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From isolated structures to continuous networks: A categorization of cytoskeleton-based motile engineered biological microstructures

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Abstract

As technology at the small scale is advancing, motile engineered microstructures are becoming useful in drug delivery, biomedicine, and lab-on-a-chip devices. However, traditional engineering methods and materials can be inefficient or functionally inadequate for small-scale applications. Increasingly, researchers are turning to the biology of the cytoskeleton, including microtubules, actin filaments, kinesins, dyneins, myosins, and associated proteins, for both inspiration and solutions. They are engineering structures with components that range from being entirely biological to being entirely synthetic mimics of biology and on scales that range from isotropic continuous networks to single isolated structures. Motile biological microstructures trace their origins from the development of assays used to study the cytoskeleton to the array of structures currently available today. We define 12 types of motile biological microstructures, based on four categories: entirely biological, modular, hybrid, and synthetic, and three scales: networks, clusters, and isolated structures. We highlight some key examples, the unique functionalities, and the potential applications of each microstructure type, and we summarize the quantitative models that enable engineering them. By categorizing the diversity of motile biological microstructures in this way, we aim to establish a framework to classify these structures, define the gaps in current research, and spur ideas to fill those gaps.

This article is categorized under:

Nanotechnology Approaches to Biology > Nanoscale Systems in Biology

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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Keywords

active matter; biomimicry; biomolecular motor protein; cytoskeletal networks; microscale devices

1 | INTRODUCTION

The challenges of biomedical research and development often lie at the interface between the medical intervention and the biological material. The relevant scale of this interface is on the order of nanometers (molecular scale) or smaller for drug developers, and the relevant scale of this interface is on the order of millimeters (tissue scale) or larger for biomedical device designers. However, there is an emerging class of biomedical solutions with scale on the order of micrometers (cellular scale). Most biological processes critical to human health have effects at the cellular scale, including cancer metastasis, immune system response, early embryonic development, stem cell differentiation, and fertility, for example. Researchers and engineers working to enable medical diagnoses and interventions at the cellular scale are developing the ability to evaluate the contents and status of individual cells (Tsioris, Torres, Douce, & Love, 2014) and deliver drugs with single-cell accuracy (Chen et al., 2017), for example.

The theoretical framework behind traditional engineering methods fails to predict behaviors of structures when applied at the cellular scale. The physical limits of fundamental laws (Murray, Edwards, Tindall, & Maini, 2009), the predominating physical phenomena, and the underlying assumptions (Phillips, Kondev, & Theriot, 2013) all differ when applied at the cellular scale.

Nearly all microstructures are subject to low Reynolds number flows (Taylor, 1951). Because fluid flows at low Reynolds number exhibit reversibility, microstructures must go through a configuration-space loop to do useful work against fluids. This phenomenon, rarely seen at the macroscale, is known as “The Scallop Theorem” (Purcell, 1977). Low Reynolds number also means that inertial effects are insignificant at the cellular scale, that is, Newton’s second law is $F = \mathrm{ma} = 0$ pretty much everywhere. Instead, the governing law of cell-scale mechanics is that the force of drag everywhere balances the net forces on a structure. Thus, Stokes drag, F_D, dominates mechanics at the cellular scale, that is, that $F_D = C_D \mu v$, where C_D is the drag coefficient, μ is the fluid viscosity, and v is the characteristic velocity. Additionally, fundamental thermodynamic principals dictate that motile microstructures must remain in an out of equilibrium steady-state. Pairwise transitions between thermodynamic microstates must break detailed balance for the processes they perform to yield time-averaged net effects (Battle et al., 2016). Maintaining a nonequilibrium steady-state in cellular scale systems is challenging because of the importance of thermal fluctuations. Moreover, other fundamental physical phenomena that are mainly ignorable at large scale become critical at small scale, including the finite

wavelength of light, the magnitude of Brownian motion, and importance of chemical diffusion.

The practical nature of shaping and assembling structures using traditional manufacturing techniques at the cellular scale is also nontrivial. Micron-scale conventional manufacturing techniques (milling, drilling, turning, and grinding) are difficult, slow, and expensive (Gao & Huang, 2017). These challenges arise because cutting forces can exceed the physical limits of tools made from conventional materials and assumptions about the homogeneity of materials at the micron scale, which is the length scale of grains in many materials, are no longer valid (Bissacco, Hansen, & De Chiffre, 2006; Câmara, Rubio, Abrão, & Davim, 2012). Even if engineers overcome these manufacturing challenges, it is still challenging to manipulate parts to assemble them into machines on the cellular scale. Lithography offers additional opportunity to make small parts, but it is limited in its ability to make arbitrary 3D parts (Waits, Morgan, Kastantin, & Ghodssi, 2005). Micro-electrochemical micromachining is also being developed to remove material from workpieces by the controlled dissolution of surface atoms without direct contact between the tool and the workpiece material, potentially alleviating some of the physical limits of conventional machining. Thus, there remains a gap between traditional manufacturing techniques, even considering modern advances, and nanoscale and chemical techniques. This gap is on the cellular scale, and it remains a challenge.

Beyond the manufacture of such structures, there are multiple practical complications around the motility of cellular scale structures made from traditional materials. The conversion of stored forms of energy to mechanical work poses challenges. For example, the spent fuel of Janus microswimmers can be highly toxic to cells (Sridhar, Park, & Sitti, 2018), and electromagnetic-permanent magnet or induction motor technology is difficult to miniaturize. The smallest traditional micromotors are only just smaller than the mm size scale (Büttgenbach, 2014). Also, many traditional propulsion technologies rely on inertial effects, which are significantly attenuated by viscous forces in low Reynolds number fluid environments. With force proportional to velocity, rather than acceleration, efficient motility mechanisms at the macroscale become much less practical at the microscale. Finally, while motility mechanisms at the macro scale tend to be deterministic and well predicted, the large relative magnitude of Brownian motion must be either overcome or taken advantage of in ways that traditional motility mechanisms are not designed to do.

While traditional engineering methods and materials face limitations, biological materials are often already capable of performing functions at the cellular scale that are unachievable with current technology. For example, proteins self-assemble into complex structures, that is, tubulins readily fold and dimerize (Khoury, Smadbeck, Kieslich, & Floudas, 2014), dimers polymerize into microtubules (Desai & Mitchison, 1997), and microtubules assemble into the mitotic spindle (Hinchcliffe, 2014), for example. Additionally, motor proteins outperform the precision of their macroscale counterparts (Block, 2007; Coy, Wagenbach, & Howard, 1999; DeWitt, Chang, Combs, & Yildiz, 2012; Qiu et al., 2012) and energy efficiency (J. Wang, 2013), that is, kinesin-1 converts chemical energy to mechanical work at 50% efficiency (Block, 1995; J Howard, 1996) and muscle myosins are 30–40% efficient (He, Bottinelli, Pellegrino, Ferenczi, & Reggiani, 2000; Reggiani et al., 1997). Cells exploit

the functionalities of natural molecular materials to produce dynamic microscale structures, overcoming the engineering challenges faced by nonbiological cellular scale machinery.

Scientists and engineers have learned much, and continue to learn more, from cells. They can directly incorporate or mimic biological materials in engineered microscale devices to create otherwise unachievable functionality. As they discover new ways to construct, use and develop microstructures, scientists and engineers need a common framework to discuss, categorize, and model them. Here we review motile engineered biological microstructures, and we provide a framework that will help the field think about them, work with them, and formulate the theoretical models necessary to engineering them.

2 | THE CYTOSKELETON AS A BASIS FOR ENGINEERED BIOLOGICAL MICROSTRUCTURES

Cells build motile microstructures using the cytoskeleton to confer shape, structure, and organization to the cell, as well as perform multiple, diverse biological functions (Alberts et al., 2002). For example, cells build the axoneme (the motile structure within cilia and flagella), lamellipodia and filopodia (motile structures behind cell migration), the mitotic spindle (the motile structure that separates genetic material during cell division) and the cytokinetic ring (the motile structure that drives the cleavage of daughter from mother during cell division), from thousands of individual cytoskeletal components (filaments, motors, and associated proteins; Figure 1). Like cells, engineers looking to build motile microstructures can find a readymade set of mostly interchangeable parts by pulling directly from, and taking inspiration in, the collection of cytoskeletal elements. However, engineers must take note that, unlike most familiar engineered structures that are at thermodynamic equilibrium, cytoskeletal structures rarely reach equilibrium. Instead, they exist in a dynamic, active steady state, far from thermodynamic equilibrium. Thus, the cytoskeleton is an ideal, active material from which to construct motile engineered biological microstructures.

Actin filaments and *microtubules* are the structural elements of the cytoskeleton (Gittes, Mickey, Nettleton, & Howard, 1993; Howard, 2001). Actin filaments are rope-like; they are flexible, with a persistence length of ~17 μm (Brangwynne et al., 2007; Gittes et al., 1993; Ott, Magnasco, Simon, & Libchaber, 1993; van Mameren, Vermeulen, Gittes, & Schmidt, 2009), but they have a high-tensile strength of ~30 MPa (Howard, 2001) resisting forces up to 400 pN (Tsuda, Yasutake, Ishijima, & Yanagida, 1996). Cells use actin in contractile structures, including muscle (Jakus & Hall, 1947) and the cytokinetic ring (Pelham & Chang, 2002). Microtubules are rod-like; they are stiff, with a persistence length of 1,000–5,200 μm (Brangwynne et al., 2007; Gittes et al., 1993) depending on parameters including microtubule length (Brangwynne et al., 2007; Pampaloni et al., 2006) and stabilization (Kikumoto, Kurachi, Tosa, & Tashiro, 2006; Mickey & Howard, 1995), but they are elastic (they buckle rather than break; Soheilypour, Peyro, Peter, & Mofrad, 2015). Cells use them in structures that bend, including cilia (Aiello & Sleigh, 1972; Gibbons, 1981; Haimo & Rosenbaum, 1981) and mitotic spindle centering asters (Garzon-Coral, Fantana, & Howard, 2016). Beyond their structural functions, actin filaments and microtubules serve as tracks for motor proteins (Ross, Ali, & Warshaw, 2008) and are inherently structurally polar (Howard,

2001), which helps direct the traffic, that is, certain motors move to the “plus ends” and others move to the “minus ends” of cytoskeletal filaments. Cells can chemically modify these filamentous tracks to regulate the motility of the motors acting on them (J. D. Alper, Decker, Agana, & Howard, 2014; Verhey & Gaertig, 2007). Additionally, both actin filaments and microtubules are dynamic structures, continually being built, disassembled and rebuilt in cells (Blanchoin, Boujemaa-Paterski, Sykes, & Plastino, 2014; Brouhard & Rice, 2018). Cells exploit filament dynamics to reconfigure their structure in response to internal and external cues (Brieher, 2013), generate forces (Driver, Geyer, Bailey, Rice, & Asbury, 2017; Peskin & Oster, 1995; Valiyakath & Gopalakrishnan, 2018), move (Nemethova et al., 2008), divide (Brugués & Needleman, 2014), and many other functions.

