Growth and site-specific organization of micron-scale biomolecular devices on living mammalian cells

Sisi Jia1, Siew Cheng Phua2,6, Yuta Nihongaki2, Yizeng Li3,4, Michael Pacella1, Yi Li1, Abdul M. Mohammed1, Sean Sun3, Takanari Inoue2 & Rebecca Schulman1,5✉

Mesoscale molecular assemblies on the cell surface, such as cilia and filopodia, integrate information, control transport and amplify signals. Designer cell-surface assemblies could control these cellular functions. Such assemblies could be constructed from synthetic components ex vivo, making it possible to form such structures using modern nanoscale self-assembly and fabrication techniques, and then oriented on the cell surface. Here we integrate synthetic devices, micron-scale DNA nanotubes, with mammalian cells by anchoring them by their ends to specific cell surface receptors. These filaments can measure shear stresses between 0-2 dyn/cm², a regime important for cell signaling. Nanotubes can also grow while anchored to cells, thus acting as dynamic cell components. This approach to cell surface engineering, in which synthetic biomolecular assemblies are organized with existing cellular architecture, could make it possible to build new types of sensors, machines and scaffolds that can interface with, control and measure properties of cells.
Micron-scale molecular assemblies including membrane-bound and membrane-less organelles, cilia, cytoskeletal networks, or the glycateX spatially organize living cells, create specialized reaction environments, serve as transport conduits, and amplify chemical and mechanical signals in ways individual molecules cannot. Assembling synthetic micron-scale cell structures and controlling their dynamics are key goals of synthetic biology and nanotechnology because these abilities could make it possible to construct, for example, new cellular reaction chambers, sensors, and information and material conduits.

A key challenge in this pursuit is that the formation and evolution of the cell’s architecture are primarily kinetically driven. The time-dependent concentrations of the assembling species must be controlled to build dynamic structures that integrate functionally into a cell’s constantly evolving architecture.

A cell’s architecture extends from its interior to its surface. Organizing and directing molecules on the cell surface is important for controlling cell phenotype and cell–cell interactions, drug and gene delivery, and building biotic–abiotic interfaces. This can be achieved, for example, by attaching nanoparticles, small molecules, and nanowire–cell–electronic interfaces to the cell surface. While controlling interactions between cell receptors and nanostructures has been studied in the context of therapeutic modulation of receptor activity and for directing import of therapeutics, less is known about creating and organizing microarchitectures that programatically modify and extend cell surface architecture.

Micron-scale filaments are ubiquitous cell motifs that serve as sensors (antennae), mechanical supports, agents for generating motion and as substrates along which molecular motors can transport cargo. Filaments must grow and be anchored in prescribed orientations to execute these functions and actively grow and reorganize to maintain their structure and respond to stimuli. Here we organize micron-scale filaments that can act as functional cellular elements on specific cell surface receptors.

We used DNA tile nanotubes, which are semiflexible filaments with persistence length 8.7 ± 0.5 μm (on the order that of actin), that polymerize via Watson–Crick hybridization. These DNA nanotubes can grow from DNA origami templates, termed seeds, and can reach 100 μm in length. Nanotube seeds serve as sites where nanotubes are anchored by their ends to specific substrates. DNA nanotube growth kinetics and hierarchical assembly pathways and diffusion rates have also been extensively characterized, allowing kinetic control over their growth and interactions with cells. Nanotubes can be functionalized with polymers, gold nanoparticles, proteins, and peptides, and thus could be templates for constructing diverse functional devices.

We sought an approach for anchoring DNA nanotube ends to specific receptors on living cells that could be easily tailored to target different receptor types and hypothesized that this anchoring could be performed by attaching receptors to nanotube seeds. The design of such an anchoring process presents key challenges. First, a nanometer-scale anchor point on a filament’s end must bind specifically to the chosen receptor, and the filament’s much larger remaining surface must not interact with the cell. Second, because nanotubes can be detached from or imported into an active cell, the attachment of nanotubes to cells must be achieved through kinetic control, where a nanotube’s anchoring rate is higher than its rate of detachment or cell import. Microparticles can be anchored to cells because their large surface areas lead to high net attachment rates; molecules or complexes can be reliably anchored when they are supplied at high concentrations (≈10 nM). Anchoring nanotubes requires interaction with a small area of nanotube surface, and because of DNA nanotubes’ large size (~50 MDa), it is only practical to present them at concentrations <100–200 pM. To overcome these challenges, we develop a method in which a DNA nanotube seed serves as an anchor and presents numerous binding sites that attach quickly and effectively reversibly to the desired receptor. This approach yields efficient attachment to multiple receptors on multiple cell types with little nonspecific binding.

Results

Reliably anchoring nanotube seeds to cell receptors. We first characterized and eliminated nonspecific interactions between DNA nanotube seeds and nanotubes and cells. We measured the rate of DNA nanotube seed/cell interaction by adding Atto488-labeled DNA nanotube seeds (final concentrations 8–64 pM) to HeLa cells in culture (Supplementary Note S5). Confocal micrograph z-stacks showed that the average fluorescence intensity of seeds at the cells’ midline increased linearly with seed concentration; the average fluorescence intensity for cells to which 64 pM seeds had been added corresponded to 107 ± 17 distinct attached seeds per cell (Supplementary Note S6, Supplementary Fig. S4a–c).

