Reversible Design of Dynamic Assemblies at Small Scales

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Emerging bottom-up fabrication methods have enabled the assembly of synthetic colloids, microrobots, living cells, and organoids to create intricate structures with unique properties that transcend their individual components. Herein, an access point to the latest developments is provided in externally driven assembly of synthetic and biological components. In particular, reversibility is emphasized, which enables the fabrication of multiscale systems that would not be possible under traditional techniques. Magnetic, acoustic, optical, and electric fields are the most promising methods for controlling the reversible assembly of biological and synthetic subunits as they can reprogram their assembly by switching on/off the external field or shaping these fields. Capabilities are featured to dynamically actuate the assembly configuration by modulating the properties of the external stimuli, including frequency and amplitude. The design principles are designed, which enable the assembly of reconfigurable structures. Finally, the high degree of control capabilities offered by externally driven assembly will enable broad access to increasingly robust design principles toward building advanced dynamic intelligent systems is foreseen.

1. Introduction

Translating the fundamentals of small-scale assembly into tunable engineering design principles has the potential to revolutionize the development of intelligent nanoscale to mesoscale systems.[11–3] The assembly of synthetic micro-/nanoparticles into multiscale structures, ranging between nano-, micro-, and mesoscale, has enabled the translation of their unique properties into large-scale materials.[4,5] In a similar fashion, living organisms can be considered as living assemblies, composed of several hierarchical layers with cells as the smallest intact single units.[6–8] In both synthetic and biological assemblies, the complex interplay of these subunits creates robust systems in which slight variations in the unit properties at the small scale can modify the whole system’s functionality at a larger scale.[9] The most common approaches of microassembly consist of bottom-up methods based on the assembly of microscale subunits into larger structures.[10–12] The forces driving this “self-organization” consist of changes that are either produced locally[13] (e.g., chemical gradient,[14,15] pH,[16,17] and temperature changes[18,19]) or driven by external power sources.[20,21]

Although recent reviews have covered the topic of self-assembly and the use of externally driven forces to drive micro-/nanoscale assembly, these works do not fully address the concept of reversible design.[22,23] In this direction, herein, we present the latest developments in reversible assembly at small scales. We define reversible design as a guided process capable of reconfiguring assemblies into new structures and the subsequent ability to restore their building blocks to their initial state. Governing the interface between the subunits, the external field functions as a vector for programmable control over the assembly. Although self-assembly methods present unique advantages including simple protocols and scalability, their major drawback is that they produce static structures that cannot be modulated. In contrast, reversible assembly offers complementary advantages to self-assembly methods, including a high degree of design flexibility, low material consumption, high packing density, when compared with self-assembly methods. Moreover, reversibility in design offers an alternative to fabricate multiscale assemblies not possible by other methods, including the ability to reconfigure the assembly in real time through the applied frequency and amplitude guide assembly formation into complex patterns without the need for scaffolds, providing...
the homogenous subunits with distinct functionalities based on their assembly and disassembly states and parallel manipulation of swarms of active particles that maintain individual self-locomotive capabilities. Furthermore, the rapid disassembly of the structure into their initial subunits can be typically induced simply by removing the applied stimuli.

Herein, we will describe the most commonly used external energy sources, including magnetic, acoustic, optical, and electrical fields, which drive and control the assembly of micro- and nanoscale programmable mesoscale structures (Figure 1). The use of external fields triggers different processes that lead to the formation of assemblies. They can i) create pressure nodes or tweezer to concentrate and trap micro-objects in a specific location, ii) modulate particle–particle interactions that can lead to attractive or repulsive behavior between the subunits that compose the assembly, or iii) use active matter (micromotors or living organisms) as a seed unit to form assemblies with larger particles, or induce swarming behavior. We thoroughly discuss each of the external fields, their mechanisms of action, and the latest applications in the synthetic and biological assembly in the following sections. Finally, we outline the challenges and opportunities for the use of external fields to control reversible assembly at small scales.