The *kinesin, dynein*, and *myosin* motor protein superfamilies are the active elements of the cytoskeleton (Hirokawa, Noda, Tanaka, & Niwa, 2009; Kato, Miyakawa, & Tanokura, 2018). All of these motors move along filaments and exert forces by converting the energy of ATP hydrolysis into mechanical work (Howard, 2001). The unique functionalities exhibited by individual members of each motor protein superfamily enables cells to perform the various functions of life.

Kinesins are molecular motors that move along and exert forces on microtubules (Endow, Kull, & Liu, 2010). Phylogenetic analyses indicate that there are about 15 families of, as well as many orphan and ungrouped, kinesins (Endow et al., 2010; Hirokawa et al., 2009; A. J. Kim & Endow, 2000; Lawrence et al., 2004). Organisms can express many kinesin family members, that is, humans have more than 50 kinesins in their genomes (Endow et al., 2010). Most kinesins are plus-end directed motors (McDonald, Stewart, & Goldstein, 1990), with the noted exceptions of kinesin-14 (McDonald et al., 1990; Walker, Salmon, & Endow, 1990), which is minus-end directed, and kinesin-13, which only diffuses along the microtubule (Helenius, Brouhard, Kalaidzidis, Diez, & Howard, 2006). Intracellular transport is the canonical functionality of kinesin, but some kinesin family members exert forces on the spindle during mitosis and regulate microtubule dynamics, for example (Endow et al., 2010). Most kinesins are homodimers, with their motor domains binding directly to the microtubule and their tail domains providing dimerization and cargo binding (Hirokawa et al., 2009; Kato et al., 2018). When they are cargo bound, many kinesins are highly processive, that is, they take many steps before dissociating from their microtubule tracks (Howard, 2001).

Dyneins are molecular motors that also move along and exert forces on microtubules (Vallee & Hook, 2006). Phylogenetic analyses indicate that there are nine families of dynein, broadly grouped as cytoplasmic dyneins, which include cytoplasmic dynein 1 (the canonical intracellular transport dynein) and cytoplasmic dynein 2 (the intraflagellar transport dynein), and axonemal dyneins, which include inner arm and outer arm axonemal dyneins (Wickstead & Gull, 2007). Dyneins are minus-end directed motors (Amos & Hirose, 2011; Roberts, Kon, Knight, Sutoh, & Burgess, 2013) except in extraordinary conditions (Ross, Wallace, Shuman, Goldman, & Holzbaur, 2006; Walter, Brenner, & Steffen, 2010). Cytoplasmic dyneins, like kinesins, are transport motors (Roberts et al., 2013), and axonemal dyneins drive the beat of cilia and flagella (Gibbons & Rowe, 1965). Cytoplasmic dyneins are homodimers that bind the microtubule through a microtubule binding domain

remote from the motor domain, and they have cargo-mediated processivity (Roberts et al., 2013). Axonemal dyneins are monomers, heterodimers or heterotrimers that form cross-bridges between the microtubule doublets within eukaryotic cilia and flagella (Roberts etal., 2013).

Myosins are molecular motors that move along and exert forces on actin filaments (Hartman & Spudich, 2012). Phylogenetic analyses indicate that there are about 35 families of myosin, as well as many orphan and ungrouped myosins (Odronitz & Kollmar, 2007). Individual organisms can express multiple members of each myosin family, that is, humans have more than 40 myosins (Berg, Powell, & Cheney, 2001). Most myosins are plus-end directed motors (Geeves, 2016), with the noted exception of myosin VI, which moves to the minus end of actin filaments (Wells et al., 1999). Because it is the only actin-associated motor superfamily, myosins perform many cellular functions, including organizing molecular components, cargo transport, and generating and sensing force (Hartman & Spudich, 2012). The myosin superfamily consists of transport homodimers and monomers that form cross-bridges between actin filaments (Geeves, 2016).

When accounting for all the individual members of these superfamilies, cytoskeletal motor proteins exhibit a broad range of motile properties and provide diverse functionalities to cells. Beyond their basal capabilities, and perhaps most importantly from an engineering perspective, the functionality of kinesin (Hirokawa & Takemura, 2004; Verhey & Hammond, 2009; Yount, Zong, & Walczak, 2015), dynein (Cianfrocco, DeSantis, Leschziner, & Reck-Peterson, 2015; Kikkawa, 2013; King, 2016), and myosin (Nishikawa et al., 2010; Reilein, Rogers, Tuma, & Gelfand, 2001) can be regulated. Engineers can, and in some cases have already begun to (K. Furuta & Furuta, 2018), build on natural regulation mechanisms that control a motor's speed (Cochran, Zhao, Wilcox, & Kull, 2011; Nomura, Uyeda, Yumoto, & Tatsu, 2006), processivity (Arpağ, Shastry, Hancock, & Tüzel, 2014; Walter, Koonce, Brenner, & Steffen, 2012), binding (A. Furuta et al., 2017), and directionality (Arpağet al., 2014; Nakamura et al., 2014) in engineered systems.

In cells, motor protein-filament systems are rarely as simple as a single motor moving on a single filament. In addition to multiple motors moving on many filaments, biological microstructures (Figure 1) contain auxiliary proteins, including passive cross-linkers (Mohan & John, 2015), filament regulators (Akhmanova & Steinmetz, 2015; Lee & Dominguez, 2010), motor regulators (Heissler & Sellers, 2016; King, 2016; Verhey & Hammond, 2009), and anchoring proteins (Burridge & Chrzanowska-Wodnicka, 1996; Jaspersen & Winey, 2004; Lüders & Stearns, 2007), for example. Engineers can include these auxiliary components in engineered microstructures to add functionality, as well. For example, Ase1, a microtubule-associated protein (MAP) that can become compacted between sliding microtubule ends, provides adaptive braking to (Braun et al., 2011), and generates directed forces in (Lansky et al., 2015), systems of sliding microtubules.

Engineers should consider using proteins from each class of cytoskeletal element (filaments, motors, regulators, and auxiliary proteins) when engineering biological microstructures. Each class can provide useful functionality to motile engineered biological microstructures,

and they each have the potential to be either directly incorporated into structures or used as inspiration for synthetic mimicry.

Having all the components is not enough; quantitative, predictive models of cytoskeletal elements are necessary to design robust motile engineered microstructures, as is true for engineered macroscale structures (Stam et al., 2017). Many models can accurately and quantitatively predict various aspects of the cytoskeleton. For example, successful models of cytoskeletal networks include treating them as active matter (Lim, Zhou, & Quek, 2006; MacKintosh & Schmidt, 2010; Needleman & Dogic, 2017) and viscoelastic networks (Brangwynne et al., 2007; Lim et al., 2006; Lin, Koenderink, MacKintosh, & Weitz, 2007; White, 2011) driven by embedded motor proteins. There are also single-molecule models that predict the mechanical properties (Feng & Mitran, 2018; Hawkins, Mirigian, Selcuk Yasar, & Ross, 2010; Memet et al., 2018; Pfaendtner, Lyman, Pollard, & Voth, 2010) and dynamics (Blanchoin et al., 2014; Bowne-Anderson, Zanic, Kauer, & Howard, 2013) of filaments, and processivity (Šarlah & Vilfan, 2017) and motility (Jülicher & Prost, 1997) of motors. Though fewer, there are some models at the mesoscale that bridge the network and molecular-scale, including how motors drive the dynamics of filament bundles (Kruse & Jülicher, 2000). Models at the network, mesoscale, and molecular scales are all necessary to further enable the engineering of motile biological microstructures.

3 | GLIDING ASSAYS: THE FIRST MOTILE ENGINEERED BIOLOGICAL MICROSTRUCTURES

Gliding assays are perhaps the most straightforward and earliest example of using the cytoskeletal components in motile engineered biological microstructures (Howard, Hudspeth, & Vale, 1989; Kron & Spudich, 1986). In gliding assays, cytoskeletal motor proteins attached to a microscope slide glide their associated filaments upon the addition of ATP (Figure 2a). Gliding assays facilitate quantitative measurements of the biophysical properties of cytoskeletal elements in a controlled environment. For example, scientists used gliding assays to quantify the speed, binding rate, and processivity of kinesin-1 (Howard et al., 1989), to discover that kinesin-1 follows a single protofilament (Ray, Meyhöfer, Milligan, & Howard, 1993) while kinesin-8 switches protofilaments (Bugiel, Böhl, & Schäffer, 2015), to demonstrate the relative strengths of kinesin-1, −2, −3, −5, and −7 (Arpağ et al., 2014), to find the step size and duty ratio of myosin II (Uyeda, Kron, & Spudich, 1990), to measure the flexural rigidity of microtubules (Martin, Yu, & Hoozen, 2012), to show that its microtubule track regulates axonemal dynein (J. D. Alper et al., 2014; J. D. Alper, Tovar, & Howard, 2013), and to demonstrate the emergence of nematically-ordered phases in two-dimensional active materials (Huber, Suzuki, Krüger, Frey, & Bausch, 2018).

Engineers can extend the utility of gliding assays beyond biophysical research by adding functionality into increasingly complicated systems. Two extended gliding assay systems, *cargo delivery* and *self-assembly*, have guided transport (Clemmens et al., 2003; Clemmens, Hess, Howard, & Vogel, 2003; Dennis, Howard, & Vogel, 1999), controllable motor activity (Cochran et al., 2011; Greene, Trent, & Bachand, 2008; E. Kim et al., 2013; Nomura et al.,

2006), and modified cargo-binding capabilities (Bachand, Rivera, Carroll-Portillo, Hess, & Bachand, 2006; Carroll-Portillo, Bachand, & Bachand, 2009; Fujimoto et al., 2013).

