaagttaaga aaagtcgaca aagctgttag ccgcgttcaa
4681 gaatcagata acttgccgaa tgtctttaat gttatcttag cagttggtaa tttcatgaac
4741 gacacttcta agcaggcaca aggtttcaaa ctttcaacct tgcagagggt gacgtttatc
4801 aaggatacta ccaatagcat gacctttcta aattacgtcg aaaaaattgt cagggttaaat
4861 tatccaagct tcaatgactt tctaagtgaa ttagaacctg tattggatgc cgtgaaagtt
4921 tccattgagc agttagttaa cgactgtaa gacttttagc aatcgattgt aaacgtagag
4981 cgttcagttg aaattggtaa tttaagcgt tcttcaaagt ttcattccatt agataaagtt
5041 ttgataaaaa cattgcccgt gttacoggaa gccagaaaaa agggggactt gttagaagac
5101 gaagttaagc tgactatcat ggaatttgaa agtctgatgc atacgtacgy tgaagattct
5161 ggtgataagt ttgcaaaaaa ctcatctctt aagaaatttg cagattttat aaatgaatac
5221 aaaaaagctc aagcgcaaaa tctagccgcg gaagaagaag aaagacttta tataaagcat
5281 aagaaaatag tggaaagaaca gcaaaaaagg gcacaagaaa aggaaaaaca aaaagagaac
5341 agcaattcgc ctcatctga aggaaatgaa gaggatgaag cagaagatag gcgcgtgtt
5401 atggataaac tattagaaca gttgaaaaat gcaggtctct ctaaaagtga cccatcttcc
5461 gccagaaaga gagccctagt gaggaaaaaa tatctttctg aaaaagacaa cgtctccaaa
5521 ttactcaacg atttggtatc tgaagaaggg tctattcttt attctccaga agctatggac
5581 cctactgccg atacagtgat tcacgctgaa tcaccactc cgcttgccac aaggggtgtt
5641 atgaatacct ctgaagattt accatcgcca tccaagacat ctgcgctgga agatcaagag
5701 gaaattagt acagagctag aatgttatta aaggagctaa gaggtctga cacaccagta
5761 aagcaaaatt ccatactga tgaacatcta gaaaaattga gggcccgcaa ggaaagatcc
5821 ataggtgagg ctagcacagg taacaggcta agtttcaaat aa

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[0078] For example, the polypeptide sequence of *S.cerevisiae* profilin is depicted in SEQ ID NO: 5. The nucleotide sequence of *S.cerevisiae* profilin is shown in SEQ ID NO: 6. Sequence information related to Pfy1p, *S.cerevisiae* profilin is accessible in public databases

by GenBank Accession numbers NP_014765.3 (protein) and NM_001183541.3 (nucleic acid).

[0079] SEQ ID NO: 5 is the amino acid sequence corresponding to *S.cerevisiae* profilin (residues 1-126):

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1 mswqaytdnl igtgkvdkav iysragdavw atsgglslqp neigeivqgf dnpaglqsnq
61 lhiqqqkfml lraddrsiyg rhdaegvvcv rtkgtviah ypptvqagea tkiveqlady
121 ligvqy

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[0080] SEQ ID NO: 6 is the nucleotide sequence corresponding to *S.cerevisiae* profilin (residues 1-381):

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1 atgtcttggc aagcatacac tgataactta ataggaaccg gtaaagtcga caaagctgtc
61 atctactcga gagcaggtga cgctgtttgg gctacttctg gtggcctatc tttgcaacca
121 aacgaaattg gtgaaattgt tcaaggcttc gacaatccag ctggtttgca aagcaatggt
181 ttgcatattc aaggccaaaa gttcatgttg ttgagagctg acgatagaag tatctacggt
241 agacatgatg ctgagggtgt tgtttgtgta agaactaagc aaaccgttat tattgtcat
301 tatccaccaa ccgtacaagc cggtagggcc accaagattg tcgagcaatt ggctgactac
361 ttgattggtg ttcaataacta a

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[0081] A protein filament monomer or protein filament binding protein can also encompass ortholog genes, which are genes conserved among different biological species such as humans, dogs, cats, mice, and rats, that encode proteins (for example, homologs (including splice variants), mutants, and derivatives) having biologically equivalent functions as the human-derived protein. Orthologs of protein filament monomer or protein filament binding protein include any eukaryotic ortholog inclusive of the ortholog in humans and other primates, experimental mammals (such as mice, rats, hamsters and guinea pigs), mammals of commercial significance (such as horses, cows, camels, pigs and sheep), and also companion mammals (such as domestic animals, e.g., rabbits, ferrets, dogs, and cats) or any other eukaryote (such as birds, *S.cerevisiae*, *D.melanogaster*, *C.elegans*, and *D.discoideum*). A protein filament monomer or protein filament binding protein can comprise a protein encoded by a nucleic acid sequence homologous to the human nucleic acid, wherein the nucleic acid is found in a different species and wherein that homolog encodes a protein similar to a protein filament monomer or protein filament binding protein. For example, sequence information related to human formin, is accessible in public databases by GenBank Accession numbers NP_001264242.1 (protein isoform a), NP_001096654.1 (protein isoform b), or NP_001264243.1 (protein isoform c) and NM_001277313.1 (nucleic acid isoform a), NM_001103184.3 (nucleic acid isoform b), or NM_001277314.1 (nucleic acid isoform c).

For example, sequence information related to human profilin is accessible in public databases by GenBank Accession numbers NP_005013.1 (protein) and NM_005022.3 (nucleic acid).

[0082] The invention utilizes conventional molecular biology, microbiology, and recombinant DNA techniques available to one of ordinary skill in the art. Such techniques are well known to the skilled worker and are explained fully in the literature. See, e.g., Maniatis, Fritsch & Sambrook, "DNA Cloning: A Practical Approach," Volumes I and II (D. N. Glover, ed., 1985); "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984); "Nucleic Acid Hybridization" (B. D. Hames & S. J. Higgins, eds., 1985); "Transcription and Translation" (B. D. Hames & S. J. Higgins, eds., 1984); "Animal Cell Culture" (R. I. Freshney, ed., 1986); "Immobilized Cells and Enzymes" (IRL Press, 1986); B. Perbal, "A Practical Guide to Molecular Cloning" (1984), and Sambrook, et al., "Molecular Cloning: a Laboratory Manual" (2001).

[0083] One skilled in the art can obtain a protein filament monomer or protein filament binding protein, (e.g., actin, formin, profilin) in several ways, which include, but are not limited to, isolating the protein via biochemical means or expressing a nucleotide sequence encoding the protein of interest by genetic engineering methods.

[0084] The invention provides for a protein filament monomer or protein filament binding protein that are encoded by nucleotide sequences. The protein filament monomer or protein filament binding protein can be a polypeptide encoded by a nucleic acid (including genomic DNA, complementary DNA (cDNA), synthetic DNA, as well as any form of corresponding RNA). For example, a protein filament monomer or protein filament binding protein can be encoded by a recombinant nucleic acid encoding a human or chicken protein filament monomer or protein filament binding protein, or fragment thereof. The protein filament monomers or protein filament binding proteins of the invention can be obtained from various sources and can be produced according to various techniques known in the art. For example, a nucleic acid that encodes a protein filament monomer or protein filament binding protein can be obtained by screening DNA libraries, or by amplification from a natural source. The protein filament monomer or protein filament binding protein of the invention can be produced via recombinant DNA technology and such recombinant nucleic acids can be prepared by conventional techniques, including chemical synthesis, genetic engineering, enzymatic techniques, or a combination thereof. A protein filament monomer or protein filament binding protein of this invention can also encompass variants of the

protein filament monomers or protein filament binding proteins. The variants can comprise naturally-occurring variants due to allelic variations between individuals (e.g., polymorphisms), mutated alleles, or alternative splicing forms.

[0085] In one embodiment, a fragment of a nucleic acid sequence that comprises a protein filament monomer or protein filament binding protein (such as, e.g., actin, formin, or profilin) can encompass any portion of at least about 8 consecutive nucleotides of SEQ ID NO: 2, 4, or 6. In one embodiment, the fragment can comprise at least about 10 nucleotides, at least about 15 nucleotides, at least about 20 nucleotides, or at least about 30 nucleotides of SEQ ID NO: 2, 4, or 6. Fragments include all possible nucleotide lengths between about 8 and about 100 nucleotides, for example, lengths between about 15 and about 100 nucleotides, or between about 20 and about 100 nucleotides.

[0086] A protein filament monomer or protein filament binding protein, can be a fragment of a protein filament monomer or protein filament binding protein, such as, e.g., actin, formin, or profilin. For example, the protein filament monomer or protein filament binding protein fragment can encompass any portion of at least about 8 consecutive amino acids of SEQ ID NO: 1, 3, or 5. The fragment can comprise at least about 10 consecutive amino acids, at least about 20 consecutive amino acids, at least about 30 consecutive amino acids, at least about 40 consecutive amino acids, at least about 50 consecutive amino acids, at least about 60 consecutive amino acids, at least about 70 consecutive amino acids, at least about 80 consecutive amino acids, at least about 90 consecutive amino acids, at least about 100 consecutive amino acids, at least about 110 consecutive amino acids, or at least about 120 consecutive amino acids of SEQ ID NOS: 1, 3, or 5. Fragments include all possible amino acid lengths between about 8 and about 80 amino acids, for example, lengths between about 10 and about 80 amino acids, between about 15 and about 80 amino acids, between about 20 and about 80 amino acids, between about 35 and about 80 amino acids, between about 40 and about 80 amino acids, between about 50 and about 80 amino acids, or between about 70 and about 80 amino acids.

[0087] Tethering Protein Filaments and Filament Binding Proteins

[0088] As described herein, the protein filaments can be coupled to a filament binding protein which can be attached to the substrate, to the lipid bilayer, or to the diffusion barrier, to form an array. In another embodiment, the protein filament can be attached to the

substrate, to the lipid bilayer, or to the diffusion barrier, to form an array. The protein filaments can be attached by a linkage or by coupling to a filament binding protein either at one end of the protein filament or at both ends. For example, when a protein is coated on the substrate prior to the deposition of the lipid bilayer, the protein filament or filament binding protein can be linked to a cognate protein that binds to the protein coated on the substrate. In one embodiment, the substrate is coated with neutravidin and the protein filament or filament binding protein linker is biotin. Linkers can be added to the protein filament or filament binding protein using standard molecular biology techniques known to those of ordinary skill in the art.

[0089] Alternatively, the protein filament or filament binding protein can be linked to the lipid bilayer. In one embodiment, the lipid bilayer is deposited onto the substrate and a protein, e.g., neutravidin or streptavidin, is linked to the lipid head groups. Biotinylated protein filaments or filament binding proteins are then introduced, linking the protein filament or filament binding protein to the lipid bilayer. In one embodiment, biotinylated lipids are bound to streptavidin which links to a biotinylated filament binding protein, such as, biotinylated formin.

[0090] In other embodiments, the protein filament or filament binding protein can be linked to the diffusion barriers. In one embodiment, the diffusion barrier is a protein, e.g., biotinylated bovine serum albumin (BSA), deposited on the substrate. Neutravidin is then bound directly to the biotinylated BSA protein barriers, and biotinylated protein filaments or filament binding proteins are linked to the biotinylated BSA protein barriers.

[0091] Other known protein-cognate protein pairs can be used in the methods described herein. For example, antibodies, e.g., anti-digoxigenin antibodies, can be used as protein barriers and the cognate antigen, e.g., digoxigenin, linked to the protein filaments or filament binding proteins.

[0092] Labeling of Polypeptides

[0093] In another embodiment, the attached protein filament or filament binding protein and/or the interacting polypeptides are visualized by detecting one or more labels attached to the polypeptides. The labels may be incorporated by any of a number of means well known to those of skill in the art.

[0094] Detectable labels suitable for use in the methods and compositions described herein include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels include biotin for staining with labeled streptavidin conjugate, magnetic beads (e.g., Dynabeads.TM.), fluorescent dyes (e.g., fluorescein, Texas red, rhodamine, green fluorescent protein, and the like, see, e.g., Molecular Probes, Eugene, Oreg.), radiolabels (e.g., ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold (e.g., gold particles in the 40-80 nm diameter size range scatter green light with high efficiency) or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads. Patents teaching the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

[0095] In some embodiments, fluorescent labels are used. The protein filament or filament binding protein can all be labeled with a single label, e.g., a single fluorescent label. Alternatively, different protein filament or filament binding protein can have different labels. For example, one protein filament or filament binding protein can have a green fluorescent label and a second protein filament or filament binding protein can have a red fluorescent label.

[0096] Suitable chromogens which can be employed include those molecules and compounds that absorb light in a distinctive range of wavelengths so that a color can be observed or, alternatively, which emit light when irradiated with radiation of a particular wave length or wave length range, e.g., fluorescers.

[0097] A wide variety of suitable dyes are available, being primary chosen to provide an intense color with minimal absorption by their surroundings. Illustrative dye types include quinoline dyes, triarylmethane dyes, acridine dyes, alizarine dyes, phthaleins, insect dyes, azo dyes, anthraquinoid dyes, cyanine dyes, phenazathionium dyes, and phenazoxonium dyes.

[0098] A wide variety of fluorescers can be employed either by alone or, alternatively, in conjunction with quencher molecules. Fluorescers of interest fall into a variety of categories having certain primary functionalities. These primary functionalities include 1- and 2-aminonaphthalene, p,p'-diaminostilbenes, pyrenes, quaternary phenanthridine salts, 9-aminoacridines, p,p'-diaminobenzophenone imines, anthracenes, oxacarbocyanine,

marocyanine, 3-aminoequilenin, perylene, bisbenzoxazole, bis-p-oxazoly benzene, 1,2-benzophenazin, retinol, bis-3-aminopyridinium salts, hellebrigenin, tetracycline, sterophenol, benzimidazolyphenylamine, 2-oxo-3-chromen, indole, xanthen, 7-hydroxycoumarin, phenoxazine, salicylate, strophanthidin, porphyrins, triarylmethanes and flavin. Individual fluorescent compounds that have functionalities for linking or that can be modified to incorporate such functionalities include, e.g., dansyl chloride; fluoresceins such as 3,6-dihydroxy-9-phenylxanthidrol; rhodamineisothiocyanate; N-phenyl 1-amino-8-sulfonatonaphthalene; N-phenyl 2-amino-6-sulfonatonaphthalene; 4-acetamido-4-isothiocyanato-stilbene-2,2'-disulfonic acid; pyrene-3-sulfonic acid; 2-toluidinonaphthalene-6-sulfonate; N-phenyl, N-methyl 2-aminoaphthalene-6-sulfonate; ethidium bromide; stebrine; auromine-0,2-(9'-anthroyl)palmitate; dansyl phosphatidylethanolamine; N,N'-dioctadecyl oxacarbocyanine; N,N'-dihexyl oxacarbocyanine; merocyanine, 4(3'pyrenyl)butyrate; d-3-aminodesoxy-equilenin; 12-(9'-anthroyl)stearate; 2-methylanthracene; 9-vinylanthracene; 2,2'(vinylene-p-phenylene)bisbenzoxazole; p-bis[2-(4-methyl-5-phenyl-oxazoly)]benzene; 6-dimethylamino-1,2-benzophenazin; retinol; bis(3'-aminopyridinium) 1,10-decandiyl diiodide; sulfonaphthylhydrazone of hellibrienin; chlorotetracycline; N(7-dimethylamino-4-methyl-2-oxo-3-chromenyl)maleimide; N-[p-(2-benzimidazoly)-phenyl]maleimide; N-(4-fluoranthyl)maleimide; bis(homovanillic acid); resazarin; 4-chloro-7-nitro-2,1,3-benzooxadiazole; merocyanine 540; resorufin; rose bengal; and 2,4-diphenyl-3 (2H)-furanone.

[0099] The label may be a "direct label", i.e., a detectable label that is directly attached to or incorporated into the protein filament or filament binding protein. Alternatively, the label may be an "indirect label", i.e., a label joined to the protein filament or filament binding protein after attachment to the substrate. The indirect label can be attached to a binding moiety that has been attached to the protein filament or filament binding protein prior to attachment to the substrate.

[00100] Polypeptides can be visualized by coupling them to, e.g., fluorescent labels described herein, using known methods. Alternatively, other labels, such as Quantum dots (Invitrogen) can be used, as described herein. In one embodiment, actin monomers can be labeled with the fluorescent dye Oregon green. In another embodiment, actin-binding proteins can be labeled with quantum dots or other fluorescent probes would permit visualization of interactions. In one embodiment, polypeptides can be labeled with

fluorescent proteins by cloning of a protein filament monomer or filament binding protein into vectors encoding fluorescent proteins.

[00101] Purified polypeptides

[00102] One skilled in the art understands that polypeptides (for example, monomers of protein filaments (e.g., actin monomers, alpha tubulin, beta tubulin, lamin, vimentin, neurofilament, keratin) and filament binding proteins (e.g. profilin, formin)) can be obtained in several ways, which include but are not limited to, expressing a nucleotide sequence encoding the protein of interest, or fragment thereof, by genetic engineering methods.

[00103] In one embodiment, the nucleic acid is expressed in an expression cassette, for example, to achieve overexpression in a cell. The nucleic acids of the invention can be an RNA, cDNA, cDNA-like, or a DNA of interest in an expressible format, such as an expression cassette, which can be expressed from the natural promoter or an entirely heterologous promoter. The nucleic acid of interest can encode a protein, and may or may not include introns. Any recombinant expression system can be used, including, but not limited to, bacterial, mammalian, yeast, insect, or plant cell expression systems.

[00104] Host cells transformed with a nucleic acid sequence encoding a protein filament monomer or filament binding protein (e.g., actin monomers, alpha tubulin, beta tubulin, lamin, vimentin, neurofilament, keratin, profilin, formin) can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. Expression vectors containing a nucleic acid sequence encoding a protein filament monomer or filament binding protein can be designed to contain signal sequences which direct secretion of soluble polypeptide molecules encoded by a protein filament monomer or filament binding protein (e.g., actin monomers, alpha tubulin, beta tubulin, lamin, vimentin, neurofilament, keratin, profilin, formin), through a prokaryotic or eukaryotic cell membrane.

[00105] Nucleic acid sequences comprising a protein filament monomer or filament binding protein (e.g., actin monomers, alpha tubulin, beta tubulin, lamin, vimentin, neurofilament, keratin, profilin, formin) that encode a polypeptide can be synthesized, in whole or in part, using chemical methods known in the art. Alternatively, a protein filament monomer or filament binding protein can be produced using chemical methods to synthesize

its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques. Protein synthesis can either be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of a protein filament monomer or filament binding protein can be separately synthesized and combined using chemical methods to produce a full-length molecule.

[00106] A synthetic peptide can be substantially purified via high performance liquid chromatography (HPLC). The composition of a synthetic protein filament monomer or filament binding protein can be confirmed by amino acid analysis or sequencing. Additionally, any portion of an amino acid sequence comprising a protein encoded by a protein filament monomer or filament binding protein (e.g., actin monomers, alpha tubulin, beta tubulin, lamin, vimentin, neurofilament, keratin, profilin, formin) can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

[00107] The invention further encompasses methods for using a protein or polypeptide encoded by a nucleic acid sequence of a protein filament monomer or filament binding protein, such as the sequences shown in SEQ ID NOS: 1, 3, or 5. In another embodiment, the polypeptide can be modified, such as by glycosylations and/or acetylations and/or chemical reaction or coupling, and can contain one or several non-natural or synthetic amino acids. An example of a protein filament monomer or filament binding protein has the amino acid sequence shown in either SEQ ID NO: 1, 3, or 5. In certain embodiments, the invention encompasses variants of a human protein encoded by a protein filament monomer or filament binding protein (e.g., actin monomers, alpha tubulin, beta tubulin, lamin, vimentin, neurofilament, keratin, profilin, formin).