2. Magnetic Reversible Dynamic Assembly

Magnetic assembly has been investigated and successfully applied in guiding the reversible assembly of small-scale structures, typically by a simple change in the magnetic field or removal of it, enabling dynamic maneuvering and reconfiguration by guiding the migration of subunits, inducing magnetic levitation or exploiting dipole–dipole interactions. The individual subunits can be magnetized or be inherently magnetic, such as magnetic nanoparticles or paramagnetic iron-containing objects. Moreover, microrobots have been widely used for directing the assembly at a small scale. To this end, researchers have worked on developing techniques to code 3D materials with various programmable features, by shape, composition, and surface properties. Reconfigurable swarms of microrobots are one example of assembly involving dynamic group behavior. For instance, alternating magnetic fields have been used to program fast and reversible transformations between different swarm organizations of hematite colloidal particles with permanent magnetic moments, including liquids, chains, vortices, and ribbons. Another study explored the latter swarm configuration, using oscillating magnetic fields to program and restructure assemblies of paramagnetic nanoparticles enabling complex behavior, including locomotion, splitting and merging, and passing through channel networks.

In another example, an alternating magnetic field was used to guide the microrobot assembly into aster shapes at an immiscible liquid interface. Patchy microcube clusters were assembled using an external magnetic field, self-folding, and self-reconfiguring into a number of possible structures, while releasing energy in the process. In reconfiguration, the original sequence encoded the subsequent function as a result of equilibrium and nonequilibrium states of the cubes. Another example in the microrobot field is the use of robotic microgrippers made of flexible-patterned magnetic material, remotely actuated by a magnetic field, to perform programmed 3D assembly of microstructures. Diamagnetically levitated millirobots were also used for 2D-assembly. The use of an optical microscope was used for feedback, achieving open-loop stability up to 78 h in this demonstration of microfabrication and manipulation, also show autonomous microassembly. Another example for the assembly of superparamagnetic particles combined magnetic fields to assemble a microrobot swarm and an acoustic field to make it rotate demonstrating a behavior similar to a neutrophil rolling on a vessel wall.

The question of assembly in different dimensions and scales presents unique challenges by adding further complexity, whereas throughput is another factor in implementation. While assembly in two dimensions is well studied, assembly in three dimensions tends to be more challenging. 3D cultures have been particularly important in complex cell culture models because they more closely resemble biological phenomena than the traditional 2D cultures. Toward this goal, guided assembly has been used on tunable gels using the inherent paramagnetic properties of free radicals as an alternative to magnetic nanoparticle-embedded hydrogels. This rendered the gels “magnetoceptive” and thus capable of constructing complex 3D structures, by

![Figure 1](image-url) Externally driven reversible assembly at small scales. Schematic of the different external fields (magnetic, acoustic, optical, and electrical) used for assembling synthetic and biological structures.
combining different building blocks in a Tetris-like fashion. The block interacted via magnetic interaction produced by the embedded magnetic nanoparticles. Addition of vitamin D eliminated the magnetic reactive oxygen species, resulting in the loss of magnetic properties, thus disassembly can be seen. In this case, the process is not fully reversible but the erasibility of magnetic properties is another potential method for modulating the assembly process (Figure 2c).

Magnetic fields have also been used to guide superparamagnetic colloidal crystals into tunable chromatic arrays that map to structural colors. The guided assembly of colloidal matter thus encompasses a wide variety of actuation techniques and applications.

The magnetic susceptibility of biological objects has been explored as a means for safe and biocompatible dynamic control over biological assembly processes. For example, magnetotactic bacteria, a rare example of living cells with naturally occurring magnetic dipoles, have been manipulated to direct the assembly of synthetic microstructures. These flagellated bacteria occur in aquatic sediments and achieve directed mobility in a magnetic field by a cytoskeletal organization of their characteristic organelle: iron-containing magnetosomes. Such magnetic properties serve to guide their assembly at low Reynolds environment with controlled magnetotaxis, to create a pyramid shape as an example. Others have harnessed the unique properties of assemblies of magnetotactic bacteria to explore their potential to generate mechanical energy, via confinement into a water-in-oil droplet under a constant magnetic field generated by a Helmholtz coil.

Magnetic levitation is also used to assemble cells. This approach can direct the assembly of subunits with low magnetic susceptibility when they are suspended in a paramagnetic substance within a magnetic gradient. Uniquely, even cells that are diamagnetic can be assembled via this technique without the need for labeling or functionalization, by being suspended in a paramagnetic solution within a magnetic gradient. The structures can be disassembled by removing the applied magnetic field. Magnetic levitation has been combined with nanopatterned cell sheets for fast and reversible interactions with their base structure to build thermoresponsive constructs for tissue engineering. Furthermore, cell encapsulating microgel assemblies were generated via control of the magnetic field as well as tuning the paramagnetic medium (Figure 3a). 3D scaffolds for bone repair in a clinically oriented application have also