In engineered cargo delivery systems, functionalized gliding filaments, which are sometimes called “molecular shuttles” (Dennis et al., 1999), carry cargo to a specific site. Antibodies (Carroll-Portillo et al., 2009), lipid vesicles (Hiyama, Moritani, Gojo, Takeuchi, & Sutoh, 2010) and viruses (Bachand et al., 2006) are integral pieces of drug-delivery systems (Bachand et al., 2006; Jia et al., 2014; Malcos & Hancock, 2011). In one specific example, a cargo delivery system with antibody-functionalized microtubules conjugated to rod-shaped viruses, which were additionally functionalized to target specific cargos (Bachand et al., 2006), replaced pressure-driven or electrophoretic flow within microfluidic devices used in the capture and separation of specifically targeted molecules from a complex mixture. In another set of examples, delivery location specificity was engineered into cargo delivery systems by creating a specific track design (Clemmens, Hess, Howard, & Vogel, 2003; Dennis et al., 1999), light-dependent ATP activation (Hess, Clemmens, Qin, Howard, & Vogel, 2001), and localized ATP regeneration systems (Figure 2b) (Jia et al., 2014).

In engineered self-assembly systems, gliding assays drive filaments into specific, controllable geometric structures. Examples include systems that assemble rings (H. Liu et al., 2008; VanDelinder, Brener, & Bachand, 2016) and other patterns (Doot et al., 2007) using confined channels (Figure 2c). These self-assembled structures could be used as directed tracks for micro-scale factories (Hess & Vogel, 2001), in lab-on-a-chip diagnostic devices (Hiyama et al., 2010), as a template for building synthetic structures (Payne, Rosi, Xue, & Mirkin, 2005), or even as a parallel-computation system (Nicolau et al., 2016).

3.1 | THERE ARE 12 MAJOR TYPES OF MOTILE BIOLOGICAL MICROSTRUCTURES

Building on the idea of using cytoskeletal elements in motile microstructures pioneered by gliding assays, engineered motile biological microstructures can be classified into four “categories” ranging from entirely biological to synthetic on one axis and three “scales” ranging from networks to isolated structures on the other axis, creating 12 “types” of motile biological microstructures (Figure 3).

The approaches used to build them define the categories of engineered motile biological microstructures. At the “entirely biological” end of the “category” range are structures that combine naturally occurring biological components into novel structures that generate desired functionalities. At the “synthetic” end are structures comprised of nonbiological, but often biomimicking, materials. These approaches can be used alone or in combination, leading to intermediate categories. “Modular” structures are biological, but they use genetically engineered components. For example, using a motor protein chimera that replaces the microtubule binding domain of cytoplasmic dynein with the actin-binding domain of muscle myosin (A. Furuta et al., 2017) in a structure would make it modular because it is biological, that is, comprised of proteins expressed by cells, but not naturally occurring, that is, comprised of genetically engineered chimeras. “Hybrid” structures contain a mixture of biological and synthetic components. For example, a structure using a polymeric microsphere embedded with a drug but otherwise comprised entirely of biological components (Jia et al., 2014) is a hybrid structure.

The characteristic size of engineered motile biological microstructures defines their scale. At the “network” end of the “scale” range are structures with material properties and densities that vary point by point, in a continuous manner. Networks need not be isotropic, but the characteristic length scale of granularity must be fine enough, as compared to the structure size, to be essentially smooth. At the “isolated” structure end of the scale range are structures that contain on the order of 10 or fewer filaments. Isolated structures do not interact, or interact only transiently, with surrounding structures. Isolated structures can be entirely substrate-bound, like gliding assays, fixed at a single point, like a cilium or filopodium, or freely floating in solution. Between networks and isolated structures are “clusters.” Toward the isolated structures end of the spectrum, clusters are easily identifiable but may interact with their neighbors. Either they tend to form a node with filaments protruding, which are reminiscent of biological asters, or they tend to coalesce into parallel or anti-parallel bundles. Toward the network end of the spectrum, clusters run together, but with local areas of low filament density. Across the range of scales, motile engineered microstructures can behave like fluids because motors can cause the filaments to exhibit large-scale flow patterns. They can behave like solids because the motors generate forces that either elastically (recoverable, often leading to fluctuations that are much stronger than thermal fluctuations) or inelastically (permanent rearrangement or fracturing) deform the structure. They can also behave like viscoelastic materials, which exhibit properties of both fluids and solids. The level of crosslinking within a structure, and the chemical nature of those crosslinking elements, often determines a structure’s behavior.

In the following sections, we systematically highlight select examples of each type of structure, discuss the unique advantages of each type of structure, and mention some current and future applications of each type of structure to both the fields of basic science and engineering.

3.2 | Category: Entirely biological structures

“Entirely biological structures” is a category of motile engineered biological microstructures that make use of natural biological components, including motor proteins, filaments, and associated proteins, only. However, these biological components need not be used exclusively with their natural substrates. Indeed, entirely biological structures often include components from multiple organisms or cell types that are recombined in novel ways.

3.2.1 | Structure type: Entirely biological networks—At the largest scale, “entirely biological networks” (Figure 3a) primarily exhibit isotropic, affine contractility (Braun, Lansky, Hilitski, Dogic, & Diez, 2016; Weisenberg & Cianci, 1984). Microtubule-dynein networks (Figure 4a) (Foster et al., 2015), microtubule-kinesin networks (Hentrich & Surrey, 2010), and actin-myosin networks (Bendix et al., 2008; Ennomani et al., 2016; Gardel et al., 2004) all tend to contract in the presence of ATP, similar to some dynamic behaviors of cells. For example, isotropic two-dimensional entirely biological networks contract in a way that resembles the cell cortex (Figure 4b,c) (Murrell & Gardel, 2012), and anisotropic networks can be built to model the motile behaviors of the mitotic spindle (Foster et al., 2015; Sawin, LeGuellec, Philippe, & Mitchison, 1992).

Entirely biological networks achieve their advantage from their biological nature and the possibility of simple construction. Entirely biological networks can contain a minimal number of component types; some are built of motor proteins and filaments alone (Hentrich & Surrey, 2010). Additionally, construction of entirely biological networks can be straightforward because motor proteins and filaments self-assemble (Surrey et al., 2001). Moreover, quantitative rules governing the properties of entirely biological networks help engineers build networks with specific properties (Hentrich & Surrey, 2010; Sonn-Segev, Bernheim-Groswasser, & Roichman, 2017; Surrey et al., 2001) and tune those properties with crosslinking and nucleating proteins, like Arp2/3 and formins (Fritzsche, Erlenkämper, Moeendarbary, Charras, & Kruse, 2016), for example.

Entirely biological networks promise to provide functionality to biocompatible and biodegradable medical devices requiring force generation. Their entirely biological nature makes them ideal for one-time use applications and as clean, biodegradable alternatives to synthetic gels (Gong, Nitta, & Osada, 1994). Additionally, further research on this structure type will advance the basic science of active materials, ultimately helping scientists and engineers develop new quantitative models of stress fluctuations in materials out of equilibrium (Brangwynne, Koenderink, MacKintosh, & Weitz, 2008) and transport properties of such materials (Hafner & Rieger, 2018), for example.

3.2.2 | Structure type: Entirely biological clusters—At intermediate scales, “entirely biological clusters” (Figure 3b) tend to exhibit localized contractility, that is, interconnected nodes move toward one another when activated. As an example, entirely biological clusters formed from microtubules and kinesin-1 motor proteins resemble pine needle bunches that connect at their distal tips (Surrey et al., 2001). Similarly, small foci-like entirely biological clusters formed from actin filaments and myosin II motor proteins can coalesce into larger superaggregates over time (Alvarado, Sheinman, Sharma, MacKintosh, & Koenderink, 2013; Silva et al., 2011). Additionally, engineered entirely biological clusters are structurally similar to the asters that form at spindle pole bodies during mitosis (Alberts et al., 2002); thus scientists used them to test models of spindle centering mechanisms in symmetric and asymmetric cell division (Laan, Roth, & Dogterom, 2012), for example.

Engineered entirely biological clusters share multiple advantages with their larger-scale continuous network counterparts. Clusters can be made to spontaneously self-assemble from initially isotropic networks by reducing the concentration of filaments across a phase transition-like point to a density that is no longer high enough to generate affine contraction upon activation of the motors (Alvarado et al., 2013; Torisawa et al., 2016). Structural and dynamic features, including microscale heterogeneity, nanoscale features, and local contractility, enable functionality in this microstructure type not otherwise possible with continuous networks (Laan et al., 2012; Silva et al., 2011; Torisawa et al., 2016).

Engineers could use the localized contractility of entirely biological clusters in multiple applications, potentially including scaffolding for engineered cells or sacrificial templates for synthetic 3D microstructured materials. For example, they could use such microstructures to fabricate metallic microscale materials with controllable nanometer-scale features, as has been done with diatom cells (Payne et al., 2005). Additionally,

understanding the contractility of this structure type can help provide additional insight into the physics of cells, enabling the development and verification of quantitative models (Garzon-Coral et al., 2016) based on reconstituted, minimal systems.

3.2.3 | Structure type: Entirely biological isolated structures—At the small scale, "entirely biological isolated structures" (Figure 3c) are distinct and disconnected from other structures, limiting interaction. They are not widely reported in the literature, other than in gliding assays. However, we have created some structures of this type composed of axonemal dynein purified from *Chlamydomonas reinhardtii* cilia (J. Alper, Geyer, Mukundan, & Howard, 2013) and taxol-stabilized, rhodamine-labeled porcine brain microtubules (Gell et al., 2011). We find that these entirely biological isolated structures fall into two major shape subtypes: askew and parallel (Figure 5). Additionally, scientists have used such structures to study in vitro reconstitutions of mitotic spindle mid zones (Braun et al., 2011; Lansky et al., 2015; Subramanian, Ti, Tan, Darst, & Kapoor, 2013) and to demonstrate the ability to incorporate dynamically assembling and disassembling building blocks into engineered systems (Lam, Tsitkov, Zhang, & Hess, 2018).

The isolated nature of this type of structure lends itself to the study of mesoscale dynamics, enabling experiments that test models that bridge the single molecule and network scales. Future research using these structures will reveal the molecular basis for emergent properties in motile biological microstructures and enable building foundational models upon which scientist can develop cluster and network models, as has been done with gliding assays (Howard et al., 1989; Paschal, Shpetner, & Vallee, 1987; Uyeda et al., 1990).