[00108] In one embodiment, chicken skeletal muscle actin can be purified from an acetone powder of chicken breast muscle by one cycle of polymerization and depolymerization followed by gel filtration. In another embodiment, a fraction of the actin can be labeled at a cysteine residue with Oregon green 488 iodoacetamide, followed by depolymerization and purification by ion exchange and gel filtration. In one embodiment, the cysteine residue is cysteine 374. In one embodiment, the fraction of actin labeled with Oregon green is at least 33%. In another embodiment, the fraction of actin labeled with Oregon green is at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%,

at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%.

[00109] In one embodiment, *S. cerevisiae* formin Bni1p can be cloned into a vector using primers that encode an N-terminal biotinylation sequence. In one embodiment, *S. cerevisiae* profilin can be cloned into a vector. In one embodiment, the vector is pQE70. In another embodiment, the vector is pMW172-SpPRF. In another embodiment, the construct can also contained a C-terminal 6-His tag. In one embodiment, the construct can be transformed into a bacterial expression system. In one embodiment, the bacterial expression system is BL21 DE3 RP Codon Plus cells. In one embodiment, expression can be induced overnight at 16°C with IPTG. In another embodiment, expression can be induced in the presence of biotin.

[00110] In one embodiment, polypeptides (e.g., formin, profilin) can be purified by lysing cells and purifying by affinity chromatography. In one embodiment, the affinity chromatography is carried out using Ni-NTA resin and imidazole. In one embodiment, the affinity chromatography is carried out using a poly-L-proline-agarose column and urea. In one embodiment, the biotinylated can be incubated with avidin resin and eluted with biotin. In one embodiment, eluted protein can be concentrated using spin-columns followed by dialysis and flash-freezing.

[00111] Expression Systems

[00112] Bacterial Expression Systems. One skilled in the art understands that expression of desired protein products in prokaryotes is most often carried out in *E. coli* with vectors that contain constitutive or inducible promoters. Some non-limiting examples of bacterial cells for transformation include the bacterial cell line *E. coli* strains DH5 α , MC1061/p3 (Invitrogen Corp., San Diego, Calif.), and BL21 DE3 RP Codon Plus cells, which can be transformed using standard procedures practiced in the art, and colonies can then be screened for the appropriate plasmid expression. In bacterial systems, a number of expression vectors can be selected. Non-limiting examples of such vectors include multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), pQE70 and pMW172-SpPRF. Some *E. coli* expression vectors (also known in the art as fusion-vectors) are designed to add a number of amino acid residues, usually to the N-terminus of the expressed recombinant protein. Such fusion vectors can serve three functions: 1) to increase the

solubility of the desired recombinant protein; 2) to increase expression of the recombinant protein of interest; and 3) to aid in recombinant protein purification by acting as a ligand in affinity purification. In some instances, vectors, which direct the expression of high levels of fusion protein products that are readily purified, may also be used. Some non-limiting examples of fusion expression vectors include pGEX, which fuse glutathione S-transferase (GST) to desired protein; pcDNA 3.1/V5-His A B & C (Invitrogen Corp, Carlsbad, CA) which fuse 6x-His to the recombinant proteins of interest; pMAL (New England Biolabs, MA) which fuse maltose E binding protein to the target recombinant protein; the *E. coli* expression vector pUR278 (Ruther et al., (1983) *EMBO* 12:1791), wherein the coding sequence may be ligated individually into the vector in frame with the lac Z coding region in order to generate a fusion protein; and pIN vectors (Inouye et al., (1985) *Nucleic Acids Res.* 13:3101-3109; Van Heeke et al., (1989) *J. Biol. Chem.* 24:5503-5509. Fusion proteins generated by the likes of the above-mentioned vectors are generally soluble and can be purified easily from lysed cells via adsorption and binding of the fusion protein to an affinity matrix. For example, fusion proteins can be purified from lysed cells via adsorption and binding to a matrix of glutathione agarose beads subsequently followed by elution in the presence of free glutathione. For example, the pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target can be released from the GST moiety.

[00113] Plant, Insect, and Yeast Expression Systems. Other suitable cell lines, in addition to microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing coding sequences for a protein filament monomer or filament binding protein may alternatively be used to produce the molecule of interest. A non-limiting example includes plant cell systems infected with recombinant virus expression vectors (for example, tobacco mosaic virus, TMV; cauliflower mosaic virus, CaMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing coding sequences for a protein filament monomer or filament binding pr. If plant expression vectors are used, the expression of sequences encoding a protein filament monomer or filament binding protein can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from tobacco mosaic virus TMV. Alternatively, plant promoters such as the small subunit of

RUBISCO or heat shock promoters, can be used. These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection.

[00114] In another embodiment, an insect system also can be used to express encoding a protein filament monomer or filament binding protein. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding a protein filament monomer or filament binding protein can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the nucleic acid sequences of a protein filament monomer or filament binding protein will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which a protein filament monomer or filament binding protein can be expressed.

[00115] In another embodiment, a yeast (for example, *Saccharomyces* sp., *Pichia* sp.) system also can be used to express a protein filament monomer or filament binding protein. Yeast can be transformed with recombinant yeast expression vectors containing coding sequences for a protein filament monomer or filament binding protein.

[00116] Mammalian Expression Systems. Mammalian cells (such as BHK cells, VERO cells, CHO cells and the like) can also contain an expression vector (for example, one that harbors a nucleotide sequence encoding a protein filament monomer or filament binding protein) for expression of a desired product. Expression vectors containing such a nucleic acid sequence linked to at least one regulatory sequence in a manner that allows expression of the nucleotide sequence in a host cell can be introduced via methods known in the art. A number of viral-based expression systems can be used to express a protein filament monomer or filament binding protein in mammalian host cells. The vector can be a recombinant DNA or RNA vector, and includes DNA plasmids or viral vectors. For example, if an adenovirus is used as an expression vector, sequences encoding a protein filament monomer or filament binding protein can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion into a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a protein filament monomer or filament binding protein in infected host cells. Transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can also be used to

increase expression in mammalian host cells. In addition, viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, lentivirus or alphavirus.

[00117] Regulatory sequences are well known in the art, and can be selected to direct the expression of a protein or polypeptide of interest (such as a protein filament monomer or filament binding protein) in an appropriate host cell as described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Non-limiting examples of regulatory sequences include: polyadenylation signals, promoters (such as CMV, ASV, SV40, or other viral promoters such as those derived from bovine papilloma, polyoma, and Adenovirus 2 viruses (Fiers, et al., 1973, *Nature* 273:113; Hager GL, et al., *Curr Opin Genet Dev*, 2002, 12(2):137-41) enhancers, and other expression control elements. Practitioners in the art understand that designing an expression vector can depend on factors, such as the choice of host cell to be transfected and/or the type and/or amount of desired protein to be expressed.

[00118] Enhancer regions, which are those sequences found upstream or downstream of the promoter region in non-coding DNA regions, are also known in the art to be important in optimizing expression. If needed, origins of replication from viral sources can be employed, such as if a prokaryotic host is utilized for introduction of plasmid DNA. However, in eukaryotic organisms, chromosome integration is a common mechanism for DNA replication.

[00119] For stable transfection of mammalian cells, a small fraction of cells can integrate introduced DNA into their genomes. The expression vector and transfection method utilized can be factors that contribute to a successful integration event. For stable amplification and expression of a desired protein, a vector containing DNA encoding a protein of interest (for example, a protein filament monomer or filament binding protein) is stably integrated into the genome of eukaryotic cells (for example mammalian cells, such as HEK293 cells), resulting in the stable expression of transfected genes. An exogenous nucleic acid sequence can be introduced into a cell (such as a mammalian cell, either a primary or secondary cell) by homologous recombination as disclosed in U.S. Patent 5,641,670, the contents of which are herein incorporated by reference.

[00120] A gene that encodes a selectable marker (for example, resistance to antibiotics or drugs, such as ampicillin, neomycin, G418, and hygromycin) can be introduced into host

cells along with the gene of interest in order to identify and select clones that stably express a gene encoding a protein of interest. The gene encoding a selectable marker can be introduced into a host cell on the same plasmid as the gene of interest or can be introduced on a separate plasmid. Cells containing the gene of interest can be identified by drug selection wherein cells that have incorporated the selectable marker gene will survive in the presence of the drug. Cells that have not incorporated the gene for the selectable marker die. Surviving cells can then be screened for the production of the desired protein molecule (for example, a protein filament monomer or filament binding protein).

[00121] A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed a protein filament monomer or filament binding protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a “prepro” form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, Va. 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

[00122] An exogenous nucleic acid can be introduced into a cell via a variety of techniques known in the art, such as lipofection, microinjection, calcium phosphate or calcium chloride precipitation, DEAE-dextrin-mediated transfection, or electroporation. Electroporation is carried out at approximate voltage and capacitance to result in entry of the DNA construct(s) into cells of interest. Other methods used to transfect cells can also include modified calcium phosphate precipitation, polybrene precipitation, liposome fusion, and receptor-mediated gene delivery.

[00123] Animal or mammalian host cells capable of harboring, expressing, and secreting large quantities of a protein filament monomer or filament binding protein of interest into the culture medium for subsequent isolation and/or purification include, but are not limited to, Human Embryonic Kidney 293 cells (HEK-293) (ATCC CRL-1573); Chinese hamster ovary cells (CHO), such as CHO-K1 (ATCC CCL-61), DG44 (Chasin et al., (1986) *Som. Cell Molec. Genet.*, 12:555-556; Kolkekar et al., (1997) *Biochemistry*, 36:10901-10909; and WO 01/92337 A2), dihydrofolate reductase negative CHO cells (CHO/*dhfr*⁻, Urlaub et al., (1980)

Proc. Natl. Acad. Sci. U.S.A., 77:4216), and dp12.CHO cells (U.S. Pat. No. 5,721,121); monkey kidney CV1 cells transformed by SV40 (COS cells, COS-7, ATCC CRL-1651); human embryonic kidney cells (e.g., 293 cells, or 293 cells subcloned for growth in suspension culture, Graham et al., (1977) *J. Gen. Virol.*, 36:59); baby hamster kidney cells (BHK, ATCC CCL-10); monkey kidney cells (CV1, ATCC CCL-70); African green monkey kidney cells (VERO-76, ATCC CRL-1587; VERO, ATCC CCL-81); mouse sertoli cells (TM4; Mather (1980) *Biol. Reprod.*, 23:243-251); human cervical carcinoma cells (HELA, ATCC CCL-2); canine kidney cells (MDCK, ATCC CCL-34); human lung cells (W138, ATCC CCL-75); human hepatoma cells (HEP-G2, HB 8065); mouse mammary tumor cells (MMT 060562, ATCC CCL-51); buffalo rat liver cells (BRL 3A, ATCC CRL-1442); TRI cells (Mather (1982) *Annals NY Acad. Sci.*, 383:44-68); MCR 5 cells; FS4 cells. A cell line transformed to produce a protein filament monomer or filament binding protein can also be an immortalized mammalian cell line of lymphoid origin, which include but are not limited to, a myeloma, hybridoma, trioma or quadroma cell line. The cell line can also comprise a normal lymphoid cell, such as a B cell, which has been immortalized by transformation with a virus, such as the Epstein Barr virus (such as a myeloma cell line or a derivative thereof).

[00124] A host cell strain, which modulates the expression of the inserted sequences, or modifies and processes the nucleic acid in a specific fashion desired also may be chosen. Such modifications (for example, biotinylation, glycosylation and other post-translational modifications) and processing (for example, cleavage) of protein products may be important for the function of the protein. Different host cell strains have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. As such, appropriate host systems or cell lines can be chosen to ensure the correct modification and processing of the foreign protein expressed, such as a protein filament monomer or filament binding protein. Thus, eukaryotic host cells possessing the cellular machinery for proper processing of the primary transcript, biotinylation, glycosylation, and phosphorylation of the gene product may be used. Non-limiting examples of mammalian host cells include HEK-293, 3T3, W138, BT483, Hs578T, CHO, VERY, BHK, Hela, COS, BT2O, T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7O3O, MDCK, 293, HTB2, and HsS78Bst cells.

[00125] Various culturing parameters can be used with respect to the host cell being cultured. Appropriate culture conditions for mammalian cells are well known in the art

(Cleveland WL, et al., *J Immunol Methods*, 1983, 56(2): 221-234) or can be determined by the skilled artisan (see, for example, Animal Cell Culture: A Practical Approach 2nd Ed., Rickwood, D. and Hames, B. D., eds. (Oxford University Press: New York, 1992)). Cell culturing conditions can vary according to the type of host cell selected. Commercially available medium can be utilized.

[00126] Cells suitable for culturing can contain introduced expression vectors, such as plasmids or viruses. The expression vector constructs can be introduced via transformation, microinjection, transfection, lipofection, electroporation, or infection. The expression vectors can contain coding sequences, or portions thereof, encoding the proteins for expression and production. Expression vectors containing sequences encoding the produced proteins and polypeptides, as well as the appropriate transcriptional and translational control elements, can be generated using methods well known to and practiced by those skilled in the art. These methods include synthetic techniques, *in vitro* recombinant DNA techniques, and *in vivo* genetic recombination which are described in J. Sambrook et al., 2001, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. and in F. M. Ausubel et al., 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

[00127] Purification of recombinant proteins

[00128] A protein filament monomer or filament binding protein can be purified from any human or non-human cell which expresses the polypeptide, including those which have been transfected with expression constructs that express a protein filament monomer or filament binding protein. A purified protein filament monomer or filament binding protein can be separated from other compounds which normally associate with the protein filament monomers or filament binding proteins, in the cell, such as certain proteins, carbohydrates, or lipids, using methods practiced in the art. For protein recovery, isolation and/or purification, the cell culture medium or cell lysate is centrifuged to remove particulate cells and cell debris. The desired polypeptide molecule (for example, a protein filament monomer or filament binding protein) is isolated or purified away from contaminating soluble proteins and polypeptides by suitable purification techniques. Non-limiting purification methods for proteins include: size exclusion chromatography; affinity chromatography; ion exchange chromatography; ethanol precipitation; reverse phase HPLC; chromatography on a resin, such as silica, or cation exchange resin, e.g., DEAE; chromatofocusing; SDS-PAGE;

ammonium sulfate precipitation; gel filtration using, e.g., Sephadex G-75, Sepharose; protein A sepharose chromatography for removal of immunoglobulin contaminants; and the like. Other additives, such as protease inhibitors (e.g., PMSF or proteinase K) can be used to inhibit proteolytic degradation during purification. Purification procedures that can select for carbohydrates can also be used, e.g., ion-exchange soft gel chromatography, or HPLC using cation- or anion-exchange resins, in which the more acidic fraction(s) is/are collected.

[00129] Detecting Polypeptides

[00130] As discussed above, the use of a fluorescent label is an embodiment of the invention. Standard procedures are used to determine the positions of the protein filaments or filament binding proteins and/or a target, e.g., a second protein filament or filament binding protein or a polypeptide. For example, the position of a protein filament or filament binding protein on an array described herein can be detected by the signal emitted by the label. In other examples, when a protein filament or filament binding protein on the array and a target polypeptide are labeled, the locations of both the protein filament or filament binding protein on the array and the target will exhibit significant signal. In addition to using a label, other methods may be used to scan the matrix to determine where an interaction, e.g., between a protein filament or filament binding protein on an array described herein and a target, takes place. The spectrum of interactions can, of course, be determined in a temporal manner by repeated scans of interactions that occur at each of a multiplicity of conditions. However, instead of testing each individual interaction separately, a multiplicity of interactions can be simultaneously determined on an array, e.g., an array described herein.

[00131] In certain embodiments, the array is excited with a light source at the excitation wavelength of the particular fluorescent label and the resulting fluorescence at the emission wavelength is detected. In certain embodiments, the excitation light source is a laser appropriate for the excitation of the fluorescent label.

[00132] Detection of the fluorescence signal can utilize a microscope, e.g., a fluorescent microscope. The microscope may be equipped with a phototransducer (e.g., a photomultiplier, a solid state array, or a ccd camera) attached to an automated data acquisition system to automatically record the fluorescence signal produced by the protein filaments or filament binding proteins and/or targets on the array. Such automated systems are known in the art. Use of laser illumination in conjunction with automated confocal

microscopy for signal detection permits detection at a resolution of better than about 100 μm , better than about 50 μm , and better than about 25 μm .

[00133] The detection method can also incorporate some signal processing to determine whether the signal at a particular position on the array is a true positive or may be a spurious signal. For example, a signal from a region that has actual positive signal may tend to spread over and provide a positive signal in an adjacent region that actually should not have one. This may occur, e.g., where the scanning system is not properly discriminating with sufficiently high resolution in its pixel density to separate the two regions. Thus, the signal over the spatial region may be evaluated pixel by pixel to determine the locations and the actual extent of positive signal. A true positive signal should, in theory, show a uniform signal at each pixel location. Thus, processing by plotting number of pixels with actual signal intensity should have a clearly uniform signal intensity. Regions where the signal intensities show a fairly wide dispersion, may be particularly suspect and the scanning system may be programmed to more carefully scan those positions.

[00134] Total Internal Reflection Fluorescence Microscopy

[00135] Total internal reflection fluorescence microscopy (TIRFM) is used to detect the protein filaments or filament binding proteins and polypeptides described herein. For TIRFM, a laser beam is directed through a microscope slide and reflected off the interface between the slide and a buffer containing the fluorescent sample. If the angle of incidence is greater than the critical angle [$\theta_c = \sin^{-1}(n_2/n_1)$; where n_1 and n_2 are the refractive indexes of the slide and aqueous samples, respectively], then all of the incident light is reflected away from the interface. However, an illuminated area is present on the sample side of the slide. This is called the evanescent wave, and its intensity decays exponentially away from the surface [N2, N3]. For most applications the evanescent wave penetrates approximately 100 nm into the aqueous medium. This geometry reduces the background signal by several orders of magnitude compared to conventional fluorescence microscopy and readily allows the detection of single fluorescent molecules, because contaminants and bulk molecules in solution are not illuminated and do not contribute to the detected signal. [N3]. By using total internal reflection fluorescence microscopy to visualize the arrays described herein, it is possible to simultaneously monitor hundreds of aligned protein filaments within a single field-of-view.

[00136] The methods described herein use microfluidic flowcells composed of substrates that are rendered inert by deposition of a lipid bilayer as described herein. By applying a hydrodynamic force to the arrays described herein, the attached protein filaments are aligned in a desired orientation that is optimal for detection by, e.g., TIRFM.

[00137] A microfluidic flowcell that can be used in the methods described herein is depicted in Figures 14A-B. Generally, a substrate described herein is overlaid with a coverslip, e.g., a glass coverslip, to form a sample chamber, and the substrate contains an inlet port and an outlet port, through which a hydrodynamic force is applied. The hydrodynamic force can be mediated by, e.g., a buffer solution that flows over the lipid bilayer described herein. An exemplary microfluidic flowcell can be constructed from 76.2 x 25.4 x 1 mm (L x W x H) fused silica slides (ESCO Products, Oak Ridge, N.J.). Inlet and outlet holes can be drilled through the slides using, e.g., a diamond-coated bit (1.4 mm O.D.; Eurotool, Grandview, Mo.). A sample chamber can be prepared from a borosilicate glass coverslip (Fisher Scientific, USA) and, e.g., double-sided tape (~25 μ m thick, 3M, USA) or a polyethylene gasket. Inlet and outlet ports can be attached using preformed adhesive rings (Upchurch Scientific, Oak Harbor, Wash.), and cured at 120°C. under vacuum for 2 hours. The dimensions of the exemplary sample chamber are 3.5 x 0.45 x 0.0025 cm (L x W x H). The total volume of the exemplary flowcell is ~4 μ l. A syringe pump (Kd Scientific, Holliston, Mass.) is used to control buffer delivery to the sample chamber. This exemplary apparatus is not meant to be limiting, and one of skill in the art would appreciate modifications that could be made.