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Figure 2. Magnetic-induced reversible assembly of synthetic micro-/nanoparticles. a) Fast transitions between different collective formations of hematite colloidal microrobots by alternating magnetic fields. The scale bar is 20 μm. Reproduced with permission. Copyright 2019, AAAS. b) Reconfiguration patterns of microcube chains controlled by dipole–field and dipole–dipole interactions. The scale bar is 20 μm. Reproduced with permission. Copyright 2017, AAAS. c) Directed magnetic assembly of magnetoceptive gels into Tetris-like structures. The scale bar is 1 mm. Reproduced with permission.
used magnetic levitation to guide the assembly of the subunits. Moreover, magnetic levitation assembly has been carried out at the international space station, to assemble 3D tissue constructs in a setting without actual gravity (Figure 3b).

Magnetic assemblies allow for remote control and manipulation of building blocks that respond to external magnetic fields. Nevertheless, some magnetic assembly systems are complex and dependent on multiple kinds of equipment that can pose a challenge for widespread use. Overall, the magnetic properties of the materials, the applied field, and their magnitudes, are the main parameters governing externally driven magnetic assembly.

3. Acoustic Reversible Dynamic Assembly

Acoustic actuation technologies have been used as a noninvasive and highly biocompatible power source for rapid and tunable assembly of micro-/nanoparticles. Acoustic assembly offers noncontact manipulation of objects based on the difference in density between the subunits and their enclosing medium. The acoustic field can drive the migration of dispersed micro-/nanoparticles toward a specific location along a pressure node. Different types of acoustic fields result in distinct methods for manipulating micro-objects, including the use of surface
Surface acoustic waves are commonly used for assembling synthetic colloids of different sizes. These types of acoustic fields are generated using an interdigitated microelectrode that converts electrical signals by propagating them as mechanical stress that travels along the surface of a piezoelectric substrate material. These generate pressure nodes that serve as traps to manipulate and assemble micro-objects into predetermined patterns (Figure 4a). 1D micrometer-sized arrays were constructed by directing the assembly of silver nanoparticles using surface acoustic waves. The nanoparticles were flowed through a microfluidic chip. Upon application of surface acoustic waves, nanoparticles accumulated at pressure nodes forming 1D structures. Turning off the applied field resulted in the dispersion of the 1D assemblies, enabling reversible assembly without any noticeable change even after multiple cycles. Once a final design was selected, the silver nanoparticles were subjected to a sintering process to enable the formation of a stable structure (Figure 4b). Similarly, surface acoustic waves applied in nanoscale pulses enhanced the pattern resolution. This pulsed ultrasound minimized both the noise and acoustic streaming generated by the continuous application of the acoustic field, providing a great degree of control over the formation of the trapping area, leaving nearby areas unaffected. In another case, surface acoustic waves were used to generate 2D acoustic nodes capable of large-scale assembly of nanowires suspended in solution into well-controlled patterns with tunable geometry and spacing. The process enabled real-time dynamic patterning of large-scale assemblies. The acoustic field could be modulated to assemble the nanowires into parallel rows with the same orientation or to generate hybrid patterns of line assemblies with spherical patterns at the junction nodes. The nanowire dispersion was induced by turning off the applied power. The alignment and pattern type were simultaneously switched by changing the direction of propagation of the surface acoustic waves. Apart from this reversible behavior, by evaporating the solution and transferring the pattern into another substrate, an irreversible structure was obtained. Despite the advantages of acoustic fields for the induction of reversible assembly, the manipulation of submicrometer structures with a high spatiotemporal resolution has remained a challenge. New methods are starting to show the possibility of driving the assembly of submicrometer particles down to 200 nm. To this end, a cylindrical chamber was used, the structure was aligned orthogonally to the wave propagation direction and parallel to the substrate. This approach served to limit the formation of competing standing waves or microstreaming forces inside the chamber, and allowed the assembly of smaller nanoparticles.