Entirely biological isolated structures are small-scale (nearly molecular), yet they exhibit emergent properties and functionalities that have the potential to be used in engineering applications at the cell or subcellular scale. The shape, size, and motility of these structures can be controlled and regulated by auxiliary components, including motor- and filament-associated proteins, post-translational modifications, small molecules, and ions. Such control gives them the necessary functionality to serve as the motile elements of engineered, autonomous cells, for example. Possible other engineering applications include scaffolding for single cells and micro-scale manipulators for cell-scale devices.

3.3 | Category: Modular structures

"Modular structures" are a category of motile engineered biological microstructures consisting of rationally designed and genetically engineered proteins. Scientists and engineers construct the genetically modified protein chimeras used in this category of structures by mixing, matching, and recombing protein domains from across biology in novel ways. For example, *D. melanogaster* kinesin-1 motors functionalized with *E. coli* biotin carboxyl carrier protein (BCCP) domains (Berliner, Young, Anderson, Mahtani, & Gelles, 1995), which effectively tag the kinesin with biotin, combine to form dimeric, trimeric, or tetrameric motors upon binding to avidin. In another example, the lever arm and linker domains of myosin and kinesin motors can be modified with light-oxygen-voltage-sensing (LOV2) domains from plant phototropins to change their speed and directionality upon light irradiation (Nakamura et al., 2014). The possibilities for using genetically

engineered proteins in modular structures are nearly limitless, and they provide engineers with the means to design nearly arbitrary functionality into microstructures.

3.3.1 | Structure type: Modular networks—At the largest scale, "modular networks" (Figure 3d) can behave quite similarly to entirely biological networks. For example, the multimeric kinesin-BCCP chimera-avidin complexes mentioned above drive affine contractions of microtubule networks (Hess & Vogel, 2001). Additionally, myosin V and VI motors that are complexed together using DNA origami and modulated by swapping lever arms can traverse a filamentous actin network in unique ways (Hariadi, Cale, & Sivaramakrishnan, 2014). This type of structure provides a platform to ask and answer fundamental questions about motor protein biophysics (Hariadi et al., 2014) and materials science at the fluid/solid/gel transition (Wu et al., 2017).

By exploiting the modular nature of their components, these structures have enabled the engineering of functionalities, including tunability, into networks that are not available from purely biological networks. In the specific examples above, multimeric motor protein assemblies can change the speed, directionality and persistence of transport through the network and the contractile properties of the network itself (Hariadi et al., 2014; Hess & Vogel, 2001). The multimeric kinesins enable engineers to tune the network-cluster phase transition, effectively lowering the critical concentration of filaments necessary to exhibit the affine contractions, as compared to entirely biological networks (Hess & Vogel, 2001).

However, these examples scratch the surface of what is possible with modular networks. Other possibilities enabled by picking from the vast set of properties exhibited by protein domains include the engineering of external, reversible control over network contractility, the coupling of microtubule- and actin-based networks, the incorporation of functional, dynamical anisotropies into the networks, and easier integration of these microstructures into larger engineered systems, for example. The purely biological macromolecular nature of modular components gives this type of structure the potential to be used in biodegradable and biocompatible systems, similar to entirely biological networks. Additionally, carefully designed experiments using modular components will lead to discoveries of fundamental mechanisms in cell biology, biophysics, and materials science otherwise untestable with purely biological networks.

3.3.2 | Structure type: Modular clusters—At the intermediate scale, "modular clusters" (Figure 3e), like modular networks, have additional tunability and functionality, as compared to entirely biological clusters. For example, scientists and engineers can vary the concentration of multimeric kinesin-1 clusters to control the degree to which a mixture of microtubule filaments form clusters (Figure 6a) (Surrey et al., 2001). Moreover, they can add multimeric minus-end kinesin-14 (ncd) motor proteins to regulate the size and shape of the clusters, further demonstrating this type of structure's tuneability (Surrey et al., 2001). Additionally, scientists used modular clusters to determine the fundamental principles of self-organization. For example, they used $KIF5B_{head}$-$Eg5_{tail}$ chimeras to understand the nonaffine contractile nature of networks that form clusters (Figure 6b) (Torisawa et al., 2016).

Similar to the advantages of modular networks, the additional parameter space afforded by using genetically engineered protein components enables engineering additional functionality and complexity into clusters. Modularity not only expands the range of sizes and shapes of clusters afforded by using modular motor assemblies but also it enables the engineering of structures with emergent properties that are unique to each modular combination and with no naturally occurring analogs.

Owing to the added functionality provided by their modular nature, modular clusters have the potential to be used in engineering applications requiring the biocompatibility of natural structures but with functionalities beyond what is found in nature. The morphology of scaffolding for engineered cells and self-assembling templates for synthetic materials could take additional forms and be increasingly temporally and spatially controllable with modular components. Additionally, modularity broadens the design possibilities for experiments on in vitro reconstituted cytoskeletal systems, for example, minimal functional models of mitotic spindles.

3.3.3 | Structure type: Modular isolated structures—At the small scale, "modular isolated structures" (Figure 3f) can take a variety of forms. Some modular isolated structures expand directly on gliding assays. For example, molecular motors can be genetically modified to stick to a surface in a specific orientation to shuttle filaments (A. Furuta, Yagi, Yanagisawa, Higuchi, & Kamiya, 2009), to carry a non-native biological cargo (Hiyama et al., 2010), or to act cooperatively in multi-motor assemblies (Diehl, Zhang, Lee, & Tirrell, 2006). Other modular isolated structures can more closely resemble engineered cilia-like structures. For example, kinesins genetically modified to dimerized can bundle microtubules. With these bundles adhered to a surface and upon the addition of ATP, the cilia-like structures spontaneously oscillate (Darnton, Turner, Breuer, & Berg, 2004; Sanchez, Welch, Nicastro, & Dogic, 2011; Sasaki et al., 2018).

The ability to genetically engineer various domains and tags into proteins, like biotins with BCCP domains (A. Furuta et al., 2009), facilitates functionality not available in entirely biological microstructures. Such additional functionalities include the ability to swap out components of, add components to, or construct arrays of modular isolated structures. For example, engineers can design novel hybrid motors that self-assemble with the molecular precision using domains with multifunctional binding sites. This precision, in turn, enables the engineering of specifically desired functionalities into modular isolated structures, that is, bidirectionality imparted by structures containing both plus-end directed and minus-end directed motors. Additionally, modularity enables the assembly of multiple copies of each structure with high fidelity. When combined with templating, it is possible to create well-defined arrays of modular isolated structures all working together.

Either alone, or in arrays, modular isolated structures are particularly useful for engineering applications such as the sorting of target components from an individual cell (e.g., vesicles, Figure 3f) in lab-on-a-chip diagnostic devices (Hiyama et al., 2010). Arrays of the cilia-like structures, for example, could provide a means to drive nanofluidic flow when other technologies are not practical or to drive the motility of autonomous, cell-scale robots (Darnton et al., 2004; Sanchez & Dogic, 2013; Shields et al., 2010; Y. Wang, Gao, Wyss,

Anderson, & den Toonder, 2013). Additionally, exploiting the ability to swap out or add components to modular isolated cilia-like structures would help scientists to understand the biophysical principles that lead biological microstructures to oscillate spontaneously. For example, adding crosslinking proteins or changing motors from kinesin to axonemal dynein might reveal essential mechanisms underlying the beat of cilia and flagella.

3.4 | Category: Hybrid structures

"Hybrid structures" are a category of motile engineered biological microstructures made from a mixture of biological (both entirely biological and modular) and fully synthetic, often biomimicking, components. Many examples of hybrid structures contain primarily biological components with fully synthetic components added to provide additional functionality. These could include microspheres, which enable larger structures with their large surface areas, inorganic nanomaterials like quantum dots, which provide enhanced fluorescent properties, and nonbiological chemicals like dyes, covalent crosslinkers, and passivating molecules, which enable covalent bioconjugate chemistry, chemical functionalization, and imaging, for example. Many possible functionalities could be added such as magnetic filaments, fluorescent dyes, synthetic scaffolding, or energy harvesting systems, leading to endless engineering opportunities.

3.4.1 | Structure type: Hybrid networks—At the largest scale, "hybrid networks" (Figure 3g) are biological networks combined with synthetic materials to provide novel functionalities. For example, multiple groups built timed drug delivery systems (Chandrasekar, Sistla, Ahmad, Khar, & Diwan, 2007; Cotí et al., 2009; Felice, Prabhakaran, Rodríguez, & Ramakrishna, 2014; Jao, Xue, Medina, & Hu, 2017) and imaging applications (Sun, Xiao, & Fang, 2012) using hybrid networks functionalized with nanoparticles. Others functionalized their structures with crowding agents for applications that require denser networks (Stam et al., 2017) and additionally confined them to liquid phase interfaces with nonbiological surfactant molecules (Zhang, Kumar, Ross, Gardel, & Pablo, 2017). Moreover, heterobifunctional linking chemicals can cross-link actin filaments to microtubules in a network. By changing cross-linker concentration, engineers can tune the viscoelasticity and linear elastic modulus of hybrid networks over at least an order of magnitude (Lin et al., 2007). Microstructure designers can control other physical properties of the network, including strain recovery and critical stress, by adding other synthetic elements to biological networks. In one example, photo-responsive DNA containing azobenzene groups allow for external control over motile behavior and the emergence of order using visible or ultraviolet light illumination (Keya et al., 2018).

The additional functionality provided by hybrid networks extends the range of possible applications beyond that of either entirely biological networks or synthetic networks alone. In addition to the specific examples mentioned above, incorporation of synthetic elements like nanoparticles, drugs, crowding agents, crosslinking molecules, can enable pharmaceutical, energy, or advanced materials applications. The hybrid nature of these structures enables more intricate and carefully controlled in vitro studies of cytoskeletal networks, for example, particle image velocimetry (Takahashi, Suzuki, Aoyama, Umezu, &

Iwasaki, 2017) and oscillating magnetic bead micro-rheometry (Ziemann, Rädler, & Sackmann, 1994) and of active matter from a materials science perspective.