Poly(ethylene) glycol (PEG) coating can reduce nonspecific interactions between nanoparticles and cell membranes. To test whether PEG coating might reduce nonspecific interaction between DNA nanostructures and cells, we hybridized 20 kDa PEG-15 nt DNA strand conjugates to seeds (Fig. 1c). Almost no PEG-coated seeds were visible on cells after 8–64 pM PEG-coated seeds were incubated with HeLa cells (Supplementary Fig. S4d–f).

We then conjugated 20 kDa PEG to nanotube monomers (Fig. 1d) and prepared seeded nanotubes by combining 415 nM PEG-conjugated monomers with 37 pM seeds and incubating them at 37 °C in TAE-Mg2+ buffer for 3 days. >40 ± 4.8% of the resulting filaments were >3 μm long (Supplementary Fig. S5), i.e. much longer than a micron. Neither PEG-coated nanotubes grown from PEG-coated or unmodified nanotube seeds attach to cells (Supplementary Note S8, Supplementary Fig. S6).

We first tried anchoring DNA nanotube seeds to cells using the SpyTag peptide and SpyCatcher protein, which form a covalent bond. We hybridized six SpyTag peptide–DNA conjugates (Supplementary Note S9 and Supplementary Fig. S7a, b) to each seed’s barrel. We then expressed a GFP–integrin–SpyCatcher fusion protein in HeLa cells via transfection (Supplementary Note S10). However, almost no nanotubes grew from SpyTag-modified seeds attached to cells (Supplementary Note S11 and Supplementary Fig. S7c, d), perhaps because of the low SpyTag–SpyCatcher reaction rate constant. Even assuming fusion receptor overexpression (104 per cell), at 64 pM nanotubes on average just one nanotube would anchor to each cell per hour (Supplementary Note S12). Anchoring nanotubes requires a much faster binding reaction, so we next considered antibody–receptor interactions, as most protein interactions have forward rate constants of 105–106 M−1 s−1.

We tried to anchor DNA nanotube seeds to epidermal growth factor receptors (EGFR) using EGFR–EGFR antibody binding. EGFR is a transmembrane receptor tyrosine kinase overexpressed up to 106 copies per HeLa cell. As expected, fluorescently
labeled secondary antibodies were attached to fixed and live HeLa cells only after EGFR primary antibodies were added (Supplementary Notes S13–S15, Supplementary Fig. S8). However, only a few DNA origami seeds with six secondary antibodies on their barrels\textsuperscript{27} attached per cell (Supplementary Note S16, Supplementary Fig. S9). We realized that while receptor–antibody binding was likely fast enough for hundreds of seeds to attach, most antibodies (including the EGFR antibody\textsuperscript{49}) have >nM affinities\textsuperscript{40}. Provided at picomolar concentrations, DNA origami seeds would thus not remain attached on average.

We thus turned to DNA hybridization. Forward rate constants\textsuperscript{50} of DNA hybridization is $10^5$–$10^6$ M$^{-1}$ s$^{-1}$, and a 15-nt DNA strand binds to its complement with sub-picomolar affinity under physiological conditions (Supplementary Note S17). We designed a process in which a DNA sequence, termed the biotin–DNA connection (BDC) tag, is first attached to a receptor using antibodies, biotin, and neutravidin. Nanotube seeds then present the BDC tag complement (BDC') which hybridizes to the tethered BDC (Fig. 1e). This approach can easily be generalized to attach different structures to different cell receptors: each of a set of antibodies could present a unique BDC sequence that would direct a particular unique nanostructure presenting that sequence's complement to bind. We termed this scheme "antibody-mediated, DNA-directed attachment" (AMDA).

![Figure 1](https://example.com/fig1)

Fig. 1 Anchoring synthetic filaments, DNA nanotubes, to specific cell surface receptors. a DNA nanotubes anchored at specific locations on the cell surface could act as dynamic, functional elements of cells. b Micron-scale DNA nanotubes self-assemble from monomer complexes by hybridization of complementary single-strand DNA overhangs or sticky ends. Arrows indicate 3' ends of DNA strands. X is complementary to X'. c Nanotube seeds are scaffolded DNA origami structures that template nanotube growth. Seeds can be coated with DNA–polyethylene glycol (PEG, molecular weight 20 kDa) conjugates (Supplementary Figs. S1 and S2). d PEG-coated DNA nanotube monomers and assembled nanotubes. e Schematic of EGFR antibody-mediated, DNA-directed attachment (AMDA) for anchoring seeded DNA nanotubes to cell surface receptors to origami seeds on nanotube ends. Primary antibodies, biotinylated secondary antibodies, streptavidin or neutravidin, and biotin–DNA connection tag (BDC tag) form complex to present a DNA sequence. This sequence hybridizes to the complementary DNA sequence on a DNA nanotube seed.
To test AMDA, we first added >10 nM each of primary EGFR antibodies, biotinylated secondary antibodies, neutravidin, and the BDC tag in steps to live HeLa cells (Supplementary Table S10). We then added 16 or 64 pM nanotube seeds. These concentrations were the same as those used in earlier experiments, providing a clear basis for comparison with those experiments, and the use of two concentrations allowed us to observe whether the process might be seed concentration-dependent. About 2-fold more seeds attached to cells after adding either 16 or 64 pM seeds presenting six sequences complementary to BDC, BDC, at their barrels’ ends than were attached after a control AMDA process where the BDC strand was not added (Supplementary Note S19, Supplementary Fig. S10).