Standing waves have also been used to induce the migration of particles toward pressure nodes, enabling the formation of small-scale assemblies. Thus, this principle has enabled ensembles of colloidal particles into “crystal structures” that are reconfigurable in real time. The fine tuning of the acoustic standing field enabled the lattice geometry modulation. Similarly, the speed of the assembly processes was controlled by modulating the applied amplitude. Another application of standing waves is the assembly of active matter. Self-propelled microswimmers move in random directions, thus making it hard to coordinate their collective behavior. In this direction, tuning the acoustic nodes has permitted to create swarms of microbots and active particles. The application of acoustic standing waves triggered the organization of chemically powered nanorobots into reversible swarm assemblies. The nanorobot swarm dispersed quickly after the acoustic field was turned off due to their autonomous catalytic locomotion, enabling a fast disassembly process. The swarm assembly generated at the nodal point was moved into different locations by changing the applied frequency (Figure 4c). The combination of acoustic fields and other external fields, including magnetic and optical fields was capable of generating more complex patterns. The assembly of the active matter was explored using nodes with...
different strengths. Strong nodes enabled closely packed assemblies, whereas weak nodes produced less-packed assemblies where the subunits suffer constant reconfiguration of the assembly. The ability to tune the strength of the node enabled the measurement of the force generated by nanorobots propelled by chemically induced locomotion.[88]

Acoustic levitation has also been used for tunable assembly of microscale units based on particle migration toward projected pressure nodes generated by arrays of acoustic transducers.[89] These create pressure nodes that trap particles, overcoming gravity forces.[90] The scattering of the ultrasound waves on the particles induced attractive interactions that led to controllable assembly. Thus acoustic levitation has been reported to enable the reconfigurable formation of small clusters of microparticles.[91] More recently, the realization of holographic acoustic tweezers was reported. Projections or holograms of acoustic patterns were generated using a mask, where the projected ultrasound wave stored the spatial distribution of the trap particles. These were further reconstructed by the interference of the projected sound waves. The microparticles were enclosed in a water container located above the transducer. Upon turning on the hologram, the particles assembled into the shape of a dove, used as an example of a projected sound pressure image. The ensemble collapsed after the system was turned off, enabling the reversible configuration of the assembly. This holographic approach demonstrated the ability to assemble structures two orders of magnitude larger than their original building blocks. Moreover, phase gradients were also generated using the acoustic hologram, which enabled trapping and transporting particles along predetermined paths. The particles followed the enclosed path indefinitely until the hologram was turned off (Figure 4d).[71] Further works demonstrated the ability to use the acoustic hologram technique to form a well-defined irreversible assembly of silicon beads by ultraviolet (UV)-induced crosslinking, illustrating the potential of the technique in fabricating permanent assemblies using this bottom-up strategy.[92]

Apart from synthetic particles, acoustic fields have shown great potential in the assembly of living organisms with high cell density and viability toward mimicking real tissue constructs.[93–97] In this direction, acoustic fields have been applied in a scaffold-free manner to drive the assembly of living cells for tissue engineering applications.[98–101] The acoustic method can assemble highly organized cell constructs without the need for conventional scaffold, showing viable promise in building larger 3D tissue-like constructs. Standing waves were applied for the assembly of micro-organisms, which enabled the study of their behavior in closely packed environments.[102] Standing waves were used to assemble microscale biomaterials into ordered structures in a scalable manner at high throughput. These accumulated a large number of cells (≈10⁴) within a few seconds (<5 s). The assembled structures were achieved by modulating the applied frequency, which permitted building structures in the macroscale range (Figure 5a).[103] In another work, 3D cell spheroids were fabricated by a similar method. The cells were first resuspended onto a low-adhesive petri dish surface and incubated for 2 days to form the cell spheroids. After collection, multiple spheroid assemblies were reversibly patterned by applying acoustic waves for a few seconds of different frequencies and amplitudes. The ability to finely tune the shape of the assembly enabled further crosslinking by thrombin and fibrinogen, enabling long-term cell culture and downstream functional characteristics (Figure 5b).[106]

Acoustic standing waves were also used to assemble dense myoblast populations in a hydrogel containing type I collagen. The generation of the muscular tissue, and myogenesis behavior, was studied when applying the external field.[107] Cardiomyocytes stem cells were assembled into pre-defined 3D assemblies using standing waves to mimic cardiac tissue. These methods enabled

![Figure 5](image-url)
high-density packing (10^9–10^10 cells mL⁻¹), which significantly improved cell activity and function, including metabolic activity and cell viability, when compared with randomly generated assemblies. Brain-like construct assemblies have also been built using standing waves. The ability to generate brain models in three dimensions is of great importance as 2D models do not sufficiently recapitulate the neural connectivity of the brain. To build these brain models, acoustic standing waves generated multiple levitation planes which matched the spacing in brain tissues. Using a similar approach, 3D neurospheroids were assembled to model Alzheimer’s disease.