3.4.2 | Structure type: Hybrid clusters—At the intermediate scale, engineers can build "hybrid clusters" (Figure 3h) by attaching filaments to nanoparticles or microspheres, which form the nodes of aster-like microstructures, that can interact at high enough concentration. For example, synthetic microspheres can be functionalized microtubule organizing center seeds from which microtubules grow. When stabilized by MAPs, for example, these structures can be used as tunable templates for synthetic nanomaterial assembly (Spoerke et al., 2013). Built under physical confinement conditions in the form of tri-block copolymer-stabilized liquid droplets, mixtures of microtubules and kinesin-14 motors functionalized with nonmotor microtubule binding domains assemble into highly regular aster-like (Figure 7a) formations (Juniper et al., 2018). Additionally, interconnected networks of aster-like actin-based microstructures (Figure 7b) formed by incubating Arp2/3 coated beads in brain extract can template nanomaterial assembly (Vignjevic et al., 2003). In one example, hybrid clusters organized nanomaterials into "nanowhiskers" (Verma, Catchmark, Brown, & Hancock, 2013).

The hybrid nature of these clusters enables increased diversity in and control over structure geometry, as compared their entirely biological or synthetic counterparts. These structures enable the design of highly controllable experiments to test models of spindle positioning and centering mechanisms derived from cells (Garzon-Coral et al., 2016), for example. Additionally, having control over the geometry of aster-like structures and the parallel or anti-parallel nature of bundles, and the ability to design novel functionalities, like magnetic, fluorescent and spectroscopic properties, into hybrid clusters, enables a broader variety of engineered applications. Potential applications for engineered hybrid clusters include medical diagnostics, therapeutics, and cell-scale micro-electromechanical systems.

3.4.3 | Structure type: Hybrid isolated structures—At the small scale, engineers and scientists have developed various hybrid isolated structures (Figure 3i). For example, one group used ring or spool-like structures functionalized with quantum dots to create a self-assembling hybrid microstructures (Figure 8a) (H. Liu et al., 2008) and another demonstrated the manufacture of electrically conducting nanowires using single, templated microtubules (Spoerke, Connor, Gough, McKenzie, & Bachand, 2014). Other examples include making the topology of a gliding assay's substrate reconfigurable with differentially thermoresponsive polymers (PMMA and PNIPAM) to control transport (Stoychev, Reuther, Diez, & Ionov, 2016), and constructing flagellar-like structures (Sasaki et al., 2018) and filopodia-like structures (Vignjevic et al., 2003) mounted to microsphere substrates (Figure 8b). Multiple groups of scientists used engineered hybrid isolated microstructures made of molecular motor proteins conjugated to plastic, silica or magnetic beads in optical and magnetic tweezers assays to discover many fundamental properties of motor proteins (K Svoboda & Block, 1994; Karel Svoboda, Schmidt, Schnapp, & Block, 1993).

The hybrid and isolated nature of these structures show promise in lab-on-a-chip devices, as electrical interfaces with cells, and for generating electromagnetic fields at the micron scale, to name a few. The size and hybrid nature of these structures are particularly advantageous

for the precise mapping of optical near-field interactions between nanostructured materials and optical emitters in biosensing, light harvesting and quantum communication applications (Groß et al., 2018). Additionally, researchers will continue to use them as a foundational technology behind the single-molecule biophysical scientific enterprise.

3.5 | Category: Synthetic structures

Entirely "synthetic microstructures" are engineered from nonbiological materials, but they are conceptualized to mimic biological materials. Biomimicry is useful in engineered systems that require innovative solutions, a level of tunability, and a lifespan well beyond what is available with biological materials. However, they are primarily useful for applications that need not be biocompatible. The focus of research is on engineering biological-like motility into fully synthetic materials to increase the variety of applications of such materials (Kuksenok & Balazs, 2015).

3.5.1 | Structure type: Synthetic networks—"Synthetic networks" are a category of motile engineered biological microstructures made from nonbiological, often biomimicking, components that behave like natural networks. For example, materials engineers built synthetic networks that mimic muscle contraction using a polymer gel comprised of spirobenzopyran (SP) chromophores and the ruthenium catalysts to drive the oscillatory Belousov-Zhabotinsky (BZ) reaction (Figure 3j) (Kuksenok & Balazs, 2015). This combination is capable of producing light-inducible autonomous motility, making these structures inherently controllable. In another example, intermediate filament-like polyisocyanopeptides grafted with polyethylene glycol side chains self-assemble into networks that mimic the characteristic mechanical properties of similar biological networks (Kouwer et al., 2013). Moreover, these networks exhibit optical properties that are thermally tunable (Kouwer et al., 2013); they transform from a translucent to a transparent hydrogel in a fully reversible manner with temperature cycling. Additionally, ethylene glycol tail length modulates the transition temperature, and specific amino acid sequence regulates the intrinsic backbone stiffness of these hydrogels.

Motile synthetic networks exhibit incredible versatility for engineering applications in environments that degrade biological molecules. Potential future applications of motile synthetic networks, particularly those that have controllable contractility, include fully synthetic cell-templates and tissue engineering scaffolds that enable in vivo-like differentiation in response to motility-based mechanical queues, like oscillations. Additionally, motor protein-filament-like networks could power microscale self-propelled, soft, reconfigurable robots, collapsible and expandable microstructures, and structures with fluid-like properties.

3.5.2 | Structure type: Synthetic clusters—Materials scientist and engineers are actively exploring synthetic microstructures at the network and isolated structure scales, however, there is not much research reported on "synthetic clusters" (Figure 3k). As mentioned in the discussion of other types of clusters, reducing the density of a filament network can result in the formation of clusters, so perhaps the same might be true of synthetic networks; more research is necessary. Potentially interesting motile synthetic

clusters could contain bioartificial filaments. For example, engineers could use carbon nanotubes both as structural and thermally conducting elements (Inoue et al., 2015), or they could use fabricated polymer, liquid metal, or ceramic filaments constructed from PDMS molds (Pokroy, Epstein, Persson-Gulda, & Aizenberg, 2009) in the construction of synthetic clusters. Clusters fabricated from synthetic filaments would require motile elements. Synthetic motors driven by spatially asymmetric chemical reactions (Paxton et al., 2004) or external electromagnetic fields, light, thermal gradients, and acoustic vibrations are all possible (Yadav, Duan, Butler, & Sen, 2015). Additionally, incorporation of other synthetic elements, including functionalized nanoparticles, energy sources, and imaging agents, would expand the possibilities for engineering unique functionalities into microstructures of this type.

The unique properties of synthetic clusters can mimic cytoskeletal structures, potentially leading to an entirely autonomous, synthetic cell. Lab-on-a-chip devices can use similar principals to sort other small materials, for example, perhaps like how a mitotic spindle separates chromosomes. As with their biological counterparts, scientists could use synthetic clusters as a tool to better understand the fundamental properties of synthetic filament and motor components.

3.5.3 | Structure type: Synthetic isolated structures—"Synthetic isolated structures" are a type of motile engineered microstructures made from synthetic, biomimicking materials (Figure 3l). There are numerous examples of engineered microstructures mimicking cellular microstructures. For example, arrays of cilia-like polydimethysiloxane (PDMS) pillars embedded with magnetic maghemite nanoparticles (Figure 9a) and actuated by external magnetic fields drive the flow of fluid (Shields et al., 2010). Another approach to biomimetic cilia uses electroactive polymers that deform mechanically in response to electric stimuli (Sareh, Rossiter, Conn, Drescher, & Goldstein, 2013). Structures built from multiple electroactive polymers, each with a distinct response curve, can recreate (Sareh et al., 2013) the required asymmetrical motion of a natural cilium (Purcell, 1977; Tam & Hosoi, 2007). Other approaches to biomimetic cilia include using silicone rubber actuators (Gorissen, de Volder, & Reynaerts, 2015) and magnetic polymers (Hanasoge, Ballard, Hesketh, & Alexeev, 2017; Hanasoge, Hesketh, & Alexeev, 2018; W. Wang, Huang, Lai, & Lu, 2017).

Beyond biomimicking cilia, synthetic isolated microstructures are becoming of increasing interest as scientists and engineers begin to require control of mechanics at the molecular and cellular scales and in harsher environments. For example, autonomous DNA walkers that rely on base-pair hybridization and use free hairpin DNA or fuel strands distributed along their tracks (Figure 9b) (Cha et al., 2014) will be useful in transport applications where the number of steps must be tightly controlled (Pan, Li, Cha, Chen, & Choi, 2015). With the vast and ever-expanding range of material options available, fully synthetic structures at this scale have the potential to function at greater extremes of temperature and in more fluids than structures containing biological components could.

4 | MODELING MOTILE ENGINEERED BIOLOGICAL MICROSTRUCTURES

This review focuses on the design elements of motile biological microstructures and a categorization scheme to help conceptualize their construction, but modeling the behaviors of these structures is too important not to address directly. Without predictive, quantitative models, the engineering of motile biological microstructures is not possible. A genuinely instructive roadmap of models that bridge molecular and tissue-scale structures is currently not available. However, there are many outstanding contributions to the development of engineering models of microscale structures (Needleman & Dogic, 2017).

At the small scale, there are excellent models of how each element of motile biological microstructures behaves. Euler's beam bending and buckling equations (Howard, 2001) describe how individual filaments behave under load, and there are multiple stochastic (Flyvbjerg, Holy, & Leibler, 1994; VanBuren, Cassimeris, & Odde, 2005) and master equations descriptions of microtubule dynamics (Bowne-Anderson et al., 2013), for instance. To capture additional motile behaviors, one must consider how filaments move through low Reynolds number fluids. Therefore, resistive flow theory, slender body theory, or Stokes law might be appropriate in certain situations (Happel & Brenner, 2009; Johnson & Brokaw, 1979). Moreover, one must make additional considerations to couple bending with fluid flows, as has been done for the case of beating cilia and flagella (Machin, 1958). The dynamic cross-bridge models of contracting muscle (Hill, 1974; Huxley, 1957), the Brownian ratchet model, or the powerstroke model of mechanochemical transduction (Howard, 1995) can be used to include the molecular descriptions of motor proteins. There are multiple models that include the effects of cross-linking molecules, including the freely-jointed chain and worm-like chain models (Saitô, Takahashi, & Yunoki, 1967) for how they extend under load, and force-dependent dissociation, slip- and catch-bond (Dembo, Torney, Saxman, & Hammer, 1988), and inter-molecular friction (Bormuth, Varga, Howard, & Schäffer, 2009) models for how they attach to filaments.