We hypothesized that seeds' PEG coating might cover the BDC sequences. We added 24 thymines to the BDC' presenting strands to increase the distance between the BDC' sequence and the PEG. We also replaced 30 fluorescently labeled DNA strands attached to a loop of DNA on the seed (Fig. 1c) with strands presenting the BDC sequence. These changes dramatically increased the number of seeds attached to HeLa cells after AMDA, without increasing nonspecific attachment (Fig. 2a, Supplementary Note S20). Cross-sectional images showed seeds on the cell membrane, consistent with receptor attachment (Fig. 2b). Elimination of any AMDA step almost completely eliminated seed attachment (Fig. 2c, Supplementary Fig. S11).

We next used AMDA to attach nanotube seeds to EGFR on suspended HEK293 cells (Supplementary Note S23). Nanotube seeds were present all over cells after AMDA, while little staining intensity over a background of HEK293 cells as determined by flow cytometry (674 ± 75) was >5-fold higher after seeds were attached by AMDA than after a control process (125 ± 55) (Fig. 2f, Supplementary Fig. S13 and Supplementary Note S24).

To verify that DNA seeds attached proximally to EGFR, we measured the colocalization of nanotube seeds with fluorescently labeled DNA strands to increase the distance between the BDC sequence and the PEG. We added 24 thymines to the BDC' presenting strands to increase the distance between the BDC' sequence and the PEG. We also replaced 30 fluorescently labeled DNA strands attached to a loop of DNA on the seed (Fig. 1c) with strands presenting the BDC sequence. These changes dramatically increased the number of seeds attached to HeLa cells after AMDA, without increasing nonspecific attachment (Fig. 2a, Supplementary Note S20). Cross-sectional images showed seeds on the cell membrane, consistent with receptor attachment (Fig. 2b). Elimination of any AMDA step almost completely eliminated seed attachment (Fig. 2c, Supplementary Fig. S11).

We next used AMDA to attach nanotube seeds to EGFR on suspended HEK293 cells (Supplementary Note S23). Nanotube seeds were present all over cells after AMDA, while little attachment was observed in controls (Fig. 2d). The fluorescence intensity over a background of HEK293 cells as determined by flow cytometry (674 ± 75) was >5-fold higher after seeds were attached by AMDA than after a control process (125 ± 55) (Fig. 2f, Supplementary Fig. S13 and Supplementary Note S24).

To verify that DNA seeds attached proximally to EGFR, we measured the colocalization of nanotube seeds with fluorescently labeled DNA antibodies (Fig. 2g, h). 76 ± 4% (N = 12 cells) of seeds were colocalized with EGFR antibodies after AMDA; Stochastic attachment would result in only 20 ± 2% (N = 12 cells) colocalization (Fig. 2i, Supplementary Note S25).

Seeded DNA nanotubes attached reliably to HeLa cell membranes via AMDA but not in controls (Fig. 3a–c, Supplementary Note S26). Cells were 35-fold more fluorescent in the nanotube seed channel (Atto488) and 46-fold more fluorescent in the nanotube (Cy3) channel over background after AMDA vs. after a control protocol (Supplementary Note S27). Seeds did not move in time-lapse movies but attached nanotubes moved freely (Supplementary Movie S1), indicating that nanotubes were anchored to cells by seeds. Nanotubes could also be anchored to EGFR on HEK293 cells (Fig. 3d–f, Supplementary Movie S2, and Supplementary Note S28). They could also be anchored to integrin receptors on HeLa cells via AMDA, demonstrating AMDA’s generality (Supplementary Note S30 and Supplementary Fig. S15).

We next asked how long nanotubes or nanotube seeds would persist on a cell’s surface at 37 °C, as receptor turnover or seed endocytosis could lead to detachment or import. DNA origami seeds and seeded nanotubes were first attached to HeLa-GFP cells at 4 °C, where detachment rates were low. The cells were then returned to 37 °C where decreases in the number of seeds or nanotubes on the cell surface were measured using time-lapse confocal microscopy (Supplementary Notes S31 and S32). The fractions of nanotubes and nanotube seeds on the surface both decreased exponentially with time (Fig. 3g, h). The time constant for seeds was six times faster than for seeded nanotubes (Fig. 3h).

These measurements did not distinguish whether structures detached from or were imported into the cell[51]. EGFR-mediated endocytosis is a receptor-mediated clathrin-dependent pathway[52] in which a membrane invagination is pinched off by the motor protein dynamin. Nanotube seeds (Fig. 1d, length: 65 nm) are small enough to conceivably be endocytosed with EGFR[53]. EGFR-mediated endocytosis takes on order 30 min[54], consistent with the rate of seeds leaving the cell surface. Nanotubes are too large to be endocytosed, but dynamin-controlled membrane closure might sever them. EGFR is a fast-turnover receptor[55] and HeLa cells are fast-growing cells so these persistence times are likely at the lower range across different receptors and cell lines.

**Nanotube shear stress sensors.** We next tested whether anchored nanotubes could measure cell surface shear stress. Shear can result from flow and is a key environmental signal in vivo. For example, the primary cilium[1] is involved in sensing flow in the kidney[56], and bends in response to flows[26,27,28], inducing signaling[29,30]. We asked whether, like primary cilia, nanotubes might bend in response to shear stress and whether the extent of this bending might indicate the magnitude of shear stress. Because nanotubes are microns in length, their bending angles could be precisely measured, suggesting that shear stress could be precisely determined as a result.