[00138] A total internal reflection fluorescence microscope is depicted in Fig. 13. An exemplary microscope is a modified Nikon TE2000U inverted microscope. [N10] A 488 nm laser (Coherent Inc., Santa Clara, Calif.) and a 532 nm laser (CrystaLaser, Reno, Nev.) were focused through a pinhole (10 μ m) using an achromatic objective lens (25X; Melles Griot, Marlow Heights, Md.), then collimated with another achromatic lens (f=200 mm). The beam was directed to a focusing lens (f=500 mm) and passed through a custom-made fused silica prism (J.R. Cumberland, Inc) placed on top of the flowcell. Fluorescence images were collected through an objective lens (100X Plan Apo, NA 1.4, Nikon), passed through a notch filter (Semrock, Rochester, N.Y.), and captured with a back-thinned EMCCD (Cascade 512B, Photometrics, Tucson, Ariz.). Image acquisition and data analysis were performed with Metamorph software (Universal Imaging Corp., Downingtown, Pa.). All protein filament

length measurements were performed by calculating the difference in y-coordinates from the beginning to the end of the fluorescent molecules. Diffusion estimates for the lipid-tethered protein filaments were performed by manually tracking the tethered ends of four different filaments, and diffusion coefficients were calculated using: $D = \text{MSD}/4t$; where MSD (the mean square displacement) is the square of the average step size measured over time interval t (0.124 sec) [N18].

[00139] Methods for Visualizing Polypeptides

[00140] The arrays described herein can be used to detect individual protein filaments or filament binding proteins, e.g., protein filaments coupled to a label. For example, an array can be constructed as part of a microfluidic flowcell described herein. The protein filaments or filament binding proteins, e.g., labeled protein filaments can be coupled to a filament binding protein which can be attached to a substrate, to a lipid bilayer, or to a diffusion barrier, as described herein. Upon the application of hydrodynamic force, e.g., introduction of a buffer as described herein, the protein filaments are aligned in direction of the hydrodynamic force, with the nonattached ends of the protein filaments extending in the direction of the flow of the hydrodynamic force. Individual protein filaments on the array can be visualized before and/or after the application of the hydrodynamic force using, e.g., TIRFM as described herein.

[00141] In some embodiments, the interactions of protein filaments on the arrays with target polypeptides are determined. The protein filaments can be visualized before and/or after the application of a hydrodynamic force, as described herein. To visualize the interactions with target polypeptides, the polypeptides can be coupled to a label and introduced into the array, e.g., a microfluidic cell including the array, as a component of the buffer that mediates the hydrodynamic force. Individual protein filaments and individual target polypeptides can be visualized, e.g., by TIRFM as described herein, and interactions can be determined by colocalization of the signals from the nucleic acid molecules and the polypeptides. Such interactions can be further analyzed by collecting signals over a period of time. Such methods can be used to visualize, e.g., the movement of polypeptides along the length of individual protein filaments, or the binding and unbinding of polypeptides to individual protein filaments, as described herein.

[00142] Methods for High-Throughput Screening of Compounds

[00143] The methods and compositions described herein can be used to screen for compounds, e.g., drug compounds, that affect, e.g., disrupt, the interactions between protein filaments and filament binding proteins, between protein filaments and polypeptides and between protein filament subunits. For example, an array can be constructed as part of a microfluidic flowcell described herein. The protein filaments or filament binding proteins, e.g., labeled protein filaments, can be coupled to a filament binding protein which can be attached to a substrate, to a lipid bilayer, or to a diffusion barrier, as described herein. To visualize the interactions with target polypeptides, the polypeptides can be coupled to a label and introduced into the array, e.g., a microfluidic cell including the array, as a component of the buffer that mediates the hydrodynamic force. In some embodiments, the polypeptides are known to interact with the protein filaments, and the interactions are visualized as described herein. For example, the polypeptides can be proteins involved in cell structure maintenance and motility. Candidate compounds can then be added to the array, e.g., as a component of the buffer that mediates the hydrodynamic force, and the effect of the compound on the interactions between individual protein filaments and the polypeptides or between protein filament subunits can be visualized. Compounds that disrupt the interactions can be visually identified. Such methods can be automated.

[00144] For example, the methods described herein can be used to screen for therapeutic compounds to treat cancer, e.g., cancer of the breast, prostate, lung, bronchus, colon, rectum, urinary bladder, kidney, pancreas, oral cavity, pharynx, ovary, skin, thyroid, stomach, brain, esophagus, liver, cervix, larynx, soft tissue, testis, small intestine, anus, anal canal, anorectum, vulva, bladder, bones, joints, hypopharynx, eye, nose, nasal cavity, ureter, gastrointestinal tract; non-Hodgkin lymphoma, Multiple Myeloma, Acute Myeloid Leukemia, Chronic Lymphocytic Leukemia, Hodgkin Lymphoma, Chronic Myeloid Leukemia and Acute Lymphocytic Leukemia.

[00145] In one embodiment, agents that bind to protein filaments can be used, which include, but are not limited to, taxanes (e.g., paclitaxel, taxol, docetaxel), epothilones (e.g., ixabepilone), nocodazole, vincristine, colchicine, erubulin, cytochalasin D, phalloidins, latrunculin A, and jasplakinolide.

[00146] Unless otherwise defined, all technical and scientific terms used herein have the

same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention.

[00147] All publications and other references mentioned herein are incorporated by reference in their entirety, as if each individual publication or reference were specifically and individually indicated to be incorporated by reference. Publications and references cited herein are not admitted to be prior art.

EXAMPLES

[00148] Examples are provided below to facilitate a more complete understanding of the invention. The following examples illustrate the exemplary modes of making and practicing the invention. However, the scope of the invention is not limited to specific embodiments disclosed in these Examples, which are for purposes of illustration only, since alternative methods can be utilized to obtain similar results.

[00149] *Example 1: Sub-picoNewton forces modulate actin filament polymerization mediated by formin FH1 and FH2 domains*

[00150] To assess the effects of tension on actin filament polymerization mediated by formins, the surface of a microfluidic chamber was coated with a lipid bilayer that was disrupted by nanofabricated metallic barrier patterns made by electron beam lithography (Figure 1B)(11-15). Formin dimers for experiments consisted of the FH1 and FH2 domains (residues 1228-1766) of *S. cerevisiae* Bni1p fused to an N-terminal tag that was biotinylated during expression in *E. coli* (Figure 1A). Streptavidin anchored formins to biotinylated lipids in the bilayer coating the surface of the chamber. In the presence of actin monomers (1.5 μM , 33% labeled with the fluorescent dye Oregon green) the formins initiated and elongated filaments. Application of hydrodynamic force aligned the lipid-tethered, formin-bound actin filaments into “actin curtains” along the leading edges of the nanofabricated barriers, which could then be visualized by total internal reflection fluorescence microscopy (TIRFM; Figures 1B and 2A).

[00151] Application of hydrodynamic force extended the actin filaments parallel to the surface, confining them within the detection volume defined by the penetration depth of the

evanescent field, thus allowing for direct visualization of the growing filaments (**Figure 2A**). The actin filaments disappeared from view when buffer flow stopped, confirming that they were linked to the bilayer only through the biotinylated formin (**Figure 2A, 2B**). Fluorescent actin filaments increased in length over time at an initial rate of ~ 12 subunits/s in buffer lacking methylcellulose and always grew in the direction of buffer flow (**Figures 2C**), and pulse-chase experiments, involving alternate injections of fluorescent and unlabeled actin, confirmed that polymerization occurred only at formin-bound barbed ends (**Figure 2D**). Tethered actin filaments could grow for >1 h without dissociating from formin, reaching lengths >40 μm , corresponding to $>16,000$ actin subunits. Subsequent experiments utilized buffer containing 0.25% methylcellulose (viscosity of 1.88×10^{-3} Pa $\cdot$ s) to maintain sufficient drag force on the filaments such that they remained extended and within the evanescent field at moderate to low flow rates.

[00152] In the absence of profilin, formin-mediated actin filament assembly occurred at approximately the same initial rates for actin curtains aligned on the bilayer-coated surfaces (13.2 ± 0.9 subunits/s when exposed to 0.05 mL/min bulk flow) compared to those anchored to myosin-coated slides (13.6 ± 1.8 subunits/s without bulk flow) (**Figure 3A** and **Table 1**). Under the same buffer conditions, free actin filament barbed ends elongated on myosin-coated slides at a rate of 17.6 ± 0.67 subunits/s in the absence of formin. The ratio of polymerization rates observed in the presence and absence of formin corresponds to a gating factor of ~ 0.75 . At higher viscosity (1.0 Pa $\cdot$ s, achieved using 0.5% methylcellulose, 4,000 cP) on myosin-coated slides, formin-bound actin filaments elongated at 6.2 subunits/s with a gating factor of 0.5, as has previously been reported for Bni1p (5, 6). These findings indicate that this new experimental platform accurately reflects the expected attributes of formin-mediated actin filament polymerization.

Table 1. Summary of Bn1l(FH1FH2)p-mediated polymerization rates

Profilin, μM	Flow rate (mL/min)						
	0.0	0.05	0.075	0.1	0.15	0.2	0.5
0	13.6 ±1.6 ^a	13.3 ± 0.9	12.7 ± 2.0	10.8 ± 1.5	10.8 ± 0.7	4.4 ± 0.6	2.3 ± 0.2
1		17.1 ± 1.9	18.1 ± 0.6	20.9 ± 2.3	20.0 ± 1.9	20.2 ± 2.0	
2.5		20.5 ± 1.6	21.3 ± 2.6	25.2 ± 1.6	25.3 ± 3.8	25.8 ± 2.6	
5		19.2 ± 2.2	19.2 ± 2.4	20.1 ± 1.7	19.9 ± 2.4	20.9 ± 1.8	
10		12.5 ± 3.1	14.8 ± 1.9	15.6 ± 1.3	15.3 ± 1.6	15.4 ± 1.7	

^aPolymerization rates are in subunits/s. Errors are standard error of the mean from the analysis of 10-30 filaments.

The hydrodynamic force exerted upon the surface-tethered formin will vary in proportion to the length of the actin filament, the flow rate and the solution viscosity (16); therefore as the filaments grow longer, the formins anchored to the surface will experience increasing amounts of force. To determine the force experienced by the anchored formins, the linear flow rates were first determined within the evanescent field based upon the velocities of broken filaments that passed through the field-of-view (**Table 2**).

Table 2. Summary of flow rates, volume explored by diffusion and drag force imposed at each bulk flow rate

Bulk flow rate	Linear flow rate	Drag force per μm filament ^b
	at height of barrier ^a	
0.05 mL/min	$3.6 \pm 0.3 \mu\text{m/s}$	$0.009 \pm 0.001 \text{ pN}$
0.075 mL/min	$5.0 \pm 0.6 \mu\text{m/s}$	$0.012 \pm 0.0015 \text{ pN}$
0.10 mL/min	$6.5 \pm 1.0 \mu\text{m/s}$	$0.016 \pm 0.0024 \text{ pN}$
0.15 mL/min	$8.8 \pm 1.9 \mu\text{m/s}$	$0.022 \pm 0.0046 \text{ pN}$
0.20 mL/min	$11.2 \pm 2.7 \mu\text{m/s}$	$0.027 \pm 0.0066 \text{ pN}$

^aFlow rates at the height of the barrier were measured by tracking the trajectories of bits of filaments that broke off filaments within the actin curtains and flowed out of the sample chamber by TIRFM. Errors are standard errors of the mean from the analysis of 10 broken filaments.

^bDrag force on a 20 μm -long actin filament was calculated for each flow rate using the following equation:

$$F_d = \eta \frac{2\pi L v}{\ln\left(\frac{L}{r}\right)},$$

where η is the solvent viscosity (0.001875 Pa·s), L is the length of the filament, v is the linear flow rate at the height of the barrier and r is the radius of the filament (8 nm).

Table 2A. Flow rates and drag force imposed at each bulk flow rate.

Bulk flow rate, mL/min	Linear flow rate, $\mu\text{m/s}$	Drag force per μm filament, pN	Height from the surface, nm
0.05	3.6 ± 0.3	0.009 ± 0.001	130.0 ± 11.0
0.075	5.0 ± 0.6	0.013 ± 0.0015	120.0 ± 14.5
0.10	6.5 ± 1.0	0.017 ± 0.0024	117.0 ± 18.0
0.15	8.8 ± 1.9	0.024 ± 0.0046	106.0 ± 23.0
0.20	11.2 ± 2.7	0.030 ± 0.0066	103.5 ± 25.0

*Flow rates within the observation plane were measured by tracking the trajectories of broken actin filaments that passed through the observation volume. Errors are SEMs from the analysis of 10 broken filaments.

†Drag force per micron of filament was estimated from a height-corrected form of the Batchelor equation.

[00153] ‡The height of the filaments from the surface was estimated by comparing the linear flow rates with the calculated flow velocity profiles. (Fig. 9B).

[00154] The Batchelor formula was then used for a force on a cylinder (16) to estimate the drag force to be ~ 0.13 pN/ μm per mL/min of bulk flow (Figure 5 and Table 2). The forces accessible in these experiments are sufficient to limit thermal fluctuations of actin filaments as expected from their stiffness (approximately 44 pN/nm for a 1 μm filament) (17), allowing them to be viewed by TIRFM, but are insufficient to deform the filaments (18) or mechanically unfold typical proteins (19, 20) ensuring the experiments remain within a biologically relevant force regime.

[00155] In the absence of profilin, the FH2 domain alone mediates actin monomer addition, allowing determination of whether FH2-mediated polymerization is sensitive to force. Under these conditions, the elongation rates of individual filaments declined over time as the drag increased with the length of the filament (Figure 3A). The elongation rate decreased linearly from ~ 13 subunits/s in the absence of force to ~ 2 subunits/s at forces ≥ 0.2 pN when using 25 nm chromium barriers (Figures 3A and 3B and 4A; Table 1). To avoid possible artifacts resulting from filaments draping over high barriers, experiments were performed using 10 nm barriers, which are expected to be short enough to allow the entire FH2-bound filament to lay parallel to the surface of the slide. In this case, the effect of force is even more dramatic and polymerization halted altogether at forces ≥ 0.2 pN (Figure 3B, closed circles), suggesting that the taller barriers might slightly insulate the formins from the effects of force. It is concluded that, in the absence of profilin, FH2-mediated filament assembly is highly sensitive to sub-picoNewton forces, suggesting that some aspects of gating, actin monomer binding, and/or processive stepping of the FH2 onto the new actin subunit were inhibited under tension.

[00156] Given the hypothesis that force would speed FH2 stepping and FH2-mediated polymerization (8, 9), the results suggest that another aspect of the process, such as the equilibrium between the “open” and “closed” conformations, is also sensitive to sub-picoNewton forces and becomes rate limiting. This hypothesis is consistent with a thermodynamic model for formin-mediated polymerization (7, 21, 22), and corresponding simulations performed using a force-sensitive gating mechanism accurately reproduce the effect of force on the FH2-mediated actin polymerization rate in the absence of profilin (Figure 3B). Taken together, this data suggest that even if force were to increase the rate at which the FH2 domain steps onto the newly added actin subunit, as predicted (8), any potential benefit of this increased stepping rate may be offset by force shifting the

conformation of the formin on the end of the filament towards the closed state that precludes elongation of the filament (Figure 3C).

[00157] How might force reduce the gating factor to favor the closed state? In a crystal structure the actin subunits bound to the FH2 domain of Bni1p were oriented at 180° along the filament short-pitch helix (*I*), a conformation where an incoming actin subunit could not bind to both terminal subunits as it may in the 167° orientation of actin subunits in free filaments. One proposal (5) is that filament ends associated with FH2 dimers undergo a rapid equilibrium between a “closed” state with a conformation similar to the 180° orientation and the 167° “open” state (Figure 1A). At any moment in time a population of filaments associated with Bni1p FH2 would have a mixture of open and closed conformations corresponding to the gating factor of 0.5 to 0.75 and thus elongate slower than free ends. A simple extension of this gating hypothesis is that application of force shifts the equilibrium towards the closed 180° conformation, thus lowering the gating factor of Bni1p to ~0 (Figure 3C). Although the extent of perturbation of the equilibrium remains constant at forces up to 0.8 pN, it is likely that much higher forces would unfold the proteins.

[00158] Profilin-stimulated elongation of actin filament barbed ends associated with formins having FH1 and FH2 domains displays a characteristic profile, with maximum rates at intermediate profilin concentrations, and slower rates at high profilin concentrations (Figure 4A). This effect of profilin on formin-mediated polymerization arises from two competing reactions: (i) profilin binds and delivers actin monomers to the polyproline tracks of the FH1 domain, while (ii) free profilin competes with profilin-actin for binding to these same polyproline tracks (5-7). As a consequence of this competition for FH1 polyproline tracks, the formin-mediated polymerization rate increases with profilin concentration up to a maximum around the K_d of profilin-actin (~2.9 μ M for *S. cerevisiae* profilin and chicken muscle actin (23)), but is lower at higher profilin concentrations where free profilin saturates the FH1 polyproline tracks (5-7).

[00159] The data shows that FH1 domains are capable of binding and delivering profilin-actin to the barbed end of a filament even with the N-terminus of one or both FH1 domains constrained through the biotin-streptavidin-mediated attachment to the lipid bilayer. Thus this assay faithfully recapitulates known effects of profilin on formin-mediated actin filament polymerization.

[00160] Next it was investigated if formin-mediated actin polymerization is sensitive to tension in the presence of profilin. Small drag forces moderately increased the rates of formin-mediated actin filament elongation at all tested concentrations of profilin, such that long filaments elongated more rapidly than short filaments in the presence of both 25 and 10 nm barriers (**Figure 4B** and **Figure 6**). The resistance of formin-mediated polymerization to inhibition by force in the presence of profilin was striking in contrast with the strong negative effect of force on polymerization in the absence of profilin. The range of drag forces tested increased formin-mediated elongation rates by up to ~20% (**Figure 4C**) at various profilin concentrations (**Figure 6**). The effect of force on the elongation rate depended on the concentration of profilin, with the largest increase in average polymerization rates at 2.5 μM profilin (**Figures 4A**). Consistent with the opposite effect of force without profilin, the differences in polymerization rates at the various flow rates were less pronounced at high profilin concentrations where saturation of the FH1 polyproline tracks with free profilin reduced the contribution of the FH1 domain to polymerization. The positive effect of force on elongation in the presence of profilin was unexpected, both because of the force-induced inhibition of polymerization observed in the absence of profilin, and also because it was reasonable to predict (*10*) that force would stretch the natively disordered FH1 domain and compromise delivery of profilin-actin to the barbed end of the filament.

[00161] Force might have a positive effect on elongation in the presence of profilin if force increased the rate of transfer of profilin-actin from the FH1 domains to the end of the filament. However, simulations of actin filament elongation using the force-sensitive FH2 gating mechanism (as in **Figure 3C**) showed that even extremely fast transfer of profilin-actin from the FH1 domain to the FH2 domains cannot overcome the negative effect of force on the gating factor (**Figure 4C**, **Figure 7**), arguing that the effect of profilin cannot be simply explained as force-enhanced delivery of profilin-actin to the growing end of the filaments. An alternative hypothesis is that profilin-actin bound to the FH1 domain directly influences the mechanism of FH2 domain gating. The biphasic effect of profilin concentration argues that the effect of force on elongation depends on binding of profilin-actin (rather than profilin alone) to the FH1 domains. These findings suggest a model in which the binding of profilin-actin to the formin FH1 domains promotes a shift in the FH2 conformational equilibrium towards the open configuration that allows actin polymerization (**Figure 4D**).

[00162] The minimal force exerted on formin-bound actin filaments present in the contractile ring during cytokinesis is estimated to be on the order of ~ 6 pN (10). Importantly, contractile ring formation fails in *S. pombe* cells with a temperature-sensitive variant of profilin (24) or in cells harboring profilin mutations that reduce affinity for either actin or polyproline (25). Taken together with the results, these findings reveal a previously unrecognized role for profilin in promoting formin-mediated actin polymerization under physiologically relevant levels of tension. This work highlights that even small forces have dramatic and unanticipated effects on biological reactions.