At the network scale, continuum modeling is critical. The Navier–Stokes equations govern the motion of networks that behave like fluids when subjected to pressure and viscous loads. However, upon the application of other forces, for example, electrostatic and osmotic, additional coupling must be considered (Grodzinsky, 2011). Various constitutive relations may describe the mechanical behavior of networks acting like a solid (Drozdov, 2006; Drozdov & Gottlieb, 2005) including, at its simplest, Hooke's Law. However, many continuous networks might be best described using models of viscoelasticity, including the Maxwell, Kelvin-Voigt, and Standard Linear Solid models, poroelasticity, and viscoporoelasticity (Truskey, Yuan, & Katz, 2004). All these continuum models need to be coupled to the motor force generators in models that account for the nonequilibrium aspects of active materials (Needleman & Dogic, 2017). Network models, and in particular systems that require combinations of models, can get quite complex particularly in nontrivial geometries, and computational methods are often necessary to gain insight. Multiphysics modeling software, for example, COMSOL, is ideal for such scenarios.

The motile biological microstructures we discussed in this review lie between the single molecule and the continuous network scales. Therefore, successful models of motile

biological microstructures quantify the interaction of and coupling between molecular models of individual motors, filaments, and cross-linkers. Some examples include models for ciliary beating (Brokaw, 1972; Lindemann, 1994; Riedel-Kruse, Hilfinger, Howard, & Jülicher, 2007; Sartori, Geyer, Scholich, Jülicher, & Howard, 2016), how filaments are transported (Duke, Holy, & Leibler, 1995; Martin et al., 2012; Uyeda et al., 1990) and interact collectively (Schaller, Weber, Semmrich, Frey, & Bausch, 2010; Sumino et al., 2012) in gliding assays, how mechanical signaling throughout a network can regulate motor activity (Howard, 2009), and the origin of self-assembly in network and cluster-scale structures (Aranson & Tsimring, 2006; Maryshev, Marenduzzo, Goryachev, & Morozov, 2018). Many other, often very application specific, models effectively predict the behavior of particular structures (Needleman & Dogic, 2017). While the principles used by of many of these models are broadly applicable, what the field lacks are general models (e.g., Hooke's Law, Newtonian fluid, Euler's beam equations) that describe a broad swath of motile biological microstructures made from a broad range of materials.

5 | CONCLUSION

Above, we reviewed several, but certainly not all, methods of engineering motile biological microstructures, and we developed a method of categorization of these structures. By grouping motile biological microstructures into 12 types (four categories by three sizes—Figure 3), we have created a framework to understand the specific functionalities and benefits that each type of structure provides for researchers and engineers in the field of motile biological microstructures. Additionally, we have highlighted potential engineering and scientific applications, as well as areas in need of additional dedicated research, for motile biological microstructures. Finally, to advance the field significantly forward, we note that a set of quantitative, predictive and broadly applicable models are necessary.

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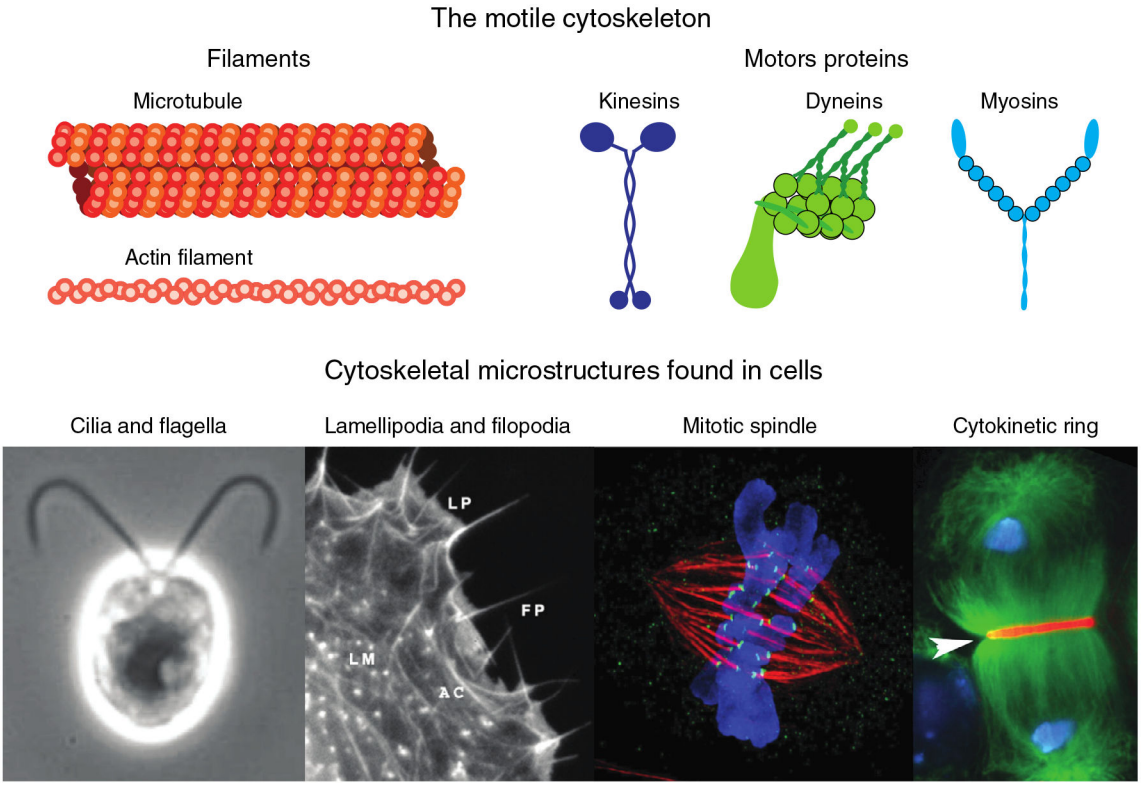

FIGURE 1.

The motile cytoskeleton. Schematics of the filaments and motors that make up the motile cytoskeleton. Microtubules are comprised of α- and β-tubulin dimers (red and orange), and actin filaments are comprised of g-actin subunits (orange). Representatives of each motor protein superfamily are shown: Kinesin-1 (dark blue) is a homodimer, outer arm axonemal dynein (green) is a heterotrimer, and a myosin V (light blue) is a homodimer that is shown decorated with associated light chains. Below are images of example cytoskeletal microstructures found in cells. Shown are the two flagella of a *Chlamydomonas reinhardtii* (green algae) cell (Geyer, Sartori, Friedrich, Jülicher, & Howard, 2016) (Reprinted with permission from Geyer et al. (2016). Copyright 2016 Elsevier); lamellipodia (LP) and filopodia (FP) in migrating goldfish fibroblast cells (Reprinted with permission from Nemethova, Auinger, and Small (2008). Copyright 2008 Royal Society of Chemistry); the mitotic spindle of a rat-kangaroo epithelial kidney cell at metaphase with microtubules (red), kinetochores (green) and DNA (blue) all stained for structured illumination microscopy (Stout & Walczak, 2013); and the cytokinetic ring (red) of a *Drosophila* sperm cell undergoing meiosis (Giansanti, Sechi, Frappaolo, Belloni, & Piergentili, 2012) used by license under a Creative Commons Attribution-NonCommercial 3.0 Unported license

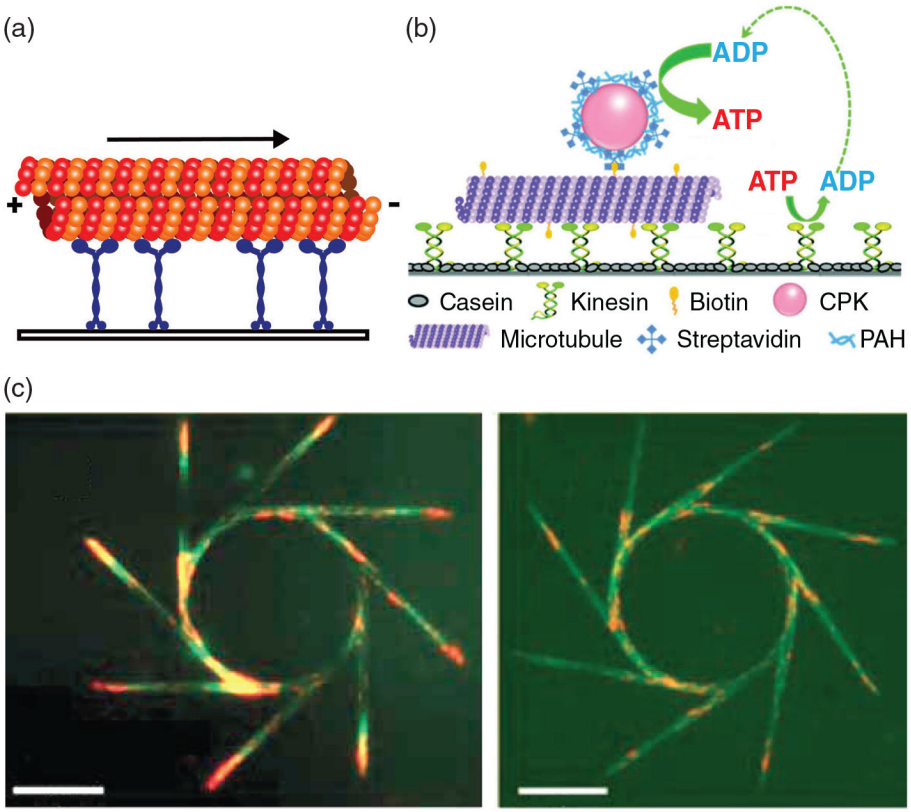

FIGURE 2.