The shape of the assemblies has also been modulated to form ring-shaped assemblies. More recent acoustic methods have utilized ionic crosslinking to rapidly remove hydrogel scaffolds, allowing the generation of tissues composed of only cellular assemblies. The use of acoustic forces was also reported toward enhancing cell packing in buckyball microstructures. Moreover, acoustic nodes were also used to induce the swarm formation of Escherichia coli bacterial suspensions. The disassembly was achieved by removal of the acoustic field, although long periods of confinement led to the formation of bacterial seed aggregates (Figure 5c). Different patterns of living cells were generated by modulating the applied ultrasound frequency without affecting cell viability. Studies of replication and cell growth under confinement were also conducted using cell assemblies exposed to ultrasound fields for more than 12 h. Other applications consisted of the directional assembly of cells into acoustic wells for localized analysis. Acoustic microstreaming around microengineered patterns were also explored using surface acoustic waves. Topographical features of the substrate interact with the traveling wave generating a localized microstreaming flows capable of trapping nearby objects. Application of this technique was carried out by building arrays of acoustic microstreaming traps that enabled the enrichment of cancer cells from whole blood around the traps. Interestingly, this methodology could also be used to generate organoids, as the cells can be released upon turning off the acoustic field (Figure 5d). Acoustic holograms have been utilized for patterning cells, enabling rapid 3D tissue fabrication.

In general, acoustic assembly methods offer the ability to concentrate diverse types of subunits into predefined large-scale patterns, controlled by the type of applied acoustic field. These methods rely on the migration of building blocks toward pressure nodes driven by difference in material properties with the medium. A limitation in the use of acoustic fields for small-scale assembly relies on restricted capabilities to assemble nanoparticles smaller than 200 nm.

4. Optical Reversible Dynamic Assembly

Optical methods have been used to assemble constructs at high speed with high spatiotemporal precision. For example, light can control the reversible assembly of nanoparticles into well-defined colloidal macrostructures. Most methods rely on the use of photoswitchable molecules that can reversibly change between two states in response to specific wavelengths, allowing to selectively trigger the assembly of responsive colloids from a heterogeneous solution. Nanoparticles functionalized with azobenzene groups reported reversible assembly. The exposure to UV light induced the isomerization of the azobenzene group from trans to cis, which made the nanoparticles lose colloidal stability, thus forming stable assemblies. This process is reversible by removing the UV irradiation. This principle was also used to create “ink” based on nanoparticle assemblies, where the final structure can be tuned to stay stable from a few seconds to days. A photomask was used to create spatial pattern designs that induce the isomerization of azobenzene groups. Exposure to visible light or sunlight resulted in the erasing of the pattern. Nanoparticles functionalized with photoswitchable azobenzene groups reported reversible assembly of large disk-like structures from 100 to 1000 nm under UV light irradiation. The application of an optical field induced the assembly and disassembly of the colloidal structure based on dipole–dipole interactions resulting from the trans–cis photoisomerization change of the azobenzene groups. Nanoparticles functionalized with 7-methylacyloxy-4-methylcoumarin (PMAMC), a photosensitive amphiphilic star-like copolymer, reported reversible self-assembly under optical stimulation. The reversible assembly was based induced by UV light irradiation, promoting the PMAMC chain photodimerization (365 nm) and photocleavage (254 nm).

The optical gradient force of a focused laser has been used to generate light traps that can assemble microparticles into well-defined micropatterns. Optical traps were generated by creating an interfering pattern between an annular-shaped laser and a reference beam. Nearby objects were trapped inside the spiral interference pattern. The assembly generation was not based on intrinsic material properties, allowing to trap and generate assemblies of diverse particle types, including silica spheres, glass rods, and chromosomes. Reversible organization of metal nanoparticle was achieved by Gaussian optical fields to generate assemblies of different geometries. The optical trap generated a focused force point that suppressed the Brownian motion and confined a group of nanoparticles into diverse tridimensional arrangements. The optical trapping and assembly of microparticles can also take advantage of the propagation of the laser force field to form assemblies outside of the focal point, resulting in the generation of horn-like structures. In another example, an array of photonic-crystal slab could assemble over 100 polystyrene nanoparticles (500 nm) by projecting a laser light from below a silicon patterned with a periodic array of holes. When the laser beam was turned on, the particles were driven to occupy the array lattice. The trapping forces arose from a strong electric-field gradient generated at the holes. Upon removing the laser irradiation, the particles disperse and diffuse away from the patterned holes. An expansion of this work reported the ability to form 1D chains that can be oriented through finely tuning the incident polarization of the light. The chain formation resulted from the competing forces consisting of particle–particle interaction and particle–hole interactions. Holographic optical tweezers have enabled the simultaneous manipulation of multiple microparticles. The assembly process has been automated using closed-loop object recognition.