[00163] *Pausing buffer flow.* The behavior of the tethered actin filaments illustrated in Figure 2A is identical to DNA molecules anchored at one end to the lipid bilayer, which stretch parallel to the sample chamber surface with the application of buffer flow, but diffuse out of the TIR excitation volume in the absence of flow (11, 12). This response of anchored DNA molecules to buffer flow is a standard control to confirm that the DNA molecules (along with any associated proteins) are anchored to the bilayer only by the biotin-streptavidin linkage, and also rules out any nonspecific interactions with either the bilayer or the underlying surface (11, 12), and also demonstrated that the actin polymers did not interact nonspecifically with the bilayer despite their close proximity to the surface.

[00164] *Influence of the buffer viscosity on actin polymerization.* Initial experiments illustrated in Figure 2 used a low viscosity polymerization buffer (10^{-3} Pa•s), which required bulk flow rates of 0.3 mL/min to confine the filaments within the evanescent field and straighten them for length measurements. This required large volumes of fluorescent actin for continuous monitoring. Fortunately, a methylcellulose polymerization buffer with a viscosity of 1.88×10^{-3} Pa•s with 15 cP (at 2% methylcellulose) maintained sufficient hydrodynamic drag on the filaments at flow rates as low as of 0.05 mL/min to confine the filaments within the evanescent field, thereby reducing the amount of protein required for experiments (Figure 3A).

[00165] *Applications of the actin curtain method.* It was demonstrated that actin filaments can be tethered to lipid-bound biotinylated Bni1(FH1FH2)p, and that applying flow aligns the formin-anchored barbed ends at nano-fabricated diffusion barriers resulting in the formation of actin curtains. Several groups have utilized buffer flow to apply force to surface-tethered molecules (27, 28). However, the actin curtain approach facilitates data collection and analysis by greatly increasing the number of simultaneously imaged, formin-bound

filaments and eliminating unbound filaments altogether. In addition, individual actin filaments are all aligned in the direction of the applied hydrodynamic force, minimizing filament overlap. The lipid bilayer also presents a more biologically relevant medium for polymerization than a glass surface.

[00166] This technique is best suited for application of small, sub-picoNewton drag forces to filaments, owing both to the large uncertainties when calculating drag forces produced at high flow rates (Figure 5), and also filament breakage over time. However, certain simple modifications can be made to increase the magnitude of the forces produced on the filaments. One potential solution would be to add beads to the ends of the filaments, and either perform the experiments as described herein or apply larger forces more accurately using an optical trap.

[00167] This technique will be especially valuable to investigate formin-mediated elongation of actin filaments but will also be useful to study other proteins. By varying the actin monomer concentration and reaction time one can grow filaments more than 40 μm long, for other purposes. For example, tagging actin-binding proteins with quantum dots or other fluorescent probes would permit visualization of interactions with many parallel actin filaments by two-color TIRFM. Because actin filaments can be physically aligned with one another into defined patterns using the nanofabricated barriers, experiments with actin binding proteins such as α -actinin, Arp2/3 complex or myosins might produce actin filament bundles or assemblies similar to the highly organized sarcomeres of striated muscles.

[00168] **Materials and Methods.**

[00169] *Buffers.* Lipid buffer (10 mM Tris-HCl (pH 7.8), 100 mM NaCl) was used for lipid bilayer deposition on the slide surface, and BSA buffer (40 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1 mM MgCl_2 , 20% (w/v) BSA) was used for the surface passivation (blocking surfaces). Monomeric actin was stored in G-buffer (2 mM Tris-HCl (pH 8.0), 0.2 mM ATP, 0.5 mM DTT, 0.1 mM CaCl_2 , 1 mM sodium azide). Formin construct Bni1(FH1FH2)p and *S. cerevisiae* profilin were stored in KMEI buffer (50 mM KCl, 1 mM MgCl_2 , 1 mM EGTA, 10 mM imidazole (pH 7.0)). Polymerization conditions were 10 mM imidazole (pH 7.0), 50 mM KCl, 1 mM MgCl_2 , 1 mM EGTA, 50 mM DTT, 0.2 mM ATP, 0.02 mM CaCl_2 , 15 mM glucose, 0.02 mg/ml catalase, 0.1 mg/ml glucose oxidase with or without 0.25% methylcellulose (15 cP at 2%).

[00170] *Purification of actin, formin and profilin.* Chicken skeletal muscle actin was purified from an acetone powder of chicken breast muscle by one cycle of polymerization and depolymerization followed by gel filtration (5). A fraction of the actin was labeled at cysteine 374 with Oregon green 488 iodoacetamide, followed by depolymerization and purification by ion exchange and gel filtration (26).

[00171] Residues 1228-1766 of the *S. cerevisiae* formin Bni1p were cloned into a pQE70 vector using primers that encoded an N-terminal biotinylation sequence (22). This construct, which also contained a C-terminal 6-His tag, was transformed into BL21 DE3 RP Codon Plus cells. Expression was induced with 0.5 mM IPTG in the presence of 50 μ M biotin and overnight incubation at 16°C. Cells were lysed by sonication in 500 mM NaCl, 50 mM Tris-HCl (pH 8.0) and clarified. The formin was purified by affinity chromatography on a 5 ml column of Ni-NTA resin (Qiagen, Valencia, CA) eluted with 250 mM imidazole (pH 8.0) in lysis buffer. The protein was then incubated with rotation for 1 hour at 4°C with 2 ml avidin resin (Promega, Madison, WI) in the elution buffer with 1 mM DTT. The resin was poured into a column, washed with lysis buffer, and biotinylated Bni1(FH1FH2)p was eluted with 5 mM biotin in lysis buffer. Eluted protein was concentrated using spin-columns (Millipore, Billerica, MA) and dialyzed into KMEI with 1 mM DTT. The protein was flash-frozen in dialysis buffer and stored at -80°C.

[00172] *S. cerevisiae* profilin was expressed from plasmid pMW172-SpPRF in BL21 DE3 cells and induced with 0.4 mM IPTG at 37°C for 4 hours (5, 23). Cells were suspended in 150 mM KCl, 20 mM Tris-HCl (pH 7.5) and lysed by sonication. After spinning the lysate, the supernatant was applied to a poly-L-proline-agarose column, washed with 2 M urea in lysis buffer and eluted with 7 M urea in lysis buffer (5). After dialyzing into KMEI, eluted protein was concentrated using spin columns and stored at 4°C.

[00173] *Nanofabrication of diffusion barrier slides.* Nanometer-scale diffusion barriers were fabricated on fused-silica slides (Finkenbeiner) (12-14). Two holes were drilled in the fused-silica slides. The slides were cleaned in Nano-Strip solution (Cyantek), then rinsed with acetone and isopropanol (IPA) and dried with N₂. A double layer of polymethylmethacrylate (PMMA) (MicroChem) was spin-coated on the slides: first 3% PMMA of 25K (molecular weight) in anisole and then 1.5% PMMA of 495 K in anisole. A conducting polymer, AquaSAVE (Mitsubishi Rayon), was coated on the PMMA layer. Each layer was spun at 4000 rpm for 45 s with 300 rpm/s acceleration. Barrier patterns were drawn using electron-

beam lithography. After the AquaSAVE was removed with a water wash, the patterns were developed using a 3:1 solution of IPA to methylisobutylketone (MIBK) (MicroChem) for 1 min with bath sonication at 4 °C. Chromium (Cr) was deposited to either 20~40 or ~10 nm on the slide using an electron-beam evaporator. Barrier height was verified by tapping mode atomic force microscopy (Veeco Dimension Icon) (**Figure 8**). PMMA layers and Cr except patterned regions were peeled off by heating and sonicating the slide in acetone for 30 min at 65 °C and for 5 min at room temperature, respectively. Then only Cr barriers were left on the slide surface.

[00174] *Flow cell preparation.* Slides were cleaned by successive treatments with 99% acetone, 2% Hellmanex detergent (Hellma Analytics, Mullheim, Germany), 99% ethanol, 1 M NaOH and water. Sample chambers with volumes of about 10 μ L were formed using double-side tape (3M) between the diffusion barrier slide and a 50 mm $\times$ 22 mm coverslip (12-548-5E, FisherSci). Nanoports (Upchurch Scientific) were glued on each hole and connected to 1/16-inch tubing (Upchurch Scientific) for injection of solutions from 1 or 3 mL Luer lock syringes.

[00175] The following solutions were injected into the sample chamber by manually pushing the syringe at room temperature: The chamber was rinsed with 3 mL of water followed by 3 mL of lipid buffer. Biotinylated liposome solution, comprised of 10 mg/ml DOPC (1,2-dioleoyl-sn-glycerophosphocholine), 0.8 mg/ml mPEG 2000-DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N- [methoxy(polyethylene glycol)-2000]) and 0.05 mg/ml biotinylated-DPPE (1,2-dipalmitoyl-snglycero-3-phosphoethanolamine-N-(cap biotinyl)), was prepared in lipid buffer (11-13). The liposome solution was diluted 25-fold in lipid buffer, and 1 mL was introduced into the sample chamber and incubated for 15 min. Excess lipids were washed out with 3 mL of lipid buffer and the chamber was incubated for 20 min to allow vesicle fusion and bilayer formation on the slide surface. Then 1 mL of BSA buffer was injected to block exposed surfaces. One milliliter of 25 μ g/ml streptavidin in BSA buffer was added to bind biotinylated lipids in the bilayer. After free streptavidin was flushed out with 3 mL of BSA buffer, 1 mL of 0.5 to 5 nM biotinylated Bni1(FH2FH2)p in KMEI was injected and incubated for less than 10 min to avoid loss of formin activity. Unbound formin was washed out with 1 mL of BSA buffer. Flow cells were connected to the flow system and placed on a microscope for TIRF imaging. Actin monomers (1.5 μ M, 33% Oregon Green-labeled) and other solutions were introduced into the sample chamber with a

syringe pump (KD Scientific) at flow rates specified in each figure caption.

[00176] *Measurement of flow rate at the height of the barriers.* Given laminar flow in the chamber, the velocity of the buffer depends on the distance from the wall. To calculate the velocity at the height of the barriers, TIRFM was used to monitor trajectories of bits of actin filaments as they broke off filaments from within actin curtains and were washed out in the direction of the flow. Using this method, it was determined that the flow rates at the height of the barriers over a range of bulk flow rates (**Table 2**).

[00177] *Calculation of drag force.* The Batchelor formula for a cylinder (16) relates the drag force (F_d) on a filament of length L to the flow velocity (v):

$$F_d = \eta \frac{2\pi L v}{\ln\left(\frac{L}{r}\right)}, \quad (3)$$

where η is the solution viscosity and r is the radius of the filament (8 nm for an actin filament). Using this equation and buffer velocity at the height of the barriers in the actin curtain experiments (**Table 2**) the drag force was calculated to be 0.009, 0.012, 0.016, 0.022 and 0.027 pN per μm filament at bulk flow rates of 0.05, 0.075, 0.1, 0.15 and 0.2 ml/min (**Figure 5**).

[00178] *TIRFM & Data analysis.* The TIRFM was built around an inverted microscope (TE-2000U; Nikon) and used a 488 nm solid-state laser (Sapphire 488 CDHR, Coherent) for illumination. The flow cell was placed on a motorized microscope stage (Ludl Electronic Products Ltd), to control the x-y-z position of the flow cell on the microscope. The laser was directed through a dove prism (ZCM1125012, ESCO) to generate an evanescent field on the sample side of the slide. Fluorescence was collected by a water-immersion objective lens (PlanApo, 60X, NA 1.2; Nikon) and imaged by an EM-CCD (Cascade 512B; Photometrics) after passing through a notch filter (NF01-488U-25, Semrock) to block the 488 nm excitation laser. Exposure times were 200 ms unless otherwise specified. Time-lapse movies of actin curtains were digitized using commercial software (NIS-Elements, Nikon) and analyzed using ImageJ software (<http://rsb.info.nih.gov/ij/>). Elongation was typically measured over a span of 300 s. Individual filament lengths were measured over time using Image J software and the “Measure Smooth Length” plugin (Pollard lab plugin). Polymerization rates were determined by applying linear fits to short spans of elongation data (30-60 s), thereby yielding multiple polymerization rates for each filament as its length increased.

Polymerization rates were determined for 10-40 filaments at each profilin concentration and flow rate.

[00179] *Simulations of formin-mediated polymerization.* A model was adapted using Virtual Cell software (National Resource for Cell Analysis and Modeling and the National Center for Research Resources; <http://nrcam.uchc.edu/>) to simulate the effects of force-sensitive gating on formin-mediated polymerization in the absence and presence of profilin (7, 21, 22). This model considers profilin, actin, FH2-associated barbed ends and FH1 domains as distinct species and the minimal components required for polymerization. **Table 3** summarizes the rate constants for the formation of intermediate species (FH1-profilin, FH1-profilin-actin, FH1-profilin-actin-barbed end, barbed end-profilin and filamentous actin).

Table 3. Rate constants used in model of FH1FH2-mediated polymerization

A. FH1-independent actin subunit addition

FH2 domain conformational changes

Gating factor ^a	$k_o/(k_o+k_c)$
k_o	$(1000 \cdot \text{gating factor}) \text{ s}^{-1}$
k_c	$(1000 - k_o) \text{ s}^{-1}$

Actin binding to FH2-bound barbed end

k_A^{B+}	^b $11.6 \mu\text{M}^{-1} \text{ s}^{-1}$
k_A^{B-}	^b 1.4 s^{-1}

Profilin-actin binding to FH2-bound barbed end

k_{PA}^{B+}	^c $10 \mu\text{M}^{-1} \text{ s}^{-1}$
k_{PA}^{B-}	$140 \text{ s}^{-1} \text{ (DB)*}$

Profilin binding to FH2-bound barbed end

k_P^{B+}	^c $10 \mu\text{M}^{-1} \text{ s}^{-1}$
k_P^{B-}	^c 2500 s^{-1}

*B. Assembly of profilin-actin onto the FHI domain**Profilin-actin binding to FHI domain***

$$k_{PA}^{F+} \quad {}^d7 \mu M^{-1} s^{-1}$$

$$k_{PA}^{F-} \quad 140 s^{-1} \text{ (DB)}$$

*Profilin binding to FHI domain***

$$k_P^{F+} \quad {}^e200 \mu M^{-1} s^{-1}$$

$$k_P^{F-} \quad {}^c4000 s^{-1}$$

Actin binding to FHI-bound profilin

$$k_A^{F+} \quad {}^f20 \mu M^{-1} s^{-1}$$

$$k_A^{F-} \quad {}^g30 s^{-1}$$

*C. FHI domain loop closure*****Closure of FHI domain loop*

$$\tau_F^+ \quad {}^h6.25 \times 10^4 s^{-1}$$

$$\tau_F^- \quad 1.25 \times 10^6 s^{-1} \text{ (DB)}$$

Closure of profilin-bound FHI domain loop

$$\tau_{PF}^+ \quad {}^i5000 s^{-1}$$

$$\tau_{PF}^- \quad {}^h1.25 \times 10^6 s^{-1}$$

Closure of profilin-actin-bound FHI domain loop

$$\tau_{PAF}^+ \quad {}^i5000 s^{-1}$$

$$\tau_{PAF}^- \quad 50,000 s^{-1} \text{ (DB)}$$

* DB indicates rate constants determined from detailed balance calculations, as described in references (7, 22).

** These rate constants were varied to produce the trends in Figure 4B,D.

*** Rate constants for loop closure were varied in combination with rates for profilin and

profilin-actin association with FH1 to produce the elongation rates for pPB₁₈, pPC₁₈ and pPD₁₈ (Figure 4D).

^a The gating factor is the fraction of time that the barbed end-bound FH2 domain remains in the open, actin binding-competent conformation without profilin. This value is close to that measured experimentally (6).

^b From measurements of elongation of free barbed ends with purified ATP-actin monomers (29).

^c From Vavylonis *et al.* (7)

^d This value was optimized to fit the experimental data as in Paul and Pollard (22).

^e Measured rate of bovine spleen profilin binding to polyproline (30).

^f From Paul and Pollard (22). To account for the reduced mobility of profilin when bound to the FH1 domain, this value is smaller than the experimentally measured association rate constant for profilin to actin.

^g This rate is the product of the K_d for profilin-actin and the profilin-actin association rate. Although the K_d for *S. cerevisiae* profilin and skeletal muscle actin has been experimentally measured to be 3 μ M, it was found that a K_d of 1.5 μ M best fit the experimental data.

^h From Paul and Pollard (22), assuming that loop closure is faster for unbound FH1 than for profilin- or profilin-actin-bound FH1, and that profilin binding to FH1 does not affect the affinity of profilin for the barbed end.

ⁱ This value was varied to fit experimental data, as in Paul and Pollard (22).

[00180] To calculate the effect of force on the gating factor, a linear fit was applied to the polymerization data for drag forces <0.2 pN in figure 3C, and divided the polymerization rates at each drag force by the polymerization rate of free (not formin-bound) filaments (measured in the absence of force to be 17.6 subunits/s). The relationship between drag force and gating factor was used in the simulations of formin-mediated polymerization in the absence and presence of 2.5 μ M profilin.

[00181] **Lipid-tethered, formin-bound actin filaments extend upon introduction of buffer flow.** Under buffer conditions: 1.5 μ M actin (33% Oregon Green-actin) in microscopy buffer (10 mM imidazole (pH 7.0), 50 mM KCl, 1 mM MgCl₂, 1 mM EGTA, 50

mM DTT, 0.2 mM ATP, 0.02 mM CaCl₂, 15 mM glucose, 0.02 mg/ml catalase, 0.1 mg/ml glucose oxidase) a movie of formin-bound actin curtains assembled at four barriers on the lipid-coated surface of a microfluidic chamber was recorded. Flow was introduced into the chamber and transiently paused.

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EQUIVALENTS

[00183] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention.

CLAIMS

1. An array comprising: a) a solid support; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.
2. An array comprising: a) a solid support, wherein the solid support comprises a barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.
3. The array of claim 2, wherein the barrier comprises a mechanical barrier, a chemical barrier, a protein barrier, or a combination thereof.
4. The array of claim 1, wherein the solid support further comprises a barrier.
5. The array of claim 4, wherein the barrier comprises a mechanical barrier, a chemical barrier, a protein barrier, or a combination thereof.
6. The array of claim 2 or 4, wherein the barrier is a scratch on the solid support.
7. The array of claim 2 or 4, wherein the barrier comprises a metal, a metal oxide, or a combination thereof.
8. The array of claim 7, wherein the metal comprises chromium, aluminum, gold, or titanium.
9. The array of claim 7, wherein the metal oxide comprises chromium oxide, aluminum oxide, or titanium oxide.
10. An array comprising: a) a solid support, wherein the solid support comprises a protein barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer.

11. The array of claim 1, 2, or 10, wherein the linkage is formed between neutravidin and biotin, or streptavidin and biotin.
12. The array of claim 1, 2, or 10, wherein the protein filament is aligned along the barrier through application of a hydrodynamic force, an electrophoretic force, or a combination thereof.
13. The array of claim 1, 2, or 10, wherein one end of the protein filament is attached by a linkage.
14. The array of claim 1, 2, or 10, wherein the protein filament is reversibly attached to the lipid bilayer along the contour of the lipid bilayer.
15. The array of claim 1, 2, or 10, wherein both ends of the protein filament are attached by a linkage.
16. The array of claim 15, wherein the ends of the protein filament are each attached by different linkages.
17. The array of claim 1, 2, or 10, wherein the protein filament is an actin filament.
18. The array of claim 1, 2, or 10, wherein the protein filament is an intermediate filament.
19. The array of claim 1, 2, or 10, wherein the protein filament is a microtubule protofilament.
20. The array of claim 18, wherein the intermediate filament comprises lamin, vimentin, neurofilament, or keratin.
21. The array of claim 19, wherein the microtubule protofilament comprises alpha tubulin, beta tubulin, or a combination thereof.
22. The array of claim 17, 18, or 19, wherein the protein filament comprises at least 10 monomer units.