To assess this possibility, we developed a model of how a nanotube anchored to the surface of a rectangular chamber would respond to shear stress induced by laminar flow of velocity U (Fig. 4a, Supplementary Note S34). A nanotube was modeled as a rigid rod anchored by a flexible linker. The chamber was much taller than a nanotube’s length (see the “Methods” section), so the flow field around the nanotube should be essentially uniform (Fig. 4b). In simulations, the polar angle between the nanotube and z-axis was close to π/2 except at very small shear stresses, so we assumed this polar angle was π/2 under external flow (see Supplementary Note S34.2, Supplementary Fig. S22). The nanotube’s response could therefore be reduced to an in-plane (xy plane) rotation, i.e. the azimuth angle, φ between the nanotube and the flow’s direction (Fig. 4b). In this case, the flow-induced viscous drag on the nanotube is $F = (a U \omega U^2, 0)$, where $a$ is the coefficient of viscous drag on the nanotube, $\mu$ is the viscosity of the fluid in the chamber, and $\ell$ is the nanotube’s length. The directional vector of the center of mass of the nanotube is $r = (\ell/2 \cos \phi, \ell/2 \sin \phi)$. Thus, the torque on the nanotube is $M = r \times F = -1/2 a U \omega U^2 \sin \phi$. We used this torque to calculate the dynamics of the nanotube, which are governed by $\gamma \dot{\phi} / \gamma = M + R$, where $\gamma$ is the nanotube’s damping coefficient, $M = |M|$, and $R$ is a random force from thermal fluctuations. $R$’s distribution is given by $P(R) \propto \exp[-R^2 \Delta t/(2k_B T)]$, where $\Delta t$ is the time step used to numerically evolve the equation. For each time step, a random $R$ was drawn. The initial value of the azimuth angle, $\phi_0$, of each nanotube, was randomly drawn from the uniform distribution $[-\pi, \pi]$. We solved the probability distribution of $\phi$ by sampling $\phi$ for a large number of nanotubes for each a set of volumetric flow rates Q = UHW, where $H$ and $W$ are the chamber’s height and width, respectively (Fig. 4c). We found that the distributions of azimuthal angles should vary for shear stresses between 0 and 1.5 dyn/cm², a range relevant for ion channel activation[25,26].

To measure the sensitivity and dynamic range of nanotube flow sensors, we anchored nanotube seeds to the bottom of a passivated glass microchannel[57] (Supplementary Notes S36 and S37) and measured their orientations under different flows using time-lapse spinning disk confocal microscopy (Fig. 4d, Supplementary Note S38). In the absence of flow, nanotubes explored all azimuthal angles and bent in the z-direction. Shear stress of only 0.05 dyne/cm² caused the nanotubes to remain in place and align with the flow (Fig. 4d). To quantify the relationship between nanotube orientation and fluid shear stress on the glass, we
measured the mean total angle of nanotube rotation over 30 frames taken every 5 s at different fluid shear stresses (Supplementary Note S41). A maximum time projection image of each nanotube was generated from these images indicating the nanotube’s total range of angular motion, \( \Phi \) (Fig. 4f). The mean \( \Phi \) for different nanotubes experiencing given shear stress decreased with increasing shear stresses between 0.05 and 2 dyn/cm², which was consistent with our model’s predictions (Fig. 4h).

Nanotubes attached to cells via AMDA (Supplementary Notes S39 and S40) also increasingly aligned with the flow as shear stress increased (Fig. 4e, g, and Supplementary Movie S3). Because cells are not flat, a nanotube’s location on a cell affects its bend direction and motion. The total angles of rotation of nanotubes on the tops of cells varied most in response to different flow rates. \( \Phi \) for nanotubes on the tops of HeLa cells was close to both the values predicted by the model and the values measured on glass for all shear stresses, suggesting how anchored nanotubes can serve as “windsocks” on cells that indicate flow direction and the magnitude of shear stress the flow induces.

Growing nanotubes on living cells. A key advantage of using self-assembled biomolecular structures as cell surface microdevices...
is that they might dynamically grow or reorganize via biomolecular reactions. To explore the possibility of constructing such dynamic microdevices, we asked whether DNA nanotubes could grow while anchored to cell receptors.

Nanotubes can grow via monomer addition but at monomer concentrations where end-on growth is preferred over homogeneous nucleation, growth occurs at <0.2 µm/h\(^{27,34,61}\). Because nanotubes persist only a few hours on EGFR, we sought instead to extend nanotubes via the end-to-end joining of pre-assembled nanotubes\(^{31,62}\). While rapid end-to-end DNA nanotube joining has been observed in vitro\(^{27}\), only 7 ± 2% (\(N = 477\)) of PEG-coated nanotubes underwent end-to-end joining within 4 h in cell buffer at physiological temperatures (Supplementary Note S42 and Supplementary Fig. S26). We hypothesized that end-to-end joining did not occur because the monomer detachment rate was very low, allowing rough facets or facets with defective monomers that cannot join to persist (Supplementary Fig. S28). To increase the monomer detachment rate, we shortened the monomers’ binding sites from 6 to 4 nucleotides\(^{63}\) to produce 4PEG nanotubes. 86 ± 3% (\(N = 803\)) of 4PEG nanotubes anchored to a glass surface grew via end-to-end joining within 3.5 h after 4PEG nanotubes (green) and 150 nM monomers that could serve as “glue” to fill in gaps between rough facets (red) were added (Supplementary Note S45 and Supplementary Fig. S29b). The average length of the joined nanotubes was 8.2 ± 0.35 µm (\(N = 774\), Supplementary Fig. S31a). The 4PEG nanotubes...
remained intact after end-to-end joining when the gentle fluid flow was applied to them (shear stress 0.32 dyn/cm²) for 35 min at 20 °C (Supplementary Fig. S29c).