The use of optical fields has been widely used to guide the assembly of active matter. For instance, TiO₂ micromotors powered by UV light illustrated a firework-like behavior based on
the diffusiophoresis of charge species gradients generated by chemical propulsion and its interaction with passive SiO$_2$ microbeads. The passive beads aggregated around the TiO$_2$ micromotors as a result of surface charges. In contrast, exposure to UV light resulted in the passive microbeads rapidly moving away from the TiO$_2$ micromotors. Upon removal of the UV light, the passive bead reaggregated near the TiO$_2$ micromotor surface (Figure 6c). $^{[132]}$ A similar firework phenomenon has been reported using optoacoustic micromotors, where the interaction of the acoustic nodes and light-induced attractive and repulsive forces resulted in tunable collective behavior. $^{[86,148]}$ In another case, a small number of UV light-powered TiO$_2$–silica Janus micromotors (1.5 μm) were used to create out-of-equilibrium microassemblies. The motile micromotors served as nucleation centers that condensed silica microbeads into 2D microassemblies based on electrostatic interactions. The assembly process was regulated by tuning the applied light intensity and by changing the active–passive particle size ratio, enabling construction of clusters of different geometries, including square, pentagonal, hexagonal, and heptagonal assemblies. The mixture of different cluster types resulted in large-scale crystal-like disordered macro/assemblies ($>40$ μm). The interaction between the micromotor and passive particle assembly remained stable until the UV light source was turned off (Figure 6d).$^{[133]}$ TiO$_2$ micromotors were functionalized with hydroxyl groups, which enabled reversible motor–motor interactions due to electrolyte diffusiophoretic attractions induced by UV irradiation. $^{[149]}$

SiO$_2$–Pt Janus micromotors functionalized with spiropyran, a photochromic functional group, reported reversible assembly under light irradiation. UV light-induced assembly by electrostatic interaction and disassembled under green light. $^{[150]}$ Photoactivated colloidal particles were assembled into 2D living crystals with dynamic behavior including reversible breaking and reassembly. Such behavior resulted from the interplay between the propulsion of micromotors and particle–particle interactions induced by light-generated osmotic and phoretic effects. $^{[151]}$ Au/TiO$_2$ micromotors with rapid on-demand reversible assembly were reported. Application of an optical field resulted in attractive and repulsive forces that drove the assembly into larger structures. The micromotors served as nucleation sites leading to reconfigurable crystal systems that went through fusion and fission transition states. The application of green light led the micromotors to start clustering into small assemblies (≈4 particles), which coalesced into medium-sized assemblies (≈10 particles) in a process similar to fusion or crystallization. The rapid switching to UV illumination led to rapid disassembly where the clusters “explode,” breaking into smaller clusters and individual motors, resembling a fission event. $^{[152]}$ Peanut-shaped hematite micromotors were assembled into twisted colloid ribbons under blue light radiation. The assemblies arose from the force competition between diffusioosmotic propulsion and phoretic attraction, resulting in the formation of large 3D chains. These assemblies diffused without blue light indicating the reversibility of the system. $^{[153]}$ Photoactive micromotors were assembled
under light activation, forming rotating gears. The system was composed of microrobots that migrated toward a hematite anchor under exposure to a blue laser focal spot. Once assembled, the laser was turned off, inducing an attractive hydrodynamic force that drove the motile micromotor into a spinning microgear. A collection of seven micromotors were shown to interact in a gear-like fashion, illustrating the ability to create engines that communicate with each other (Figure 6e).[134]

AgCl microparticles assembled into swarms under exposure to UV light. Cyclical exposure between visible and UV light resulted in the expansion and compression of the swarm area coverage. The assembly was driven by silver ions secretions generated at the AgCl microparticles.[15] The formation of small clusters between polystyrene/Ag/AgCl Janus micromotors and passive polystyrene beads was achieved based on the difference in surface charge produced under blue light illumination. The optical field was used to power the propulsion of the Janus micromotor.[154]