Gliding assays are motile engineered biological microstructures. (a) Schematic of an example gliding assay in which kinesin-1 motor proteins (dark blue) are attached to a microscope coverslip (black). Upon the addition of ATP, the kinesins walk along a microtubule (red and orange) toward its plus end (indicated), and the microtubule moves with its minus end (indicated) leading. The arrow indicates the direction of microtubule motility. (b) Schematic of a self-powered microtubule-creatine phosphate kinase (CPK) engineered cargo delivery system gliding on the kinesin-modified surface (Reprinted with permission from Jia, Dong, Feng, Li, and Li (2014). Copyright 2014 Royal Society of Chemistry). (c) Rhodamine-labeled (red) stable microtubule seeds were extended with fluorescein-labeled (green) tubulin to demonstrate that microtubules can self-assemble into a completely overlapping network in confining channels. Scale bars are 15 μm. (Reprinted with permission from Doot, Hess, and Vogel (2007). Copyright 2007 Royal Society of Chemistry)

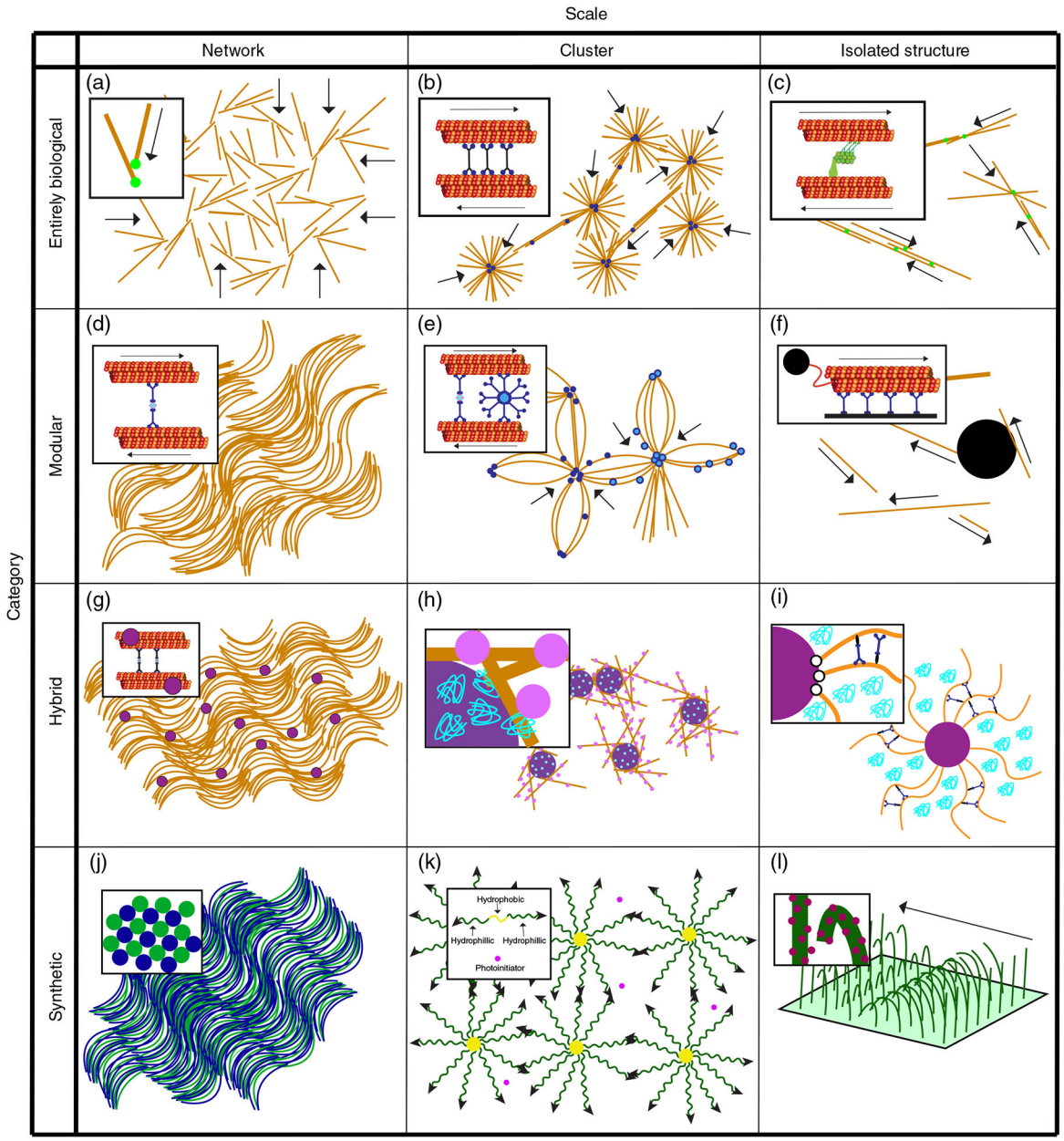

FIGURE 3.

Schematics of engineered biological microstructure types. (a) Entirely biological network: Dynein motor proteins accumulate at the minus end of microtubules (inset), which causes a microtubule network to contract as these dyneins walk toward the minus end of neighboring microtubules upon the addition of ATP (Foster, Fürthauer, Shelley, & Needleman, 2015). (b) Entirely biological cluster: Kinesin-5 motor proteins crosslink and slide neighboring microtubules (inset), which causes otherwise dissociated microtubules to coalesce into aster-like structures (Torisawa, Taniguchi, Ishihara, & Oiwa, 2016). (c) Entirely biological isolated structure: Axonemal dynein motor proteins crosslink microtubules (inset) into parallel or askew single structures. (d) Modular network: Truncated kinesin-1 motor proteins are complexed using biotin and streptavidin (inset) to form a tunable motile gel that exists in

a dynamic, turbulent state limited only by the availability of ATP (Henkin, DeCamp, Chen, Sanchez, & Dogic, 2014). (e) Modular cluster: Truncated kinesin-1 motor proteins are complexed using biotin and streptavidin and used in conjunction with multimeric minus-end-directed kinesin-14 (ncd) motor proteins complexed using GST domains and anti-GST antibodies (inset) to cause the formation of interconnected asters (Surrey, Nédélec, Leibler, & Karsenti, 2001). (f) Modular isolated structure: A liposome attached to a microtubule with single-stranded DNA in a kinesin-driven gliding assay-like system (inset) transports cargo (Hiyama et al., 2010). (g) Hybrid network: A tunable gel-like network, similar to panel (d), functionalized with synthetic nanoparticles (inset) could be used to deliver drugs conjugated to the nanoparticles throughout the network. (h) Hybrid cluster: MAPs (shown in teal) attach microtubules to synthetic microspheres that are functionalized with nanocrystals to form a non-motile hybrid cluster (Spoerke, Boal, Bachand, & Bunker, 2013). (i) Hybrid isolated structure: Artificial cilia are created by fixing microtubules with kinesin-1 motors conjugated to a polystyrene bead (purple). Neighboring microtubules are crosslinked, and motility is driven by the kinesins. Methylcellulose (teal) is used as a crowding agent to promote crosslinking of neighboring microtubules (Sasaki et al., 2018). (j) Synthetic network: Polymer gels with spirobenzopyran chromophores and ruthenium catalysts (blue and green, inset) drive the Belousov-Zhabotinsky reaction leading to oscillatory motility in the active material (Kuksenok & Balazs, 2015). (k) Synthetic cluster: Pluronic F127-DA micelles, which form in the presence of the activated photo-initiator Irgacure 2,959 (purple), crosslink to form potentially functional clusters (X. Liu et al., 2018). (l) Synthetic isolated structure: Biomimetic cilia made from a magnetic nanoparticle-polydimethysiloxane (PDMS) composite material (inset) can be actuated with a magnetic field (Shields et al., 2010). In all panels, dyneins (shades of green dots), kinesins (dark blue), synthetic particles (shades of purple dots), DNA (red lines), synthetic filaments (dark blue and dark green lines), and microtubules (orange lines) are as shown. Arrows in all panels indicate the direction of motility, and schematics are not to scale

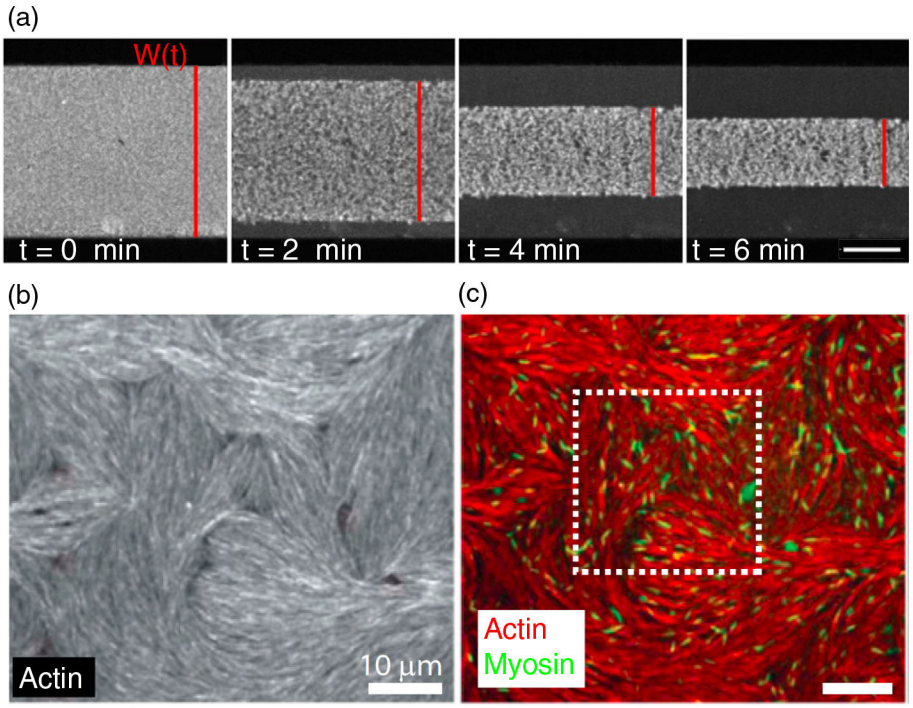

FIGURE 4.