23. The array of claim 1, 2, or 10, wherein the filament binding protein comprises cross-linking proteins, motor proteins, branching proteins, severing proteins, polymerization proteins, capping proteins, depolymerizing proteins, end tracking proteins, nucleators, monomer binding proteins, stabilizers, sidebinders or membrane anchors.
24. The array of claim 23, wherein the filament binding protein comprises formin, or profilin.
25. The array of claim 1, 2, or 10, wherein the protein filament is coupled to a label.
26. The array of claim 25, wherein the label is a fluorescent label.
27. The array of claim 1, 2, or 10, wherein the solid support comprises SiO₂.
28. The array of claim 1, 2, or 10, wherein the lipid bilayer comprises zwitterionic lipids.
29. A microfluidic flowcell comprising the array of claim 1, 2, or 10.
30. The microfluidic flowcell of claim 29, wherein the protein filament of claim 1, 2, or 10 is aligned along the barrier through application of a hydrodynamic force, an electrophoretic force, or a combination thereof.
31. The microfluidic flowcell of claim 30, wherein the hydrodynamic force is tangential, perpendicular, or a combination thereof.
32. The microfluidic flowcell of claim 30, wherein the electrophoretic force is tangential, perpendicular, or a combination thereof.
33. A method for analyzing an interaction between a protein filament and a polypeptide, the method comprising: a) providing the array of claim 1, 2, or 10, wherein the attached protein filament is coupled to a first fluorescent label that permits visualization of the protein filament; b) contacting a polypeptide to the attached protein filament, wherein the polypeptide is coupled to a second fluorescent label that permits visualization of the polypeptide; c) applying a hydrodynamic force or an electrophoretic force tangential to the surface of the support to align the attached protein filament s in a desired orientation; d) visualizing the protein filament and the polypeptide; and e) determining whether the

protein filament interacts with the polypeptide, wherein localization of the polypeptide anywhere along the length of the protein filament is indicative of interaction.

34. The method of claim 33, wherein the determining comprises observing a static localization or dynamic localization of the polypeptide along the protein filament.
35. The method of claim 34, wherein the static localization of the polypeptide along the protein filament indicates binding between the protein filament and the polypeptide.
36. The method of claim 34, wherein the dynamic localization of the polypeptide along the protein filament indicates binding and movement of the polypeptide along the protein filament.
37. A method for identifying a polypeptide sequence or compound that disrupts an interaction between a protein filament and a polypeptide, the method comprising: a) providing a first array of claim 1, 2, or 10, wherein the first array comprises a first population of identical protein filaments, and wherein the protein filaments are coupled to a first fluorescent label; b) providing a second array of claim 1, 2, or 10, wherein the second array comprises a second population of identical protein filaments, wherein the protein filaments are coupled to the first fluorescent label, and wherein the second population of protein filaments differ from the first population of protein filaments by at least one monomer unit; c) contacting a polypeptide sequence or compound to the arrays, wherein the polypeptide sequence or compound is coupled to a second fluorescent label that permits visualization of the polypeptide sequence or compound; and d) determining whether the first population of protein filaments and the second population of protein filaments interact with the polypeptide sequence or compound, wherein localization of the polypeptide sequence or compound anywhere along the length of the first population of protein filaments is indicative of an interaction between the first population and the polypeptide sequence or compound, and wherein an absence of localization of the polypeptide sequence or compound along the length of the second population of protein filaments is indicative that the second population comprises a polypeptide sequence or compound that disrupts the interaction between the first protein filament and the polypeptide sequence or compound.

38. A method for identifying a polypeptide sequence or compound that alters the structure of a protein filament, the method comprising: a) providing the array of claim 1, 2 or 10, wherein the protein filament is coupled to a first fluorescent label that permits visualization of the protein filament; b) applying a hydrodynamic force or an electrophoretic force tangential to the surface of the support to align the protein filament in a desired orientation; c) visualizing the length of the protein filament; d) contacting a polypeptide sequence or compound to the protein filament, wherein the polypeptide sequence or compound is optionally coupled to a second fluorescent label that permits visualization of the polypeptide sequence or compound; e) visualizing the length of the protein filament and, optionally, visualizing the polypeptide sequence or compound; and f) determining whether the protein filament changes length following the contacting step, wherein an increase or a decrease in the length of the protein filament is indicative of a polypeptide sequence or compound that alters the structure of the protein filament.
39. A method for identifying an agent that disrupts the interaction of a polypeptide and a protein filament, the method comprising: a) providing the array of claim 1, 2, or 10, wherein the protein filament is coupled to a first fluorescent label that permits visualization of the protein filament; b) contacting polypeptide to the protein filament, wherein the polypeptide is capable of interacting with the protein filament, and wherein the polypeptide is coupled to a second fluorescent label that permits visualization of the polypeptide; c) contacting an agent to the protein filament and the polypeptide; d) applying a hydrodynamic force or an electrophoretic force tangential to the surface of the support to align the attached protein filaments in a desired orientation; e) visualizing the protein filament and the polypeptide; and f) determining whether the agent disrupts the interaction between the protein filament and the polypeptide, wherein loss of localization of the polypeptide anywhere along the length of the protein filament is indicative of an agent that disrupts the interaction between the protein filament and the polypeptide.
40. A method for identifying one or more agents that disrupt the interactions between one or more polypeptides and a protein filament, the method comprising: a) providing the array of claim 1, 2 or 10, wherein the array comprises a plurality of identical protein filaments, wherein the protein filaments are coupled to a first fluorescent label that permits

visualization of the protein filaments; b) contacting one or more polypeptides to the protein filaments, wherein the one or more polypeptides are each capable of interacting with the protein filaments at different known locations, and wherein the one or more polypeptide are coupled to a second fluorescent label that permits visualization of the polypeptides; c) applying a hydrodynamic force or an electrophoretic force tangential to the surface of the support to align the attached protein filaments in a desired orientation and visualizing the protein filaments and the polypeptides; d) contacting a first agent to the array; e) visualizing the protein filaments and the polypeptides; f) determining whether the first agent disrupts the interaction between the protein filaments and one or more of the polypeptides, wherein loss of localization of one or more of the polypeptides along the length of the protein filaments is indicative of an agent that disrupts the interaction between the protein filaments and the one or more polypeptides; and g) optionally contacting a second agent to the array and repeating steps e) and f).

41. The method of claim 39 or claim 40, wherein the agents are from a library.
42. The method of claim 33, or 37-40, wherein the steps are automated.
43. The method of claim 33, or 37-40 optionally comprising applying continuously the hydrodynamic or electrophoretic force tangential to the surface of the support.

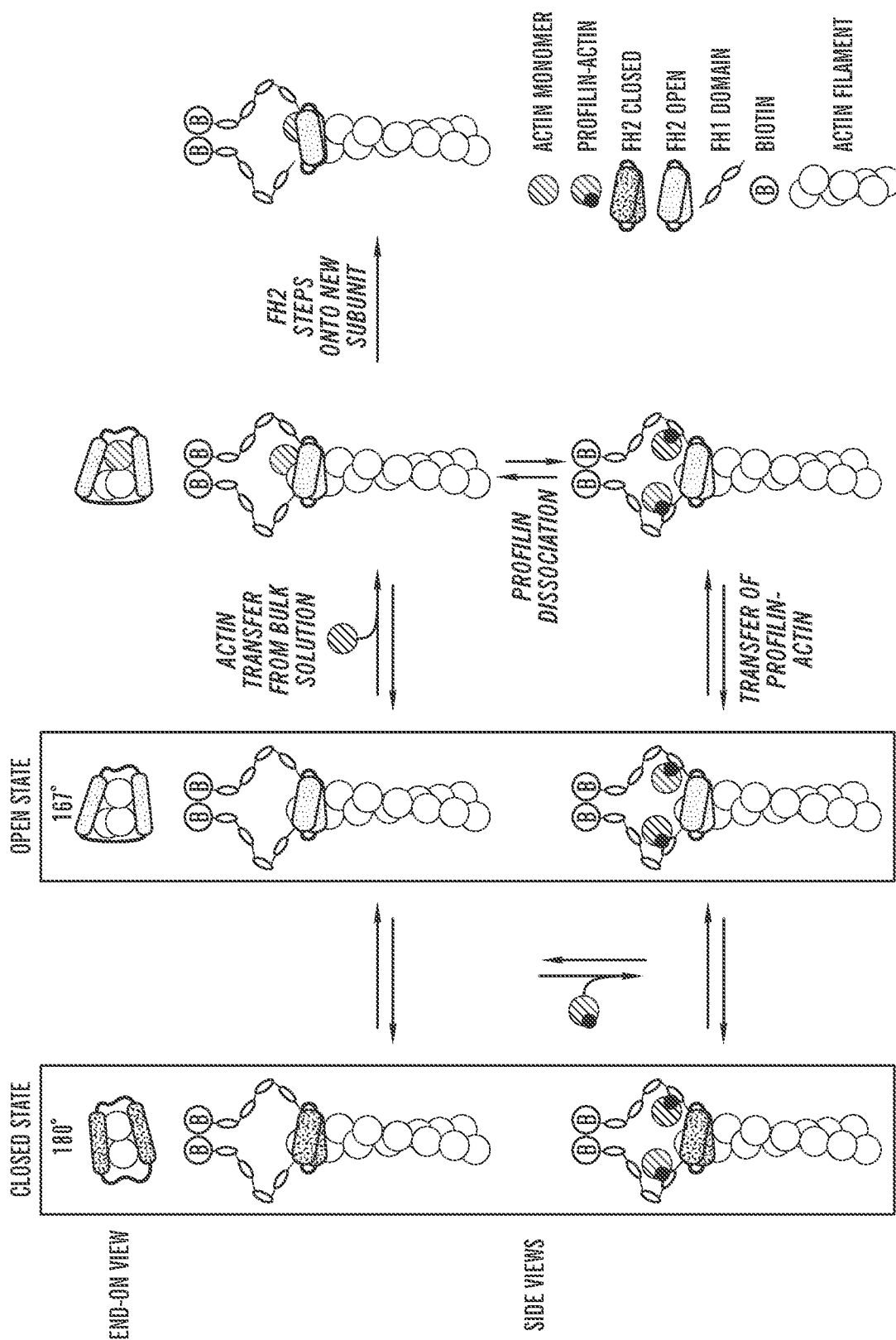


FIG. 1A

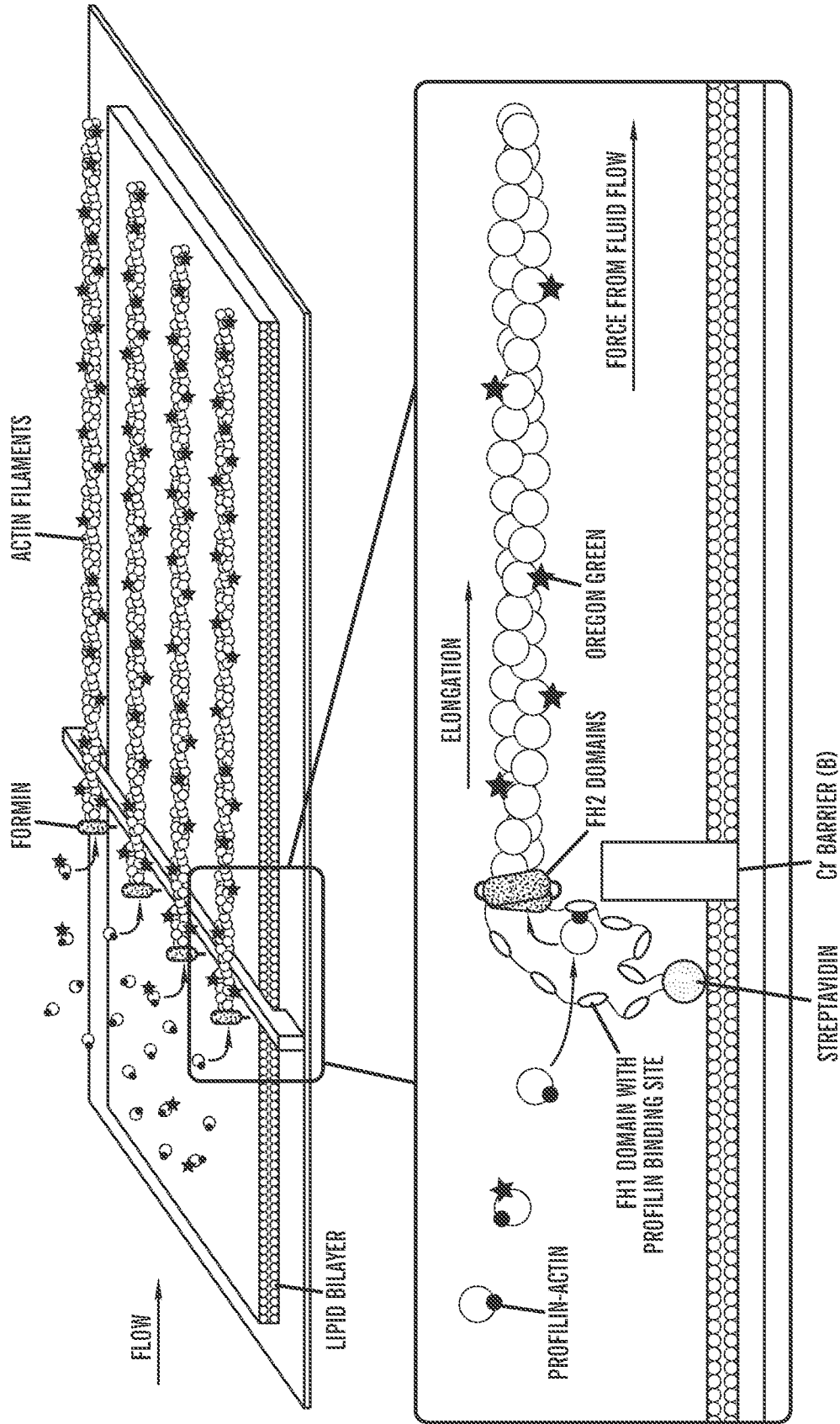
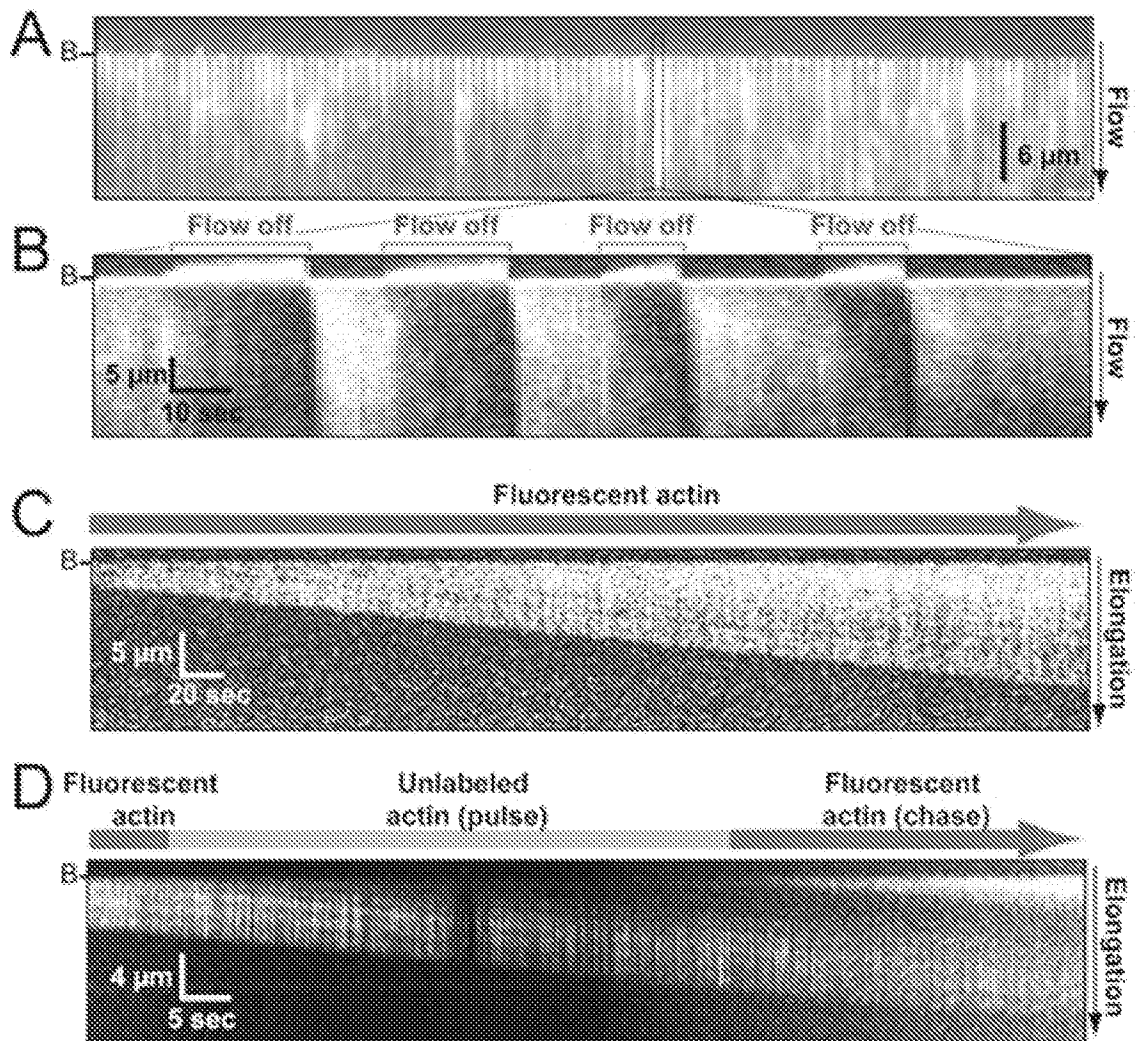
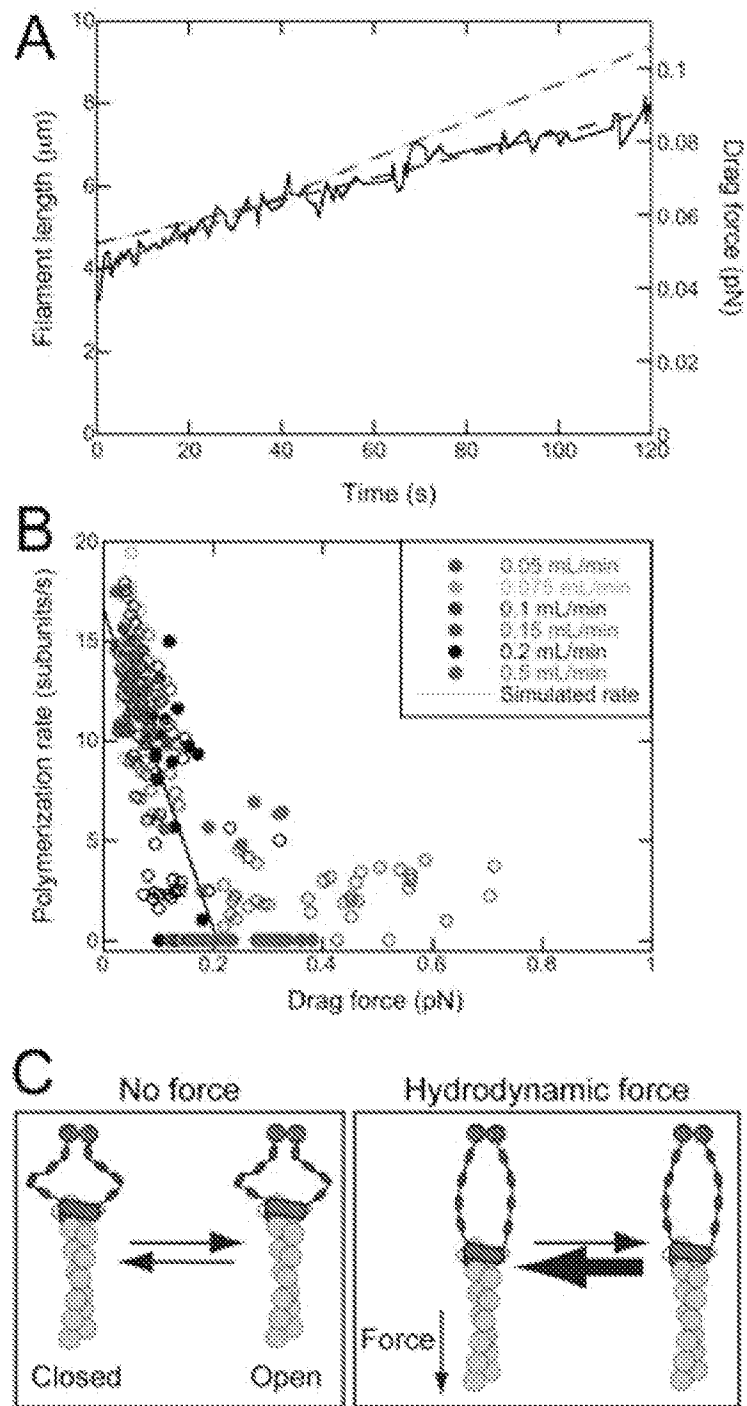


FIG. 1B



Figures 2A-D.