Living organisms can be assembled using optical forces[27,155–157] Optical tweezers generate microvortices that serve as optical traps. For example, different kinds of cells including yeast (nonmotile) and Chlamydomonas reinhardtii (motile), were assembled into micrometer-sized dynamic cellular arrays with well-defined spatiotemporal positioning (Figure 7a).[158] Holographic optical tweezers were used to generate 3D cellular microarchitectures. As a proof of concept, a different number of mouse embryonic cells were patterned into 3D assemblies (Figure 7b).[159] An optical image-driven dielectrophoresis mechanism was reported for assembling a large number of microparticles (≈15 000) using a light-emitting diode combined with a digital micromirror spatial light modulator array to generate optical patterns of millimeter-sized area. By taking advantage of the dielectric contrast between living and dead cells, this platform reported the ability to separate human cells suspended in solution. The living cells were collected in the assembly regions, whereas dead cells did not present interaction with the optical field (Figure 7c).[160]

Moreover, optical manipulation has been used to manipulate motile living organism using light patterns to rectify their locomotion. For example, phototaxis capabilities of some micro-organisms were used to steer them toward specific regions within a microfluidic chip.[162,163] In this direction, spatially patterned optical fields were used to control the assembly of genetically engineered E. coli that responded to changes in light intensity. A digital mirror device was used to generate light patterns, which induce the bacteria to swim away of the illuminated pattern, accumulating at the edges outside the boundary area of the pattern. The removal of the light pattern led to the dissolution of the bacterial assembly. The optical field was tuned to control the position and sharpness of engineered assemblies, ranging from 10 μm to a few millimeters.[164]

The use of blue light (480 nm) has been used to control engineered bacterial adhesion with functionalized substrates to build

![Figure 7. Optically induced reversible assembly of living microorganisms. a) Use of optical tweezers to induce the assembly of multiple micro-organisms into well-defined patterns. Reproduced with permission. Copyright 2020, Wiley-VCH. b) Use holographic optical tweezers to build 3D cellular microassemblies. Reproduced with permission. Copyright 2015, Springer Nature. c) Parallel assembly and isolation of live cells from dead ones using optical fields. Reproduced with permission. Copyright 2005, Springer Nature. d) Reversible assembly of bacteria with synthetic microcargoes under red/far-red light based on the bonding of and disassembly PhyB and PIF6 proteins. Reproduced with permission. Copyright 2020, Wiley-VCH.](image-url)
5. Reversible Electric Dynamic Assembly

Electric fields have been used as an external stimulus to direct multiscale assemblies of micro-/nanoparticles.\textsuperscript{166,167} Electrical-induced assemblies have demonstrated the ability to generate multifunctional assemblies in which properties arise from their structural design or by tuning the applied field. The use of such electric fields has been shown to induce positive or negative migration into organized structures based on the innate material properties of the subunit particle into assemblies of larger orders of magnitude.\textsuperscript{168} For instance, silver nanoparticles (6 nm) were assembled into micrometer-size superlattice crystals using electric fields. This assembly method was based on the migration of the charged nanoparticles toward a cathode or electrode. Similarly, the addition of tetraoctyl ammonium bromide induced the formation of silver nanocrystals macroassemblies over the surface of an anode electrode, based on the change in surface charge of the nanoparticles. Removing the electric field, while the electrodes were submerged in the solution, resulted in the silver nanoparticles super lattice to dissolve. This work generated nanocrystal assemblies into large supper lattices without resulting in a change of composition or requiring solvent evaporation (Figure 8a).\textsuperscript{169} The large-scale assembly of polymer latex nanoparticles using electrophoretic deposition was exploited to generate reversible assemblies. Upon applying a direct current (DC) electric field, the positively charged particles assembled over a conductive negatively charged indium tin oxide (ITO) electrode, generating a 20 μm-thick crystal lattice with a close-packed structure, capable of maintaining its properties for prolonged periods. The assemblies presented reversibility within 60 s. The reversibility between the ordered and disordered nanoparticles resulted in tunable structural color (Figure 8b).\textsuperscript{170} A similar approach relied on the use of predefined patterned scaffolds over an ITO electrode to generate assemblies in the centimeter scale within a few seconds.\textsuperscript{171} The electrically driven reversible assembly of plasmonic gold nanoparticles (16 nm) in between two immiscible electrolyte solutions was reported. The nanoparticles were functionalized with hydrophobic chains, which responded to negative polarization, thus accumulating in the liquid-organic interface by the induction of positive electric polarization. The assembly resulted in a change in optical response by the colloidal suspension of nanoparticles shifting to red while aggregated and blue when they were dispersed. Such capabilities were used as a proof of concept to generate electric modulated liquid mirrors based on the change in reflectivity, switching from a highly reflective to a transparent material, induced by the reversible assembly of the plasmonic nanoparticles.\textsuperscript{174}