60±9% (N = 206) of 4PEG nanotubes attached to EGFR on HeLa cells were extended to an average length of 6.9 ± 0.62 µm (N = 189, Supplementary Fig. S31b) using a similar protocol of end-to-end joining and monomer gluing (Fig. 5a–c, Supplementary Movie S4 and Supplementary Note S46). Since the flow used to image the joined nanotubes attached to cells sometimes severed the nanotubes (Supplementary Fig. S30), we repeated the joining process and then added methylcellulose to reduce nanotube diffusion (Supplementary Note 48). This process revealed alternating green-red segments indicating nanotube gluing and joining (Fig. 5c–e, Supplementary Figure S33 and Supplementary Movie S5) as well as overlapping red and green segments indicating filament bundling, which can be induced by high viscosity medium64.

Discussion
While biomolecular filaments are structurally simple, they can assemble in myriad ways to create complex functional materials...
and devices, as exemplified by cytoskeletal structures. Here we site-specifically anchored synthetic DNA filaments to living cells by determining the required binding affinities, reaction rates and avidity for efficient attachment, and mitigating nonspecific interactions. These anchored DNA filaments act as cellular "windsocks" that can sensitively measure the magnitude of shear stress at the cell surface within a physiologically significant range. The resulting precise control over attachment-in combination with our understanding of DNA nanotube growth rates, hierarchical assembly and reorganization—might be used to build a range of synthetic, dynamic filament-based devices on cells, including antennae, motion-inducing devices or conduits that connect receptors on different cells. The ability to form nanotubes from many different types of monomers could also make it possible to build more complex, heterogeneous devices. Here we showed that nanotubes could remain attached to cells for multiple hours. Longer time scales might be achieved by attaching nanotubes to receptors with long turnover times. The techniques developed here could also be combined with techniques that increase DNA nanostructures stability in biological environments, such as coating DNA nanostructures with cationic polymers, cationic block co-polymers, or lipid bilayers. Nanotube lifetime in the presence of nucleases could also be increased by adding monomers to the cell medium that incorporate into nanotubes to repair damage. We also demonstrate that, like the cells they are attached to, anchored DNA filaments can continue to grow as monomers or whole filaments can bind end-to-end to them. Growing filaments could

Fig. 5 Nanotube growth on the surfaces of living cells. a Schematic of nanotube growth via end-to-end joining and monomer addition. Seeds on anchored and capped nanotubes were unlabeled. Nanotubes were labeled with Cy3 (green), monomers with atto647 (red). b Two-color fluorescence micrograph of joined nanotubes anchored to live HeLa cells. Gentle fluid flow (shear stress 0.32 dyn/cm²) was applied to stretch the nanotubes for better characterization (Supplementary Note S46). Scale bar: 20 μm. Zoom-in images of end-to-end joined nanotube structures on the cell surface. Scale bar: 5 μm. This experiment was repeated independently more than three times with similar results. c Nanotube end-to-end joining yield quantification: 6nt seeded nanotubes incubated with capped seeded nanotubes in solution at 37 °C for 3.5 h without additional monomers (Supplementary Note S42 and Supplementary Fig. S26, N = 477 nanotubes at n = 8 random locations was analyzed), 4PEG seeded nanotubes anchored on glass surface incubated with capped nanotubes and additional 150 nM monomers added at 20 °C for 3.5 h (Supplementary Note S45 and Supplementary Fig. S29, N = 803 nanotubes at n = 11 random locations was analyzed), 4PEG seeded nanotubes anchored on cell membrane incubated with capped nanotubes and additional 150 nM monomers at 20 °C for 4 h (Supplementary Note S46, N = 206 nanotubes at n = 6 random locations was analyzed). Error bars here are 95% confidence intervals. d and e Schematic (d) and 3D projection images (e) of joined nanotubes (green and red) on a live HeLa cell after 0.6% methylcellulose (IMDM) was added (Supplementary Note S48). Cells were transfected with GFP (blue) to reveal cell shape and extent. Scale bar: 10 μm. This experiment was repeated independently more than three times with similar results.
form between cells or anchor points. More generally, the understanding of how filament binding kinetics and thermodynamics, assembly timing, and component stoichiometry affect filament growth and organization might also enable the design of genetically encoded processes to direct the assembly of filaments synthesized by the cells themselves.

**Methods**

**Reagents.** M13mp18 scaffold strand was purchased from Bayou Biolabs. All other DNA strands used in this study were synthesized by Integrated DNA Technologies, Inc. (IDT). Strands for the DNA nanotube tiles (Supplementary Note S49) and the adapter strands for the DNA nanotube seeds (Supplementary Note S50), Cy3-, ATTO647- and ATTO488-, biotin-labeled strands and amino-modified strands were HPLC purified. All other strands were simply desalted. Concentrations of DNA solutions were determined either by optical absorbance at 260 nm (using extinction coefficients supplied by IDT) or by relying on IDT to determine solution concentrations. N-hydroxysuccinimide (NHS) functionalized polyethylene glycol (molecular weight 20 kDa) (PEG-20K) was purchased from NANOCS (PG1-SVA-20K). Phosphate buffered saline (PBS) (28372) was purchased from ThermoFisher (molecular weight 20 kDa) (PEG-20K). Phosphate buffered saline (PBS) was purchased from ThermoFisher.