**Figures 3A-C.**

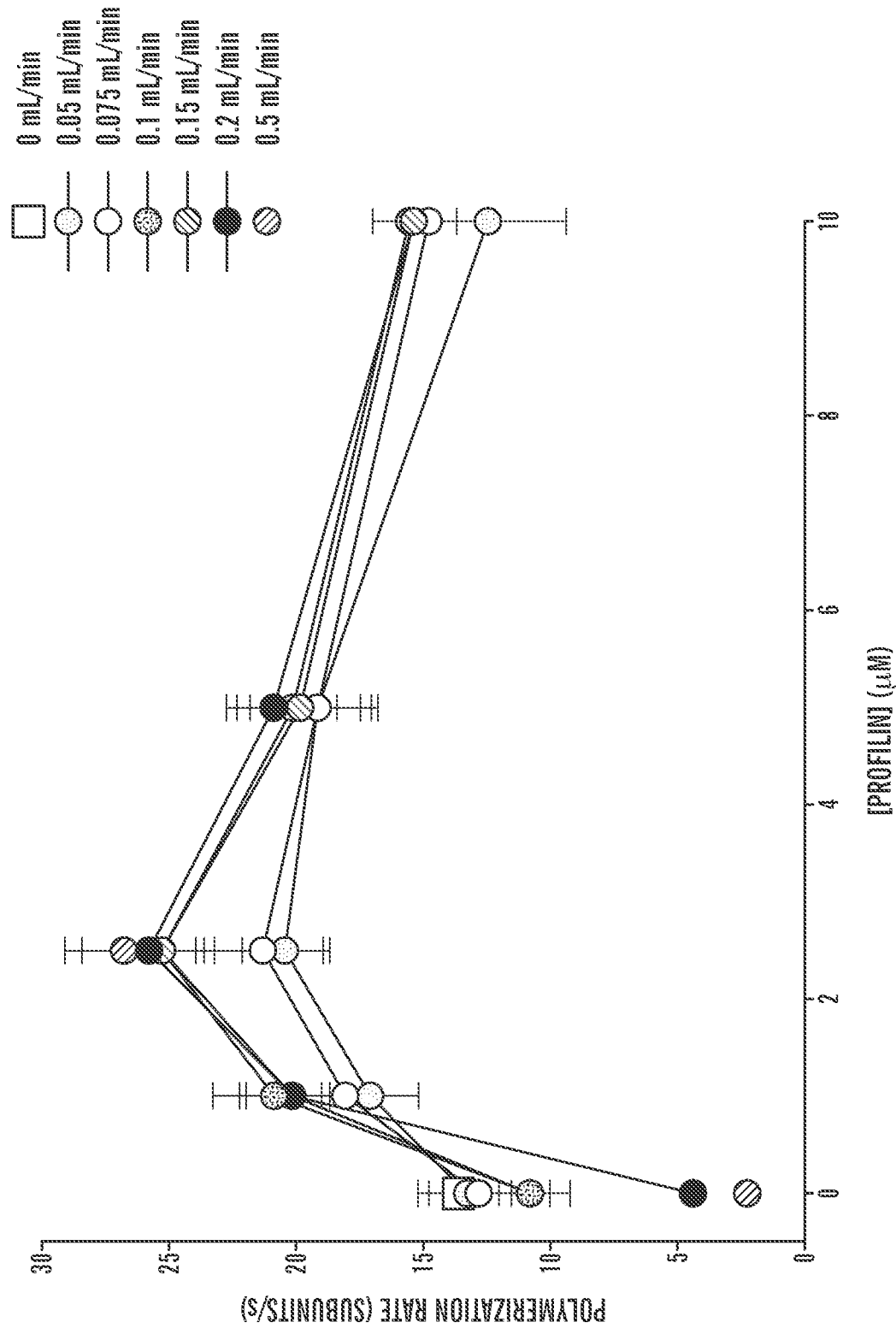


FIG. 4A

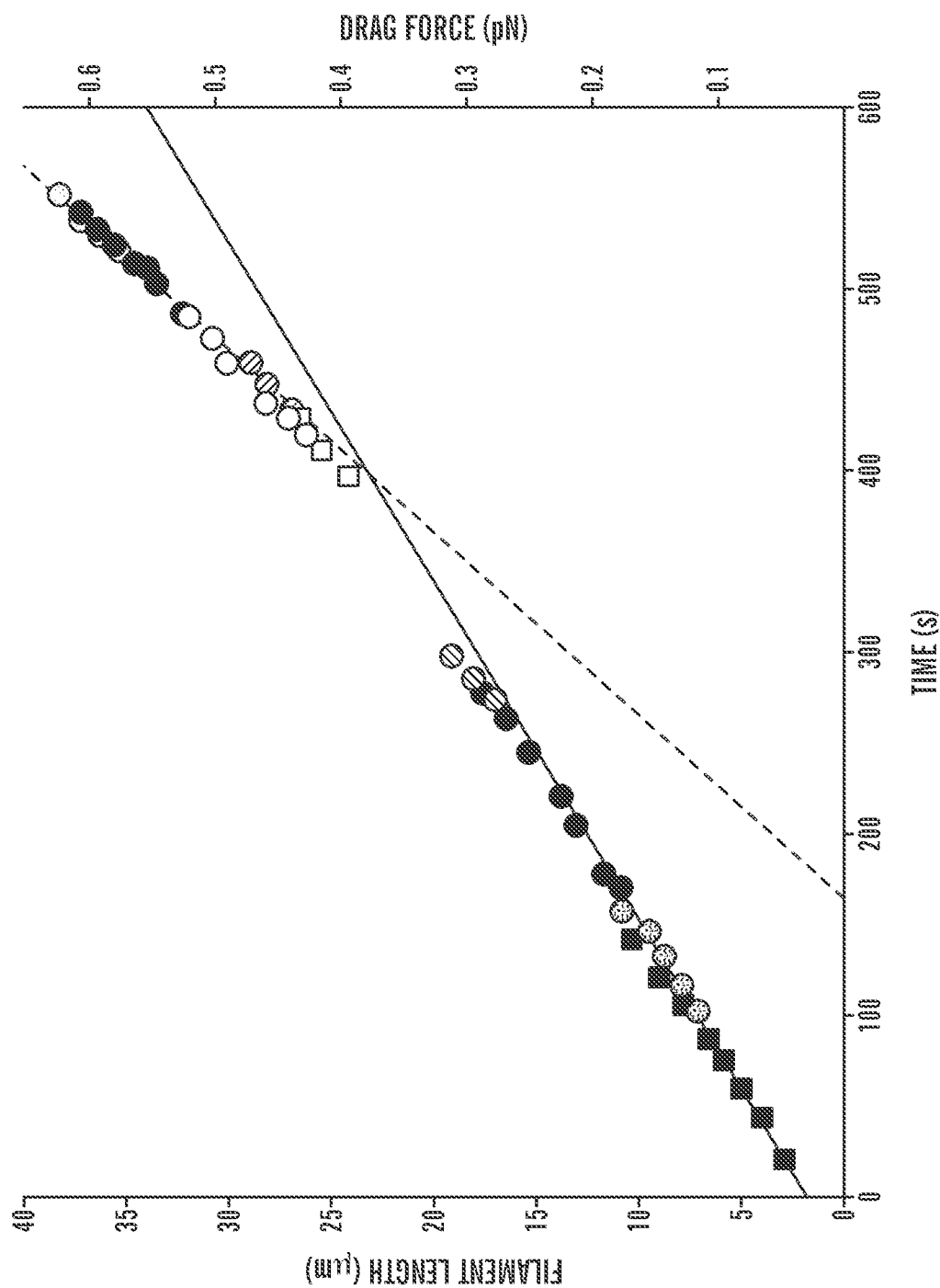


FIG. 4B

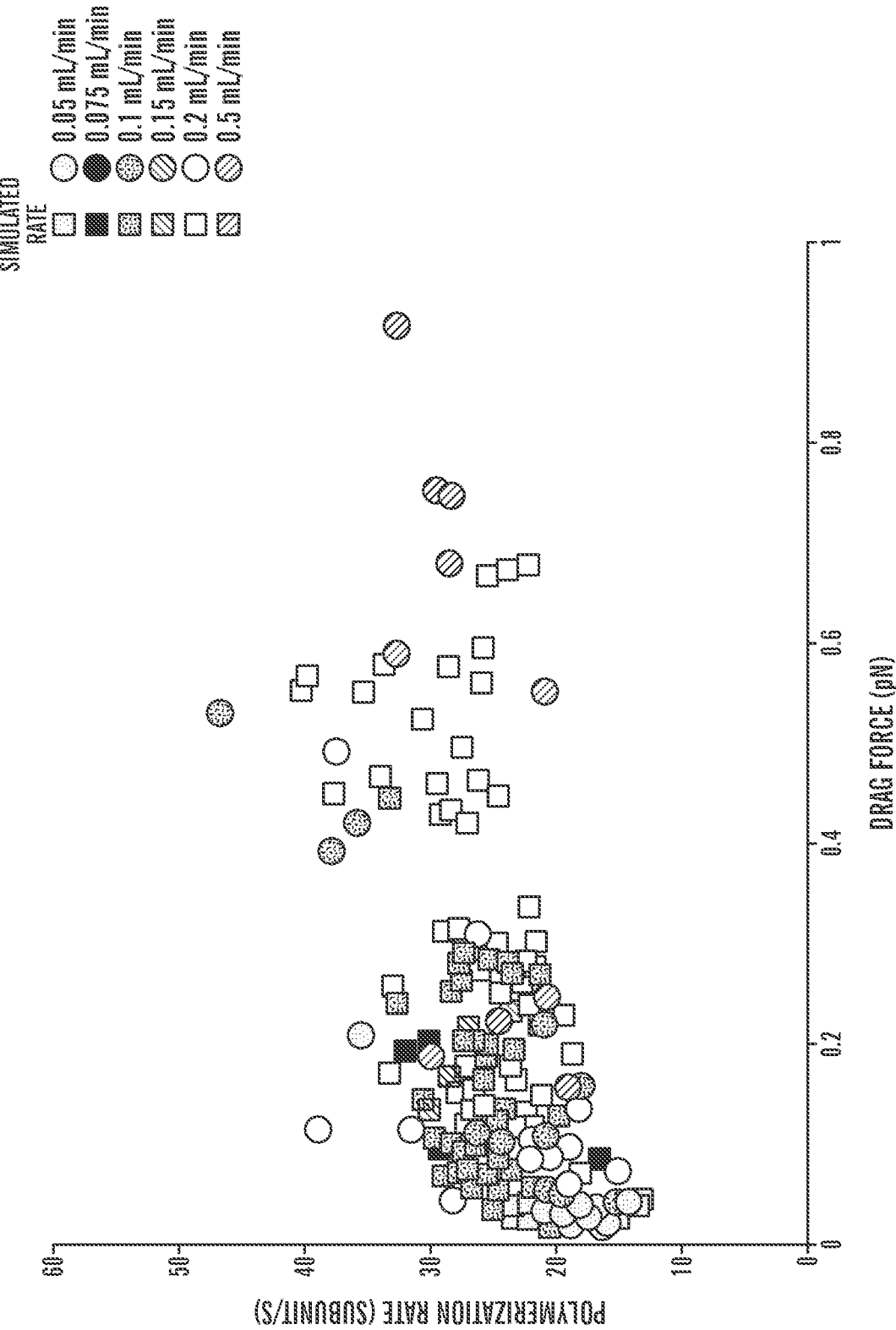


FIG. 4C

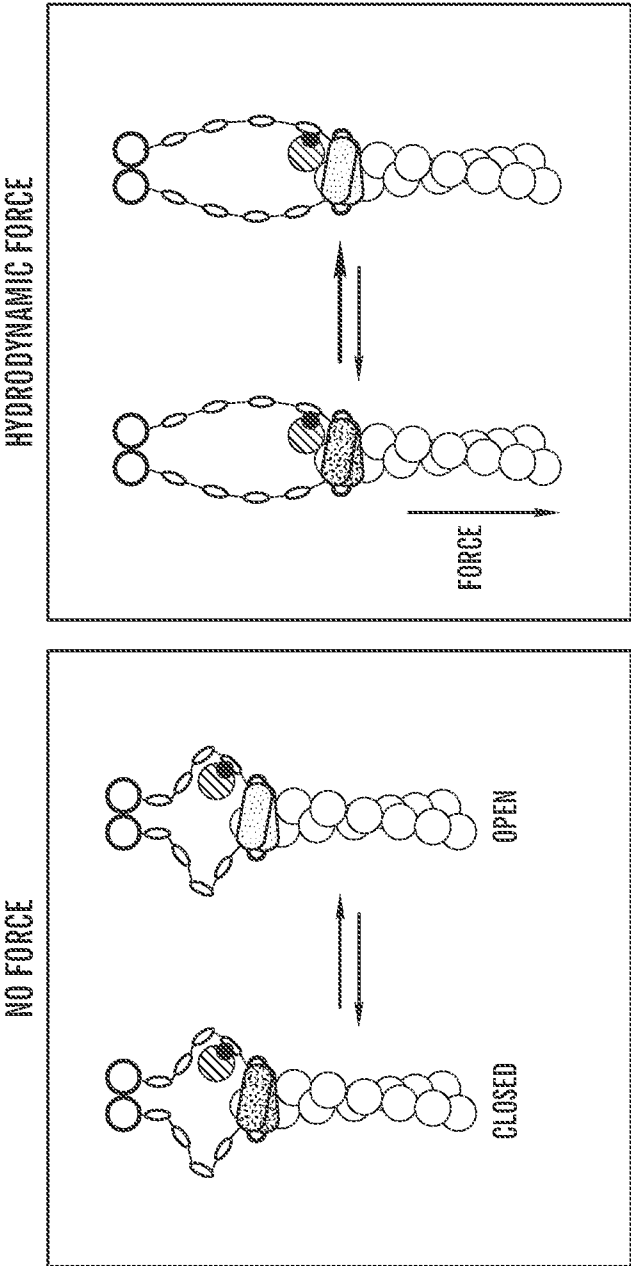


FIG. 4D

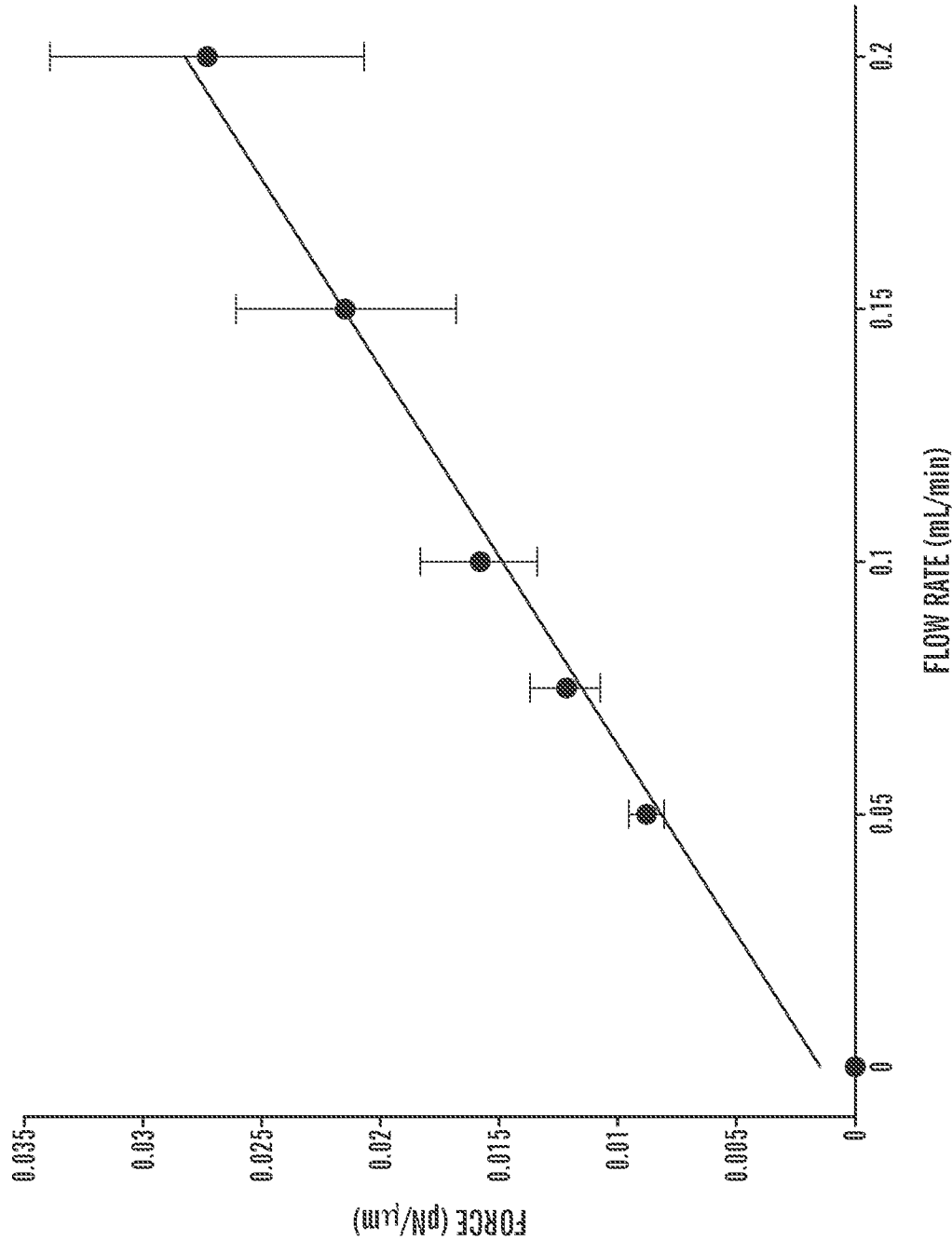
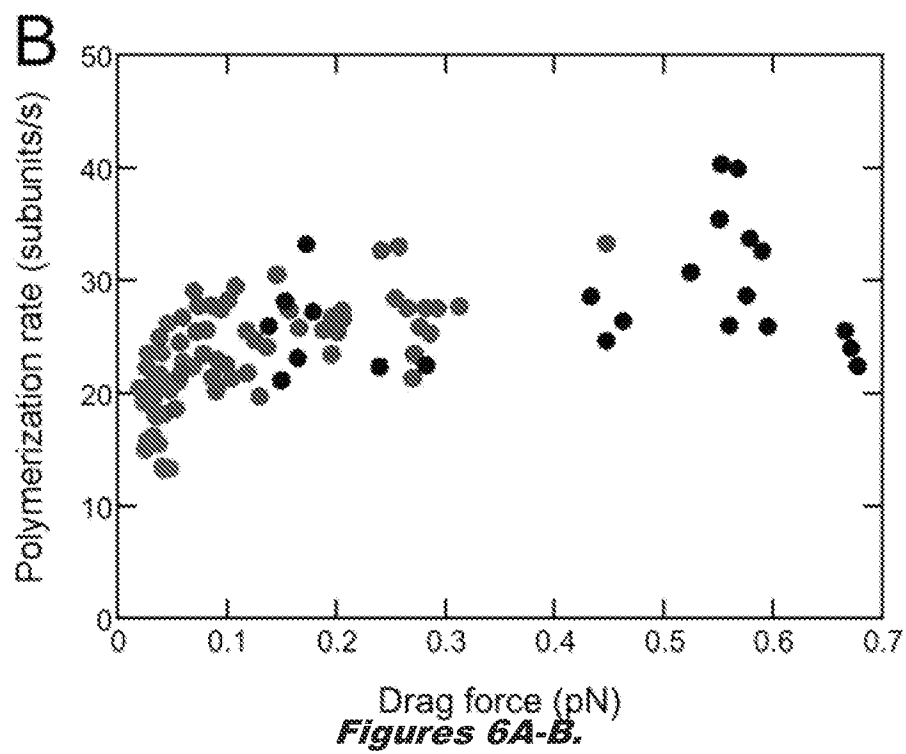
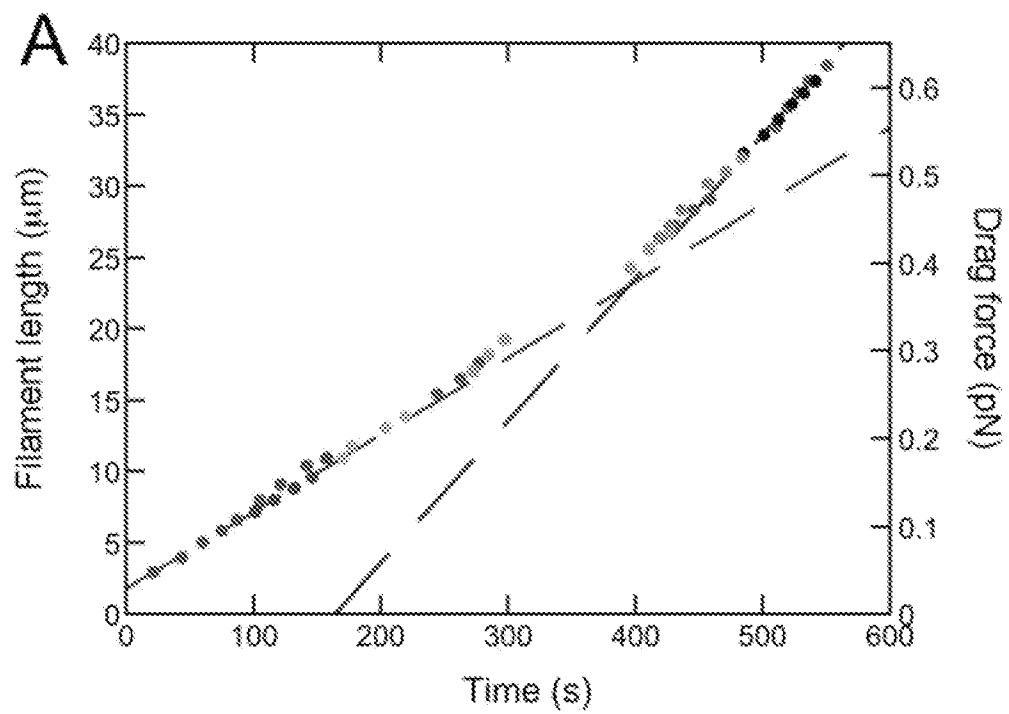