Example entirely biological networks. (a) Nearly undiluted meiotic *Xenopus* egg extract plus taxol rapidly forms an essentially continuous, stabilized microtubule network. Dynein-driven motility leads to a contraction of the microtubule network confined in a flow channel over time. W(t) is 1.51 mm, 1.22 mm, and 0.78 mm at t =0, 2, and 4 minutes, respectively, and the scale bar is 500 μm. (Reprinted with permission from Figure 2(a) of Foster et al. (2015) and reproduced under the CC by 4.0 international license - https://creativecommons.org/licenses/by/4.0/). (b) Reconstituted contractile actomyosin cortex. Alexa-568–labeled F-actin associates with a supported lipid bilayer coated surface. (c) F-actin (red) and myosin II (green) are shown in the reconstituted cortex immediately after myosin thick filament formation. Panels (b) and (c) are reprinted with permission from Figure 1 of Murrell and Gardel (2012), and the scale bars on both panels are 10 μm

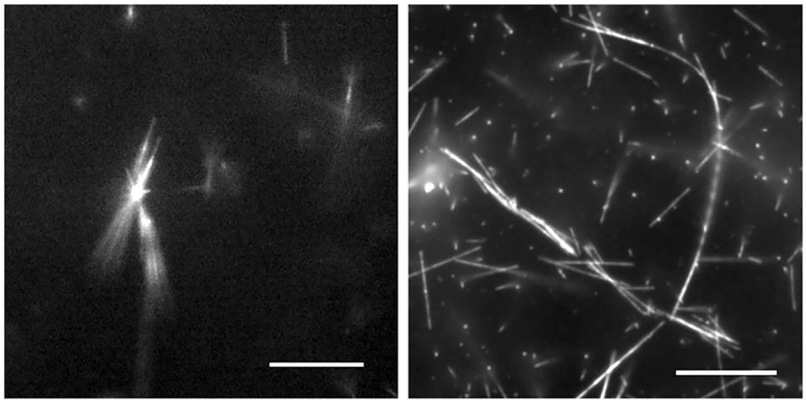

FIGURE 5.

Examples of entirely biological isolated structures. Askew (left) and parallel (right) structures made from axonemal dynein motor proteins and rhodamine-labeled microtubules. These structures form when motors and filaments are combined in solution. Imaged using fluorescence microscopy and the scale bar is 10 μm in both images

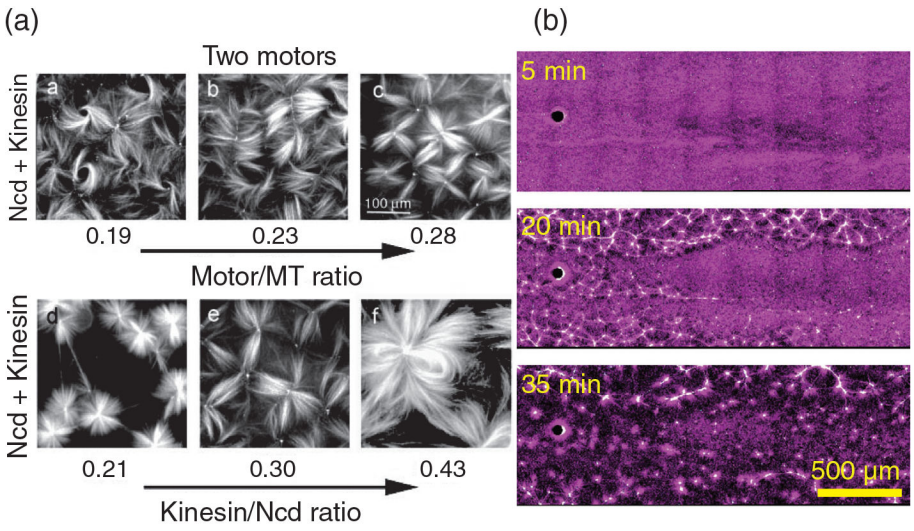

FIGURE 6.

Examples of modular clusters. (a) Multimeric kinesin-1 and minus-end directed Ncd motors crosslink microtubules and organize them into aster-like clusters in the presence of ATP. By modulating native kinesin-1 motors, which normally bind to only one microtubule, into multimeric complexes and changing the concentration of these and other elements of the system, microscale modular clusters resembling vortices, asters, and interconnected networks can be generated (Reprinted with permission from Surrey et al. (2001). Copyright 2001 AAAS). (b) Time series evolution of modular cluster formation using taxol-stabilized ATTO647N-labeled microtubules (magenta), ATTO565-labeled microtubules (yellow), and $KIF5B_{head}$-$Eg5_{tail}$ chimera motor proteins (cyan). (This panel was reprinted with permission from Torisawa et al. (2016). Copyright 2016 Attribution-NonCommercial-NoDerivatives 4.0 International Creative Commons License (CC BY-NC-ND 4.0)

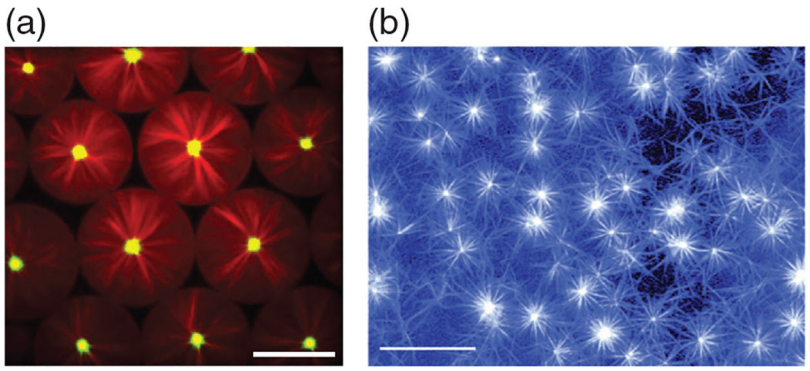

FIGURE 7.

Examples of hybrid clusters. (a) Tri-block copolymer-stabilized droplets are comprised of non-ionic PFPE–PEG–PEPE surfactant and contain microtubules (red) and kinesin-14 motor proteins functionalized with non-motor microtubule binding domains (green). Under the correct conditions, hybrid clusters that resemble asters can be formed. Scale bar is 100 μm. (Reprinted with permission from Juniper, Weiss, Platzman, Spatz, and Surrey (2018). Copyright 2018 The Royal Society of Chemistry and Creative Commons Attribution 3.0 Unported License). (b) Interconnected actin-based clusters formed from Arp2/3 coated beads in brain extract. Scale bar is 50 μm. (Reprinted with permission from Vignjevic et al. (2003). Copyright 2003 *Journal of Cell Biology* and Rockefeller University Press)

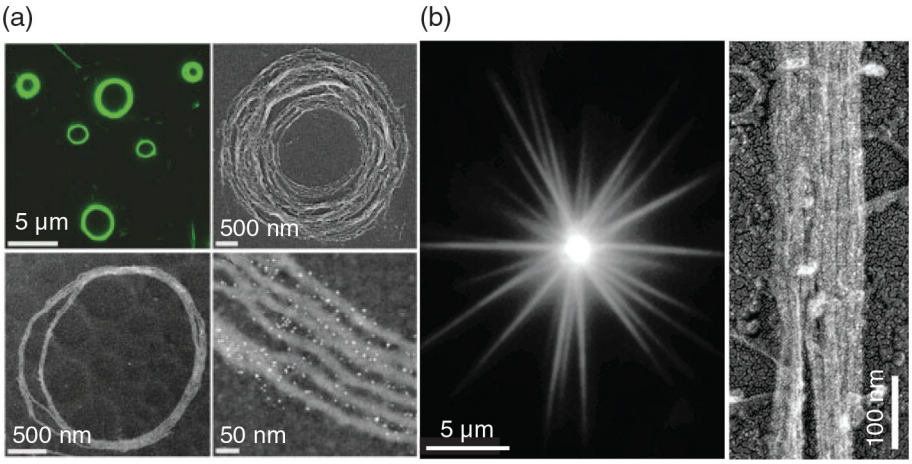

FIGURE 8. Examples of hybrid isolated structures.

(a) Structures formed by conjugation of quantum dots onto a microtubule-motor protein structure, resulting in a self-assembling ring. In this case, an otherwise entirely biological structure is being used as a carrier for a synthetic particle, making the structure a true hybrid (Reprinted with permissions from Liu et al. (2008). Copyright 2008, John Wiley & Sons., Inc.) (b) WASP-coated beads were incubated with rhodamine-labeled actin and Arp2/3 complexes to allow for actin assembly. These isolated aster-like structures formed upon the addition of fascin (left, scale bar is 5 μm). Electron microscopy of the star bundles revealed a filopodia-like isolated microstructure (right, scale bar is 100 nm). (Panels were reprinted with permission from Vignjevic et al. (2003). Copyright 2003. *Journal of Cell Biology* and Rockefeller University Press)

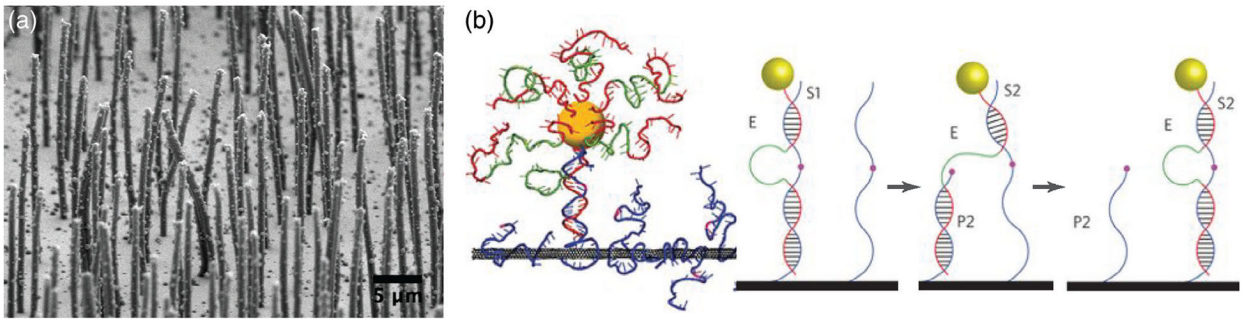

FIGURE 9.

Examples of synthetic isolated structures. (a) Artificial cilia. The cilia in this scanning electron micrograph are composed of maghemite nanoparticles embedded in PDMS pillars. While engineered to function like biological cilia, this construct contains no biological building blocks (Reprinted with permission from Shields et al. (2010). Copyright 2010 Proceedings of the National Academy of Sciences of the United States of America; PNAS). (b) a nanoparticle (yellow) functionalized with a DNA-based molecular motor comprised of a catalytic core (green) and two recognition arms (red) moves along a carbon nanotube track (black), shown as a molecular model (*left*) and series of schematics (*right*). The motor converts the chemical energy of RNA into mechanical motion through a series of DNA conformation changes, walking along the nanotube track processively and autonomously, similar to intracellular protein motors. (Reprinted with permission from Cha et al. (2014). Springer Nature Customer Service Centre GmbH: Springer Nature Nanotechnology)