**Cell culture.** HeLa cells (ATCC) and HEK 293 cells (ATCC) were grown in a DMEM medium containing 10% FBS and 1% penicillin–streptomycin. 5 mL of culture was grown in 25 cm2 culture flask at 37 °C in 5% CO2 and constant humidity. Cells were released from the flask surface using 0.05% Trypsin–EDTA and split every 2 days. The HELa cells were cultured in media with: 3, 6, 9, and 12 mM MgSO4 overnight, after which time cell viability was confirmed by phase under a bright-field microscope (Supplementary Note S4).

**Characterizing the extent of nonspecific interactions between DNA nanotube seeds or nanotubes and HEa cells.** HeLa cells were seeded in borosilicate glass Lab-Tek 8-well chambers at a density of 40,000 cells per well in 2 mL of IMDM (Supplementary Note S22). DNA nanotubes with and without PEG coating were diluted to make 8, 16, 32, and 64 PM solutions in a cold DMEM solution containing 1% BSA (w/v) and 12 mM MgSO4. The medium in each well chamber containing HeLa cell was exchanged for 250 µl of diluted solutions. The cells were then incubated in a 4 °C refrigerator for 30 min and subsequently washed with DMEM-12 mM MgSO4 buffer 3 times (Supplementary Note S5). Seeded DNA nanotubes grown from 37 PM seeds with PEG or 28 PM seeds without PEG in TAE–Mg2+ were diluted one-fold with cold 1% BSA (DMEM)–12 mM MgSO4. The medium in the wells containing HeLa cells was exchanged for diluted nanotube solution. The cells were then incubated in a 4 °C refrigerator for 2 h and subsequently washed with DMEM-12 mM MgSO4 3 times (medium in each well chamber Note S8). After both treatments, the cells were fixed using 4% paraformaldehyde before imaging.

**Transfection of HEa cells with SpyCatcher-fusion transgenes.** The GFP-integrin-SpyCatcher plasmids were constructed by inserting the SpyCatcher DNA sequence into the GFP-integrin construct at the Ndel restriction site. The backbone of the integrin-SpyCatcher is a Glucont with kanamycin resistance. For plasmid sequences (see Supplementary Note S51). The plasmid was transformed and amplified in DH5α bacteria, and amplified using a Qiagen miniprep kit. HeLa cells for transfection were cultured and passaged as described above. HeLa cells were counted using a hemacytometer and diluted to 2.4 × 103 cells per mL in DMEM 10% FBS. Each transfection process, 12 µL of Opti-MEM (1x) and 2 µL of a solution containing 1 mg/mL plasmid DNA were mixed well in a 1.5 mL Eppendorf tube via pipetting. 2 µL X-tremeGENE 9 solution was then added and the solution again mixed well via pipette, after which the mixture was incubated at room temperature for 30 min. 42 µL of the plasmid solution was then added to 500 µL of diluted cells in a 1.5 mL Eppendorf tube and mixed well by gently inverting the tube around 20 times. 250 µL of cell solution was then pipetted into a well of borosilicate glass Lab-Tek 8-well chamber. The cells were then incubated for 2 days before use (see Supplementary Note S10).

**Attachment of seeded nanotubes to cells using SpyCatcher-SpyTag attachment.** SpyTag-modified seeded DNA nanotubes were prepared by attaching an azide-modified SpyTag peptide to an amino-modified DNA oligonucleotide via a click reaction (Supplementary Note S9) and hybridizing this strand to the complementary sequence presented at the ends of nanotube seeds which were then spin-filtered to remove extra SpyTag (Supplementary Note S1 step 3). Nanotubes were then grown from seeds with and without the SpyTag modification and diluted 1:5-fold with buffer. The medium in each well containing HeLa cells expressed with GFP–integrin–SpyCatcher fusion protein was exchanged for 250 µL of diluted nanotube solution prepared as described for characterization of nonspecific nanotube–cell interactions but diluted by DPBS with 12 mM MgSO4 buffer and incubated at 37°C/5% CO2 for 30 min (Supplementary Note S11).

**Attachment of nanotubes seeds or seeded nanotubes to EGF receptors on HEa cells using AMDA.** PEG-coated nanotube seeds or seeded nanotubes were prepared as described above and HeLa cell were seeded overnight in borosilicate