FIG. 5



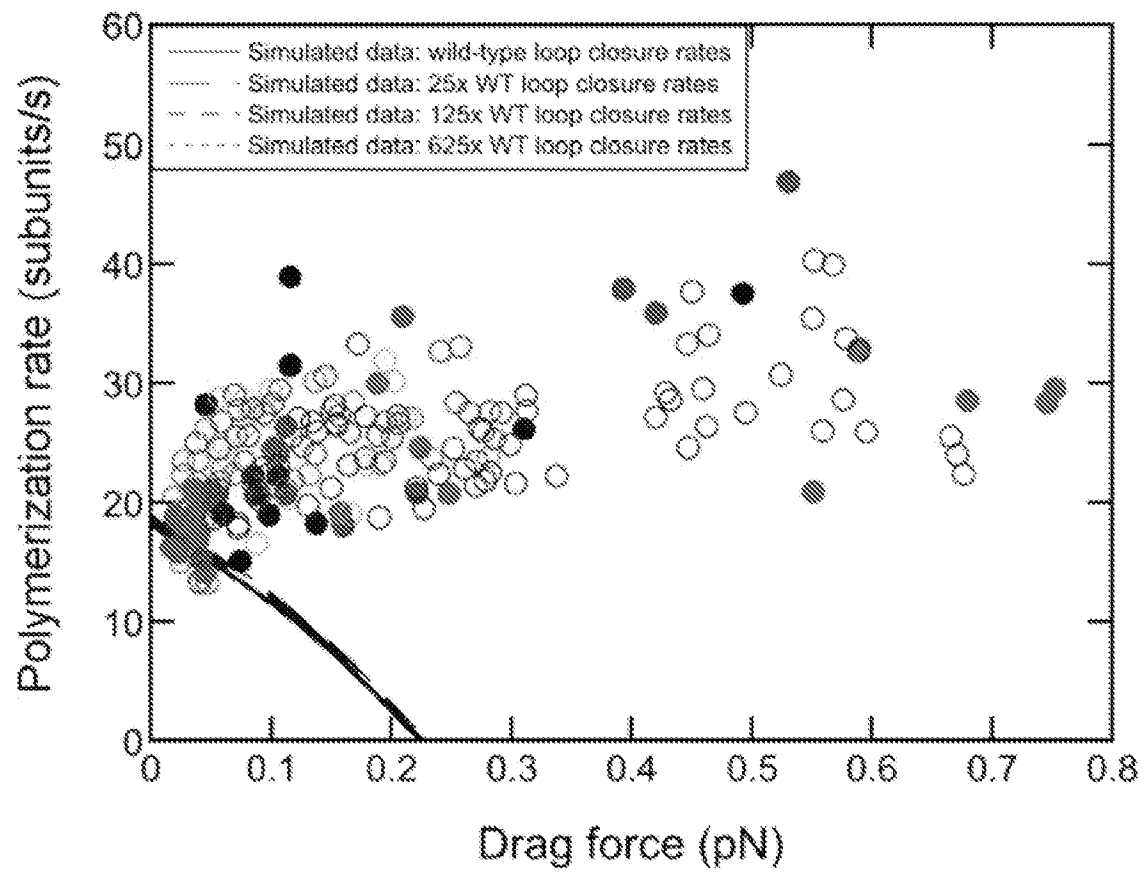
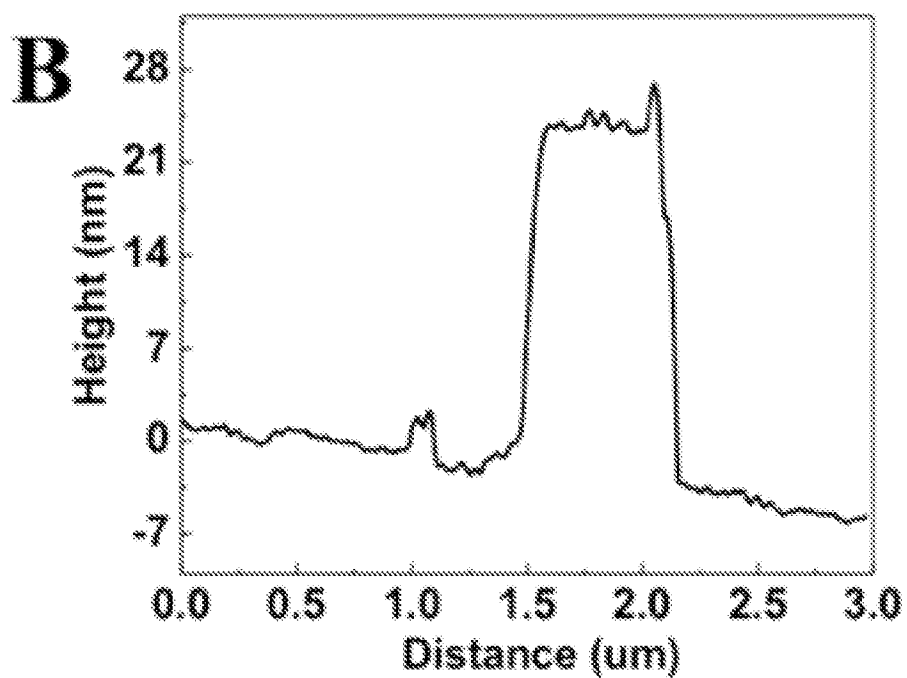
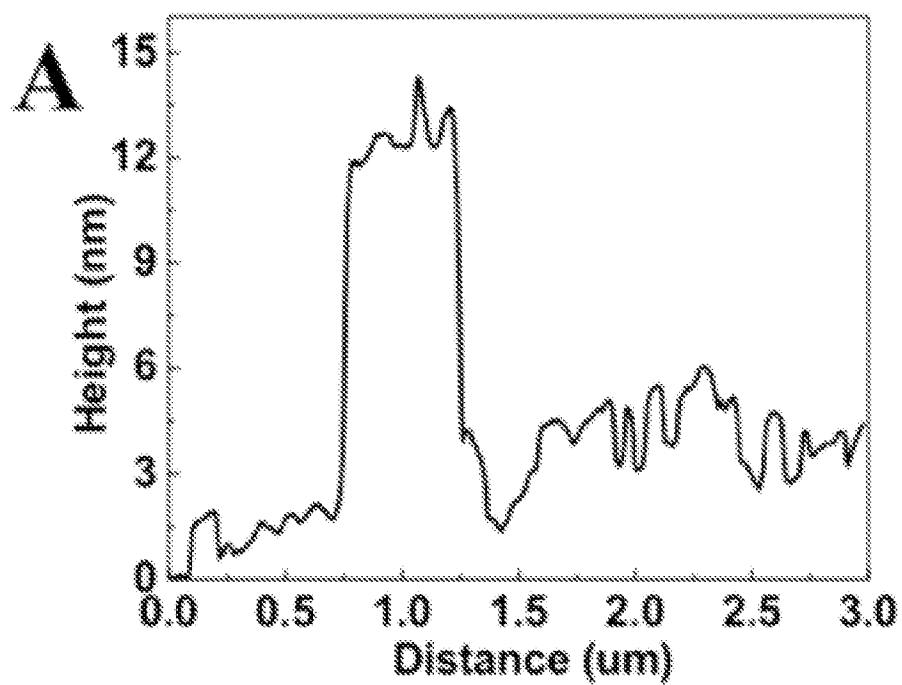


Figure 7.



Figures 8A-B.

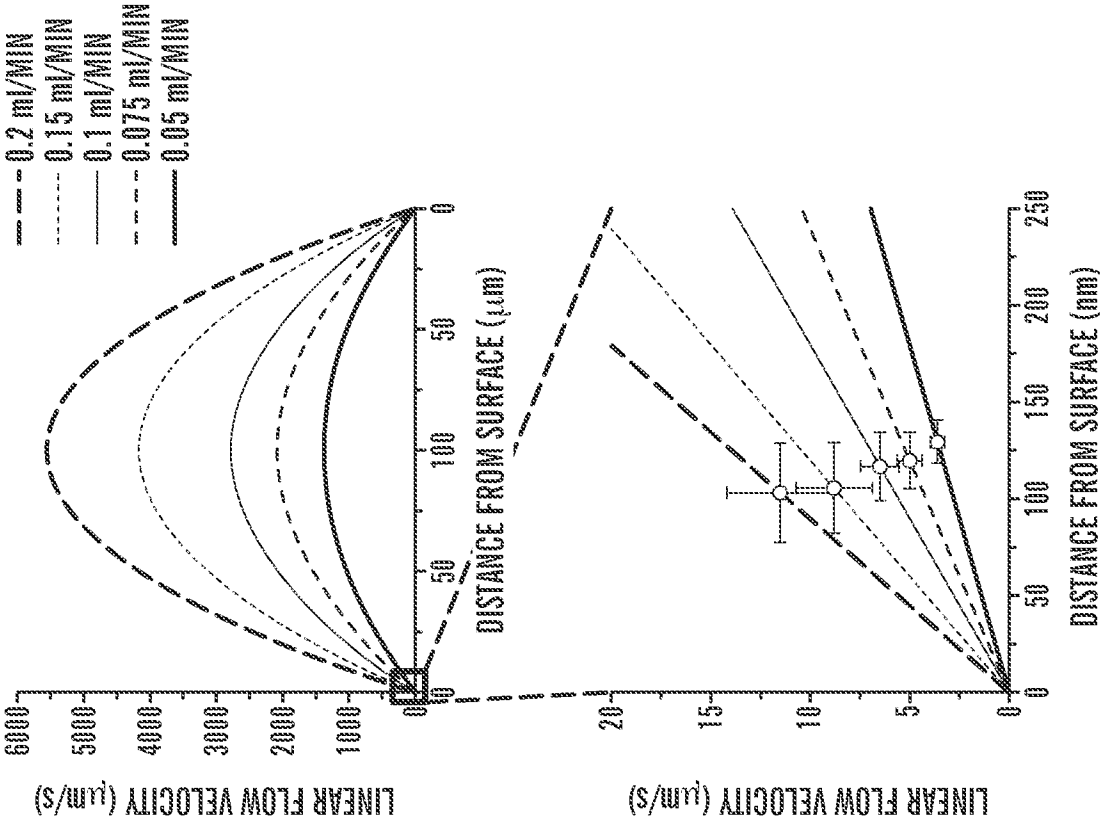


FIG. 9B

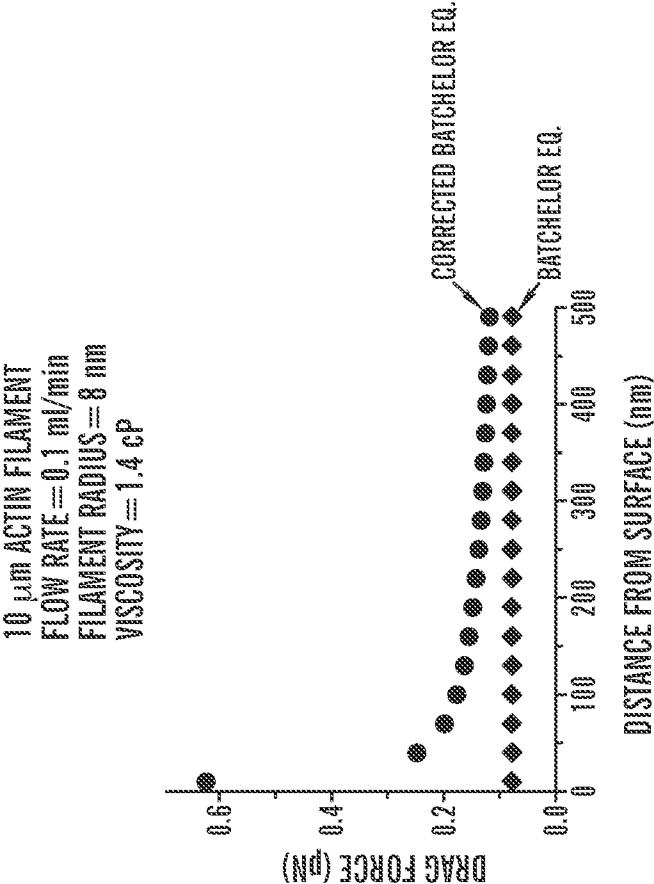
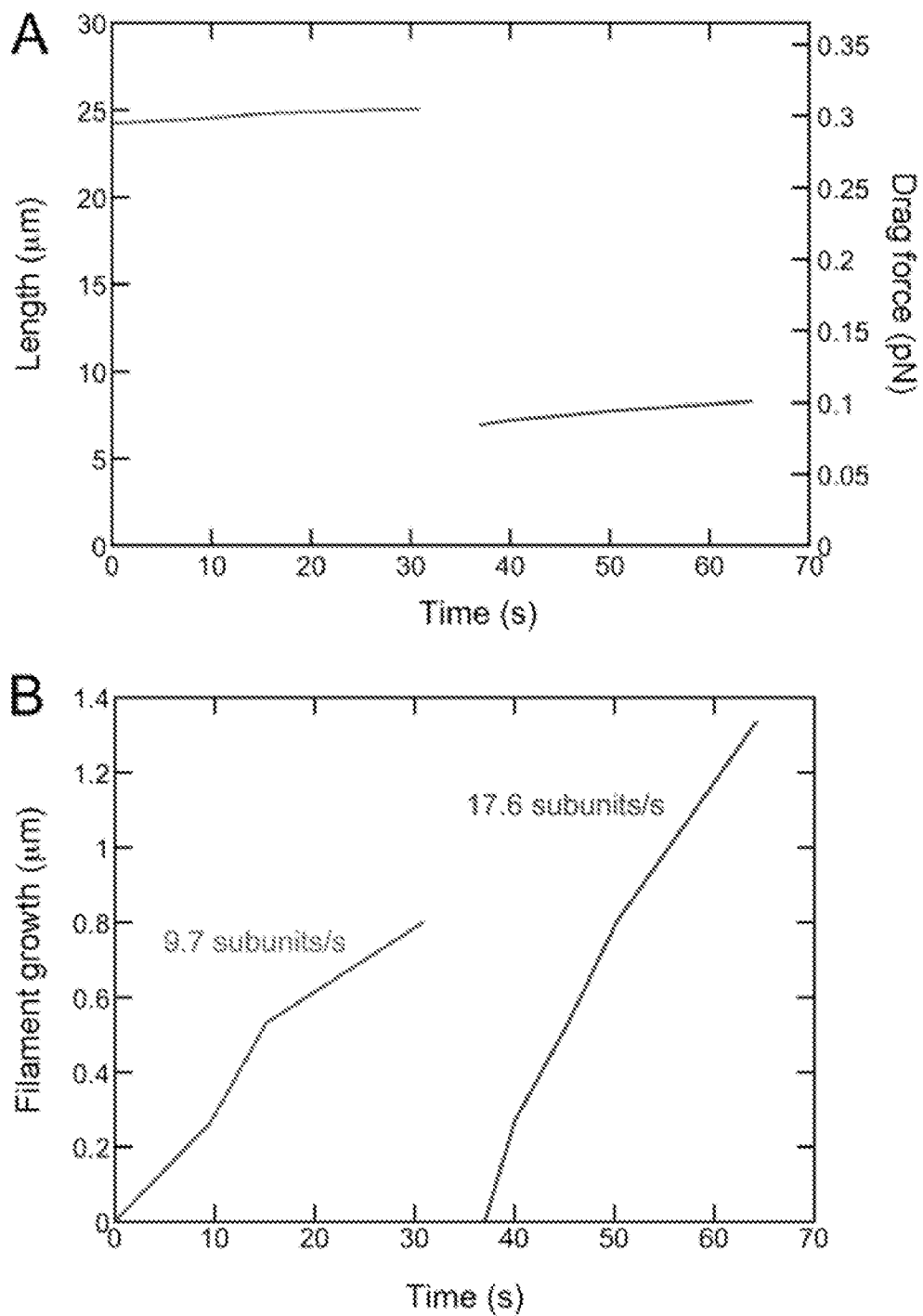
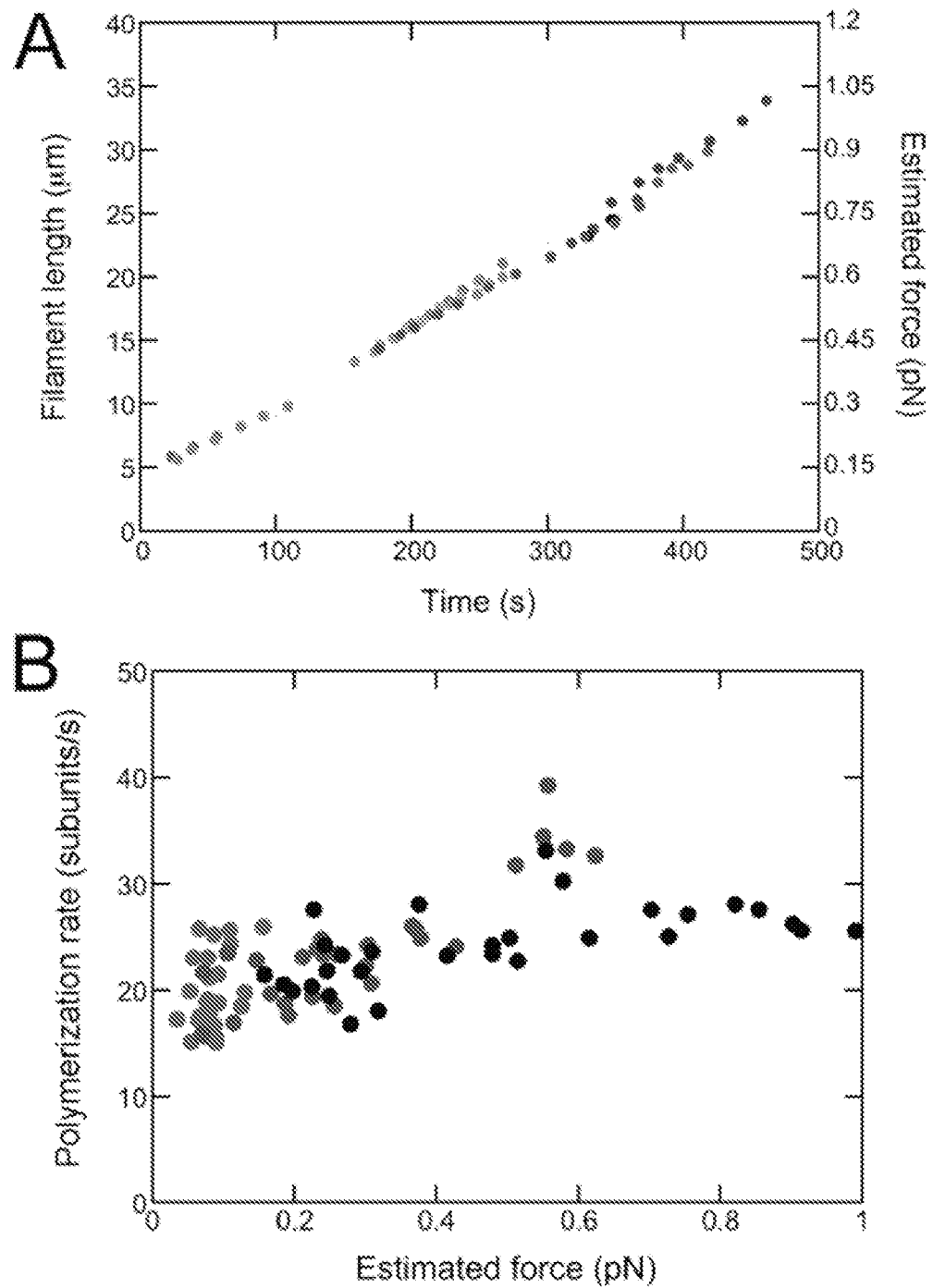