Growing PEG-coated seeded nanotubes. The structures and sequences of DNA nanotube monomers with both 6- and 4-base sticky end binding sites are given in Supplementary Note S49 and Supplementary Tables S27 and S28. To grow PEG-coated seeded nanotubes with six base sticky ends, the central SEs3 strand of the tile was conjugated with PEG as described in Supplementary Note S7. 19.7 µL of a TAE–Mg2+ solution containing 450 nM of each of the strands for the monomers was annealed from 90 to 37 °C as described in Supplementary Note S1 step 2. 2.2 µL of a solution containing 0.4 nM PEG-coated seeds was added after the monomer solution reached 57 °C. The mixture was kept at 37 °C for 3 days. To grow 4PEG nanotubes, the central SE3 and SE4 strands of the two monomer types were each conjugated with PEG (Supplementary Fig. S5) and Supplementary Table S28). 19.7 µL of a TAE–Mg2+ solution containing 180 nM of each of the strands of the two 4PEG nanotube monomers was annealed from 90 to 20 °C as described in Agrawal et al. 34, 2 µL of a TAE–Mg2+ solution containing 0.4 nM PEG-coated nanotube seeds (Supplementary Table S29) were adsorbed to 200 nM of TAE–Mg2+ coated capped nanotube seeds (Supplementary Table S22) as appropriate for experiments on nanotube joining were added after the solution reached 20 °C. The solution was then incubated at 20 °C for 3 days. Seeded nanotubes with PEG coating were imaged under an epi-fluorescence microscope (Olympus IX71) with a ×60/1.45 NA oil immersion objective lens using Andor SOLIS (Oxford Instruments) software.
glass Lab-Tek 8-well chambers at a density of 40,000 cells per well with 250 µL medium (Supplementary Note S5 step 2). The next morning, the cells were first incubated in a 4 °C refrigerator for 10 min. DMEM buffer containing 1% BSA (w/v) was then added to the cells, which were then incubated for 5 min. To attach nanotube seeds, 250 µL of a solution containing (1) 2 µg/mL EGFR primary antibody, (2) 10 µg/mL Alexa 647-labeled secondary antibody–streptavidin conjugate, (3) 1 µM BDC tag and (4) BDC–tag labeled nanotube seeds at concentration to achieve the stated concentration in cell solution were added in the order listed. After each addition the cells were incubated in the refrigerator for 30 min, then washed 3 times with cold DMEM buffer containing 1% BSA (w/v) then a solution of seeded nanotube solution (1-fold diluted after buffer). To attach seeded nanotubes, resuspension in Alexa 647-labeled secondary antibody followed by (2b) 3 µg/mL Alexa488-labeled streptavidin. After each addition cells were washed 3 times with cold DMEM buffer. To attach nanotubes to cells, the seed solution was replaced by a one-fold diluted solution of seeded nanotubes. This solution was incubated with cells for 2 h, pipetting gently every 30 min. After incubation cells were washed with DMEM–12 mM MgSO4 buffer 3 times. Attachment of nanotube seeds or nanotubes to EGFR receptors on HEK293 cells using AMDA. PEG-coated nanotube seeds and seeded nanotubes were prepared as described above and HEK293 cells were trypsinized and suspended to a concentration of 10⁶–10⁷ cells per mL. The cells were centrifuged at 300 x g for 5 min, the supernatant was removed and the cells were resuspended in cold 1% BSA in DMEM. To attach nanotube seeds to suspended HEK293 cells, the cells were centrifuged and resuspended in a solution containing (1) 2 µg/mL EGFR primary antibody, (2) 10 µg/mL Alexa 647 2AB-STA, (3) 1 µM BDC tag, and (4) PEG-coated nanotube seeds in sequence. After each addition, the cells were incubated in a 4 °C refrigerator for 30 min and pipetted-mixed every 15 min. Then, washed by centrifuging and resuspending in 1 mL cold DMEM buffer to remove unattached reagent. After this sequence, the cells were resuspended in DMEM–12 mM MgSO4 buffer. To attach seeded nanotubes, resuspension in Alexa 647–labeled secondary antibody solution was replaced by resuspension in (2a) 500-fold diluted biotinylated secondary antibody then (2b) 3 µg/mL Alexa488-labeled streptavidin. After the addition of the BDC tag and resuspension, the cells were resuspended in 1 mL cold DMEM buffer then a solution of seeded nanotube solution (1-fold diluted after preparation). The cells were incubated in a 4 °C refrigerator for 2 h during which time they were pipetted gently every 30 min. The cells were centrifuged a last time, then resuspended in DMEM–12 mM MgSO4 buffer. Attachment of nanotubes to integrin receptors on HeLa cells using AMDA. The steps for AMDA were followed above except that the EGFR primary antibody solution was replaced with a solution containing 4 µg/mL Integrin β1 antibody. After addition, cells were incubated at 4 °C for 1 h. Spinning disk confocal microscopy. Cells with attached DNA nanotube seeds or seeded nanotubes were imaged using a Zeiss AxioObserver Yokogawa CSU-X1 spinning disk confocal microscope with a x60 oil objective. Stack images were taken by ZEN2 (blue edition) from the bottom of the cell to the top of the cell with a stack depth of 0.27 µm (for HeLa cells) or 0.5 µm (for HEK293 cells) at 10–15 random locations. Quantification of the number of nanotube seeds and seeded nanotubes attached to each cell. The average fluorescence intensity per cell was used to quantify the average number of seeds/seeded nanotube attached to a HeLa cell. A z-stack of images was collected at each imaging position, and the stack image from the height closest to the center of an average-sized cell was selected for analysis. This choice was made because this image largely excluded the structures attached to the glass rather than the cell while maintaining a sufficiently large cross-sectional area for each cell for analysis. The number/total length of nanotube seeds and seeded nanotubes present was measured by characterizing the total fluorescence intensity (Supplementary Notes S22 and S27). The seeds’ intensity per cell and nanotube intensity per cell were then calculated by dividing these respective quantities by the number of cells in an image, which was also counted manually. Flow cytometry was used to characterize the number of nanotube seeds on the HEK293 cells (Supplementary Note S24). The fluorescence intensity of ~10,000 cells were collected using a FACScanto flow cytometer (BD Biosciences, USA) with BD FACSDiva software v8.0 and analyzed by software FlowJo 10.4. The number of seeded nanotubes on HEK293 cells was determined by generating a 3-dimensional projection image from each of a z-stack of images collected at multiple random locations and manually counting the number of seeded nanotubes visible on each cell (Supplementary Note S29.2). The amount reported is the average of these counts. Quantification of the dwell time of seeds and seeded nanotubes on the cell membrane after AMDA. PEG-coated DNA nanotube seeds or seeded nanotubes were anchored on HeLa–GFP cells using EGFR AMDA as described above. The cells were washed with cold (4 °C) DMEM medium-12 mM MgSO4 then placed into an incubator (37 °C, 5% CO2 and constant humidity) on a Nikon A1 confocal microscope (Nikon, Tokyo, Japan) with a x63 oil objective. Stacks of images at height closest to the center of the cell in the field of view. Image stacks of cells with attached nanotube seeds were collected by NIS-Elements AR 5.20.01 (Nikon) every 10 min over 70 min with a stack height of 0.27 µm. Image stacks of cells with attached nanotubes were collected every 15 min over 20 h with a stack height of 1 µm. The number of nanotube seeds and seeded nanotubes on each tracked cell’s membrane were counted manually. These numbers were normalized by the number of seeds or nanotubes on that cell at t = 0. Seeds were counted if they were visible at a cell’s edge. The top and bottom images of a stack were omitted from quantification because it was not possible to determine whether seeds were removed or if they were attached to the cell. The total number of seeded nanotubes on a cell’s surface at each time point was manually counted using maximum projection images (Supplementary Note S33). Use of fluid flow to apply shear stress at a glass surface or HeLa cell membrane. Shear stress was applied within flow cells (ibidi, µ-slide V1.0.5 for glass-anchored nanotubes, height 0.54 mm, length 17 mm and width 3.8 mm and µ-slide V1.0.4 for cell-anchored nanotubes, 0.4 mm, length 17 mm and width 3.8 mm) and the shear stress corresponding to a given flow rate was calculated according to the methods provided by Ibidi (Supplementary Note S35 and Supplementary Table S17). Seeded nanotubes were anchored to the glass bottoms of flow cells using a method developed previously (Supplementary Notes S36 and S37). A syringe pump (New Era, NE-1000) was used to induce controlled, unidirectional laminar flow of TAE–Mg2+ buffer as flow perfusate (Supplementary Note S38). Seeded nanotubes were anchored to the HeLa cell membrane through EGFR AMDA performed in a flow cell (Supplementary Note S39). DMEM–12 mM MgSO4 buffer was used as flow perfusate (Supplementary Note S40). Seeded nanotubes under fluid flow were imaged using a spinning disk confocal microscope with 5x intervals for 30 cycles. The nanotubes were imaged in the xy-plane in which the largest number of seeds and the largest fraction of the nanotubes were in focus. The total angle of the nanotube rotation under fluid flow was measured by first cropping the area spanned by a single nanotube from each larger image. A Gaussian filter (radius:1.00) was applied in ImageJ for all the 30 cropped images to reduce the image background. A maximum time projection image was then generated from this cropped time-lapse movie and the total angle of the nanotube rotation under fluid flow was measured manually (Supplementary Note S41). The total angle of 15 nanotubes on the glass surface >15 nanotubes on cell membrane was measured for each shear stress. Growth of seeded nanotubes anchored to the cell membrane. 4PEG nanotubes and 4PEG capped and seeded nanotubes were prepared as described in Supplementary Note S43. Here both the capped and anchored nanotube seeds were unlabeled (Supplementary Tables S20 and S22). Anchored seeded nanotubes were attached to the HeLa cell membrane through EGFR AMDA within a µ-slide channel. 50 µL of a solution containing 900 nM inactive nanotube monomers (Supplementary Note S44 and Supplementary Table S29) labeled with atto647 (Supplementary Table S25) was annealed in TAE–Mg2+ buffer from 90 °C to 20 °C. 0.27 µL of solution containing 100 µM of a strand to activate the inactive monomers (Supplementary Note 46) was added to this solution after which 25 µL of it was immediately mixed with 60 µL of a solution containing 37 µM of capped nanotubes and 65 µL of DMEM–12.5 mM MgSO4 buffer containing 1% BSA. This mixture was immediately added to the HeLa cells to which seeded nanotubes had been attached. The sample was covered with foil and incubated on the lab bench (at about 19–21 °C) for 4 h. It was then washed with DMEM–12 mM MgSO4 three times before imaging. A gentle fluid flow (0.18 mL/min) inducing a shear stress of 0.32 dyn/cm² was applied to stretch the nanotubes and allow visualization of their contours. An epi-fluorescence microscope with a x60 oil objective was used to capture continuous 20 images of each location. The yield of nanotube joining on cell membrane was calculated by counting the total number of seeded nanotubes on the cell and the number of nanotubes on the cell that had visually joining. Cells in six images were quantified. 0.6% methylcellulose media (IMDM) with 12 mM MgSO4 was added to HeLa GFP cells after the joining protocol (but not flow or imaging) was completed (Supplementary Note S48). Stacks of images at random locations were taken from the bottoms to tops of cells with a stack height of 0.27 µm using a spinning disk confocal microscope. Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article. Data availability: The data for this study are available online at: https://doi.org/10.7281/T1/CUEL0W. Source data are provided with this paper. Code availability: The simulation code and image analysis code for this study are available online at: https://doi.org/10.7281/T1/CUEL0W. Source data are provided with this paper.
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Author contributions
This study was conceived by S.J., R.S. and T.I. S.J. designed the experiments, carried out the experiments, and analyzed and interpreted the data. S.C.P. and A.M.M. designed the SpyTag-SpyCatcher fusion binding approach for nanotube-cell receptor attachment. S.C.P. constructed the GFP-integrin-SpyCatcher and GFP-integrin plasmids DNA and helped with cell culture and cell transfection. Y.N. helped with the flow experiment setup. M.P. and Yi. L. helped with data analysis. Yizeng. L. and S.S. designed the flow model, performed the simulation and analyzed the simulation results. S.J. and R.S. wrote the manuscript with input and edits from all authors.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to Rebecca Schulman.

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