FIG. 9A

**Figures 10A-B.**

**Figures 11A-B.**

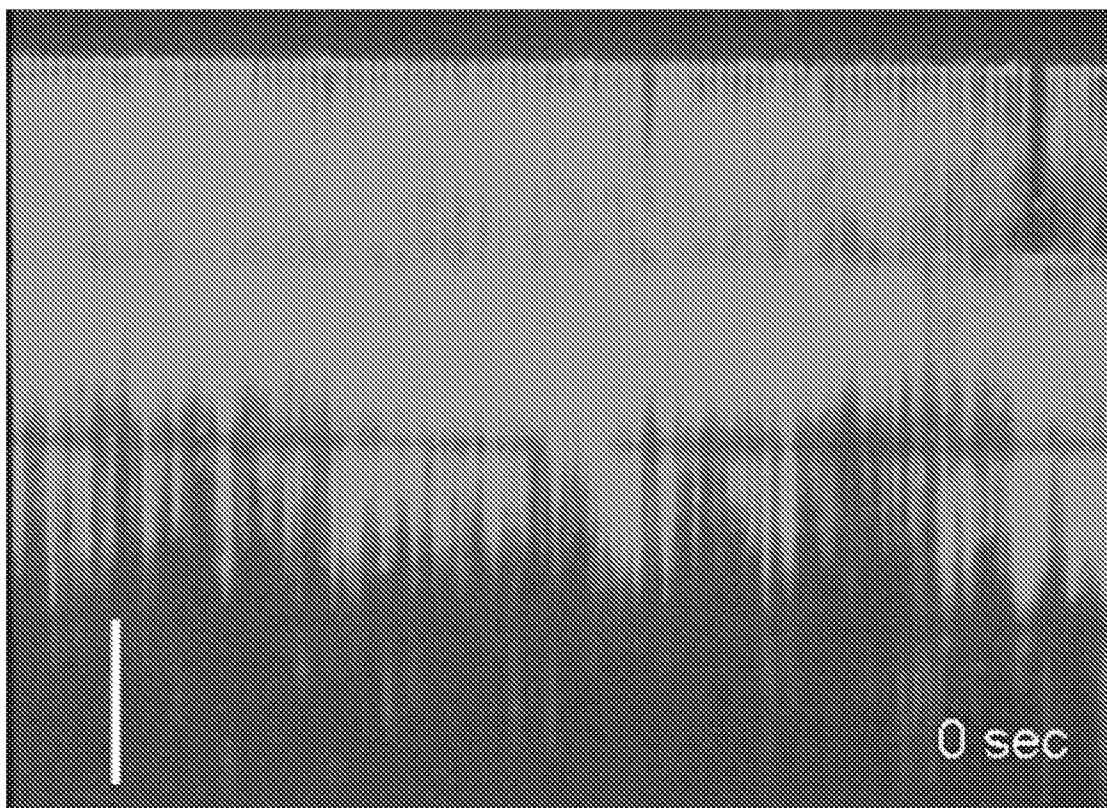
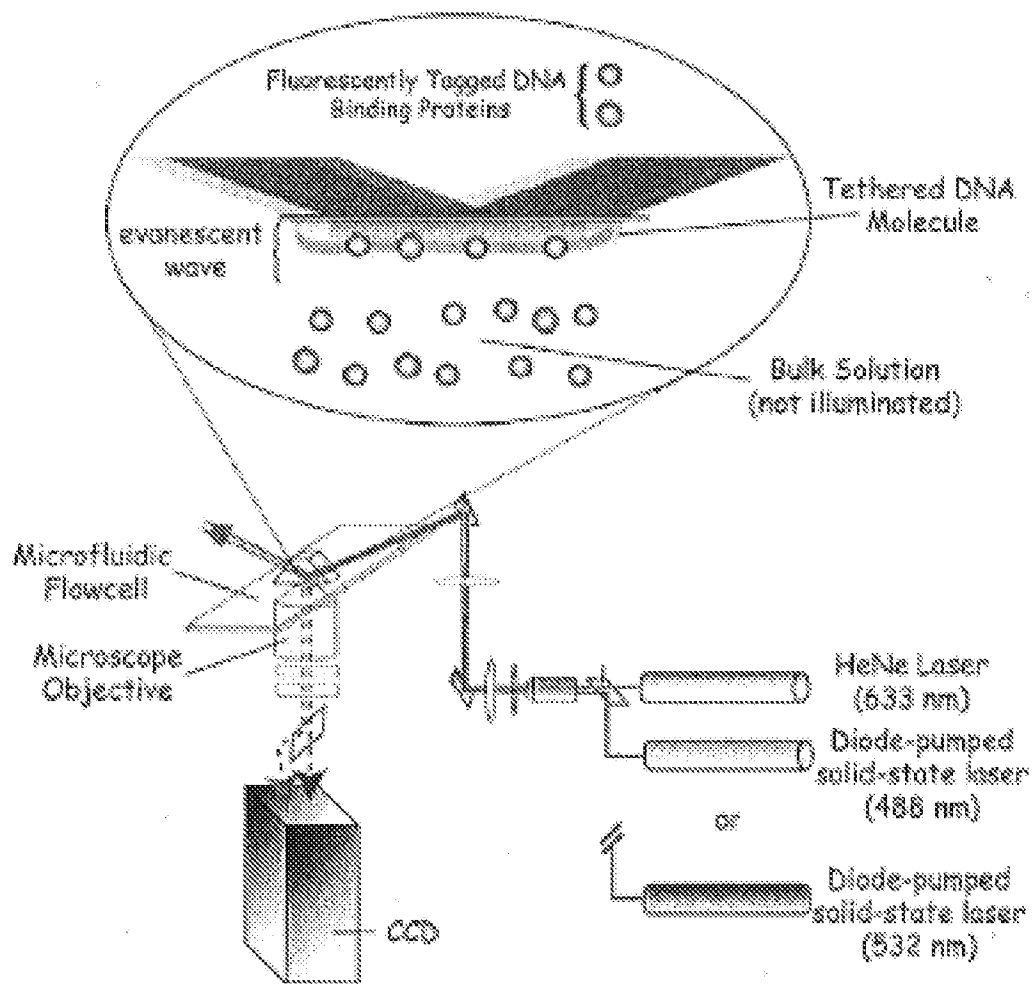
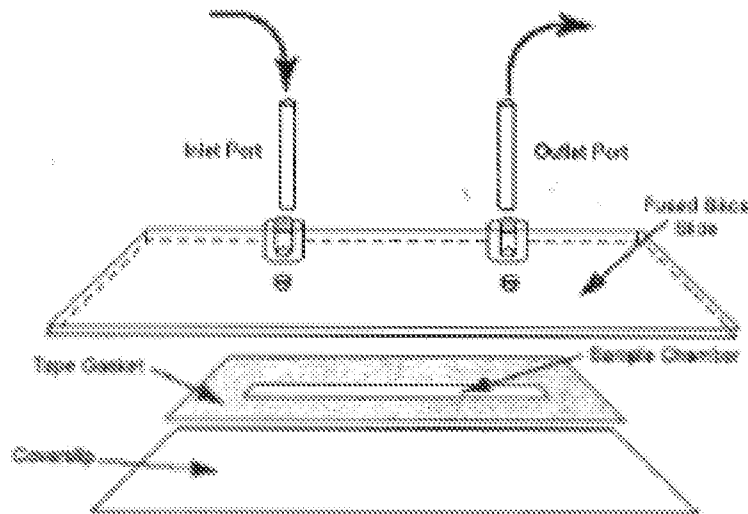


Figure 12.

**Figure 13**

A.



B.



- replace tape gasket with polyethylene (comprised a mixture of low and high density polyethylene).
- the use of polyethylene will allow us to make a stamping press that can be used to make the gaskets from sheets of polyethylene.
- assemble flowcell and heat to just above the melting point of the polyethylene, then cool to seal the junction between the coverslip and fused silica slide.

Figures 14A-B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/77583

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C40B 20/08, 30/10, 40/10; C12N 9/00 (2014.01)

USPC - 506/6, 12, 18; 435/183

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC: 506/6, 12, 18; 435/183

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
506/6, 12, 18, 435/183, 174, 317.1; 530/350 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Electronic data bases: PatBase, Google Patents, Google Scholar

Search terms: lipid bilayer array, protein filament (e.g. actin, vimentin, tubulin), filament binding protein (e.g. formin, profilin, plectin, alpha-actinin, fascin, spectrin), solid support, protein barrier, microfluidic, flow cell, hydrodynamic or electrophoretic force, fluorescent la

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2004/0018601 A1 (BOXER) 29 January 2004 (29.01.2004) Especially para [0027], [0032], [0037], [0039], [0050], [0054].	1-6, 11-17/(1,2), 23/(1,2), 27-32/(1,2)
Y	FRITZ et al. Actin Binding to Lipid-inserted alpha-Actinin. Biophys J Nov 1993 Vol 65 No 5 Pages 1878-1885. Especially abstract, pg 1883 fig 5, B.	1-6, 11-17/(1,2), 23/(1,2), 27-32/(1,2)
Y	US 2011/0136676 A1 (GREENE) 09 June 2011 (09.06.2011) Especially para [0014-0016], [0142].	12/(1,2), 28-32/(1,2)
A	WO 2012/020011 A1 (THERY et al.) 16 February 2012 (16.02.2012) Especially pg 2 ln 31- pg 6 ln 7.	1,2
A	US 2005/0106629 A1 (McGRATH et al.) 19 May 2005 (19.05.2005) Especially para [0006-0011].	1,2
A	US 2010/0304980 A1 (TAKEUCHI et al.) 02 December 2010 (02.12.2010) Especially para [0013-0038], sheet 1 fig 1.	1,2
A	DELATOUR et al. Arp2/3 controls the motile behavior of N-WASP-functionalized GUVs and modulates N-WASP surface distribution by mediating transient links with actin filaments. Biophys J June 2008 Vol 94 No 12 Pages 4890-4905. Entire document.	1,2

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

13 March 2013 (13.03.2013)

Date of mailing of the international search report

02 JUN 2014

Name and mailing address of the ISA/US

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Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

013/07/553 02.0
International application No.

PCT/US 13/77583

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 22, 41-43
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
---please go to Extra Sheet for continuation-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, 11-17/(1,2), 23/(1,2), 27-32/(1,2)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

U13/07/583 02.06.2014
International application No.

PCT/US 13/77583

Box III (Lack of Unity of Information)

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-21, 23-32, drawn to an array comprising: a) a solid support; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer. The first invention is restricted to an array, wherein the solid support comprises a barrier (claim 2) comprising a mechanical barrier (first option in claim 3) comprising a scratch on the solid support (claim 6), wherein the protein filament is actin (claim 17) and the filament binding protein comprises crosslinking protein, alpha-actinin (the first option in claim 23, see instant specification para [0074]). Group I+ will be searched to the extent that it reads on above restriction, without fee. It is believed that claims 1-6, 11-17/(1,2), 23/(1,2), and 27-32/(1,2) read on this first named invention. Applicants must indicate, if applicable, which claims read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group will result in only the first claimed invention to be examined. An exemplary election would be a scratch on the solid support (claim 6) and the intermediate filament protein vimentin (claim 20) and filament binding cross-linking protein spectrin (indicated in instant application para [0074]) (claims 1-6, 11-16/(1,2), 18/(1,2), 20/(1,2), 23/(1,2), 25-32/(1,2)).

Group II: Claims 33-40, drawn to a method of using an array composition of Group I+.

The inventions listed as Groups I+-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Specific technical features

The special technical feature of each invention of Group I+ is a unique array composition, not in any other invention in the Group I+.

Common technical features

Groups I+ and II are related as a composition (Group I+) and a method of using the composition (Group II), and share the common technical feature of (Group I+), an array comprising: a) a solid support comprising a protein barrier; b) a fluid lipid bilayer disposed on the solid support; c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer. However, said common technical features do not represent a contribution over the prior art, as being obvious over US 2004/0018601 A1 to Boxer et al. (hereinafter "Boxer"), in view of the publication titled "Arp2/3 controls the motile behavior of N-WASP-functionalized GUVs and modulates N-WASP surface distribution by mediating transient links with actin filaments" by Delatour et al. (hereinafter "Delatour") [published June 2008 in Biophys J Vol 94 No 12 Pages 4890-4905].

Boxer discloses an array comprising:

a) a solid support (para [0027]; "The terms "immobilized" or "supported" are used herein, for purposes of the specification and claims, to mean adsorption, coating or bonding the biological membrane according to the present invention to a support surface or structure"; para [0054]; "The substrate materials may comprise polymeric materials, e.g., plastics, such as polymethylmethacrylate (PMMA), polycarbonate, polytetrafluoroethylene (TEFLON.TM.), polyvinylchloride (PVC), polydimethylsiloxane (PDMS), polysulfone, and the like; para [0002]; "Supported lipid bilayers can be created by the self assembly of lipids into bilayers on solid supports, typically glass") comprising a protein barrier (fibronectin barrier in the array (para [0067]; "The flows impinge on a surface containing barriers of fibronectin that define a series of rectangular, 400 mm² regions that become "corrals" for assembly of lipid bilayer" sheet 1 fig 1C); b) a fluid lipid bilayer disposed on the solid support (para [0050]; "Devices. For the purposes of this invention, a device may comprise a supported lipid bilayer comprising pure populations of mobile membrane-associated biomolecules, e.g. in an information processing system, and may comprise multiple supported membranes, e.g. an array lipid bilayer is supported on a surface, allowing for one or more binding partners of the membrane to interact by specific binding with a mobile reactant"); Boxer does not specifically disclose c) at least one protein filament; d) a filament binding protein coupled to the protein filament; and e) a linkage for attaching the filament binding protein to the lipid bilayer. However, Boxer does disclose a linkage for attaching a protein to the lipid bilayer (para [0037]; "Specific high-affinity molecular interactions may also be employed to link selected compounds to a supported bilayer. For example, a bilayer expanse may be formed to include biotinylated lipids (available from, e.g., Molecular Probes, Eugene, OR), and a compound linked or coupled to avidin or streptavidin may be linked to the bilayer via the biotin moieties" para [0032]; "A variety of methods are available for preparing a conjugate composed of a molecule of interest and a vesicle forming lipid. For example, water-soluble, amine-containing compounds can be covalently attached to lipids, such as phosphatidylethanolamine, by reacting the amine-containing compound with a lipid that has been derivatized to contain an activated ester of N-hydroxysuccinimide"; [other methods described in para [0033-0036]). In addition, Boxer teaches specific protein binding-pairs used in the supported lipid bilayer (para [0039]; "Specific Binding pair. The term "specific binding member" as used herein refers to a member of a specific binding pair, i.e. two molecules, usually two different molecules, where one of the molecules through chemical or physical means specifically binds to the other molecule, and where the binding of the members of the specific binding pair is at a substantially higher affinity than random complex formation. Generally, the binding affinity will be at least about $K_m > 10^5$. The complementary members of a specific binding pair are sometimes referred to as a ligand and receptor. Such binding pairs may include antigen and antibody specific binding pairs, peptide-MHC antigen and T cell receptor pairs [...] peptide ligands and receptor; effector and receptor molecules; hormones and hormone binding protein; enzyme cofactors and enzymes; enzyme inhibitors and enzymes; and the like").

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Additionally, Delatour teaches (N-WASP) [indicated in instant application para [0074] as actin monomer binding protein and branching protein] mediates dynamic attachment between a membrane bilayer and the growing ends of actin filaments (in complex with Arp2/3) (pg 4907 fig 7 legend; "Relationship between the mobile states of N-WASP at the GUV surface and the elementary steps of filaments branching. N-WASP is anchored to the lipid membrane and binds G-actin in rapid equilibrium. N-WASP also binds the Arp2/3 complex. The ternary complex N-WASP actin-Arp2/3 associates with the barbed end of an actin filament, tethering it to the membrane. Once G-actin and the Arp2/3 complex have detached from N-WASP, the branched junction allows the growth of a new branch in the actin network. Elongating actin filaments push against the membrane"). Consequently, it would have been obvious to combine the array device taught by Boxer, with a binding pair of actin [protein filament] and N-WASP [filament binding protein], with the N-WASP linked to the lipid bilayer array of Boxer using the method taught therein, because it would have enabled studying the cellular cytoskeleton in a supported lipid bilayer array.

An additional common technical feature shared in Group I+ is a microfluidic flowcell (claim 29). Although Boxer discloses a microfluidic device as part of the lipid bilayer array (para [0020]; para [0055]), Boxer does not specifically disclose a microfluidic flow cell. However, said microfluidic flowcell applicable to a lipid bilayer array was known in the art at the time of the invention, as exemplified by US2011/0136676 A1 (Greene). Greene discloses a microfluidic flow cell comprising an array (para [0014]; In another aspect, the invention features a cell, e.g., a flowcell, e.g., a microfluidic flowcell, that includes an array described herein. The flowcell can be configured to allow a fluid to interact with the lipid bilayer, e.g., to flow over the lipid bilayer"; para [0015]-" molecules of the array are positioned into a desired orientation by application of a hydrodynamic force, an electrophoretic force, or combination of either, to the flowcell"). An artisan of ordinary skill in the art would have recognized the value of incorporating a microfluidic flow cell, as disclosed by Greene, into the microfluidic device and lipid bilayer array taught by Boxer because it would have enabled monitoring polymerization of a filament protein, or interaction of a filament protein with a filament binding protein, measure the effect of a compound or polypeptide on a protein interaction, etc.

Therefore, inventions of Groups I+ and Group II lack unity of invention under PCT Rule 13.