Dielectrophoretic methods have been used to perform assembly and sorting of particles with lower electric permeability. For example, the reversible assembly of diverse types of objects, including liquid droplets and cell-loaded hydrogel units, using electric fields was reported. This methodology used a two-parallel plate electrode system to induce electrical assembly based on dielectrophoresis and electrowetting. Each building block could be directed to assemble into 3D structures.\textsuperscript{175} The ability to assemble 1D structures was shown by the electrically driven assembly of Janus ellipsoids into colloidal fibers. The ellipsoid shape of the microparticles limited the interaction that enabled formation into fibrillary assemblies.\textsuperscript{176} The application of a perpendicular applied vertical electrical gradient resulted in the assembly of metal–dielectric Janus colloids into distinct types of swarms by inducing asymmetric interactions between the polarized particles. The mismatched dielectric responses of the metallic and dielectric hemispheres of the Janus colloids to the perpendicular electric field resulted in controlled locomotion and programmable particle–particle interactions. By tuning the amplitude and frequency, the Janus colloids assembled into diverse structures, including clusters, chains, swarms, and isotropic gas-like formations (Figure 8c).\textsuperscript{171} The assembly of asymmetric microparticles under electric fields reported subunits exhibiting chirality, with such behavior generated in part by asymmetric hydrodynamics flows that caused the rotational motion of the assemblies. The use of an alternating current (AC) electric field generated dipole-based assemblies between a central microparticles and orbiting dimers.\textsuperscript{177}

Moreover, electric fields have been used to power the locomotion of untethered robots, enabling them to interact and assemble into large swarms. Mixing electrically powered micrometer-sized Janus micromotors with passive silica particles resulted in the ability to create rotating cluster assemblies. For instance, under an applied AC electric field, motile micromotors served as seed particles that acted as anchors to passive microbeads, in which the steps of the cluster interaction defined the final structure. The AC electrical field induced one dipole at the center of the silica microbead, whereas the micromotor generated two dipoles at different hemisphere based on the different material properties of the Janus structure, thus generating the repulsion and attraction forces that led to the assembly of different structures with different rotation preferences. These assembled structures lost their stability upon removal of the electric field (Figure 8d).\textsuperscript{172} Janus micromotors were also used as carriers of microbeads for targeted delivery using an externally applied electric field to induce the capture or repulsion of cargo carriers without the requirement of previous functionalization or labeling of the micromotor surface. The Janus micromotor presented the ability to perform on-the-fly capture and assembly of different targets ranging from 100 to 720 nm beads, controlled by
modulating the applied voltage. The disassembly/release of the beads were achieved by changing the applied frequency.\[^{178}\] PMMA-Ag Janus micromotors were coordinated to ensemble into assemblies that periodically expand and contract by changing the applied electric field. The synchronization of the micromotors motion created a beating assembly induced by each motor-generated chemical field responding to nearby micromotors being attracted or repelled by it.\[^{179}\] Dielectrophoretic interactions have been used to enable 3D modular assembly of microrobots based on electrically induced attractive interactions generated between a structural body and multiple microengines. Specifically, the application of an electric field induced the polarization of the main body, which generated an asymmetric-tailored dielectrophoretic gradient localized at geometrical-driven bonding sites that guided the microengines assembly into the desired locations of the microrobot frame. The 3D distribution of the engines resulted in different propulsion modalities and behavior, including axial and rotational motion. Moreover, the motion was used to drive the hierarchical assembly between two microrobots (Figure 8d).\[^{180}\]

Electric fields can also assemble living micro-organisms into assemblies via electrophoresis, where the micro-organism migrate to an electrode presenting opposite charge, or by dielectrophoresis by inducing dipoles in the cells via an AC electric field. Usually, these methodologies are used for sample sorting and isolation applications. Bacterial assembly is a common process naturally happening in biofilms and plays an important role in many infectious diseases.\[^{181}\] Despite different bacteria having different shapes and sizes, the clustering can be simplified to spherical colloids as building blocks interacting between each other and with other components in the media. The motion of micro-organisms follows random run and tumble or chemotactic behavior toward nutrients. Nevertheless, the use of electrical fields has shown the ability to direct their assembly into bioassemblies based on the polarizability of their surface using an AC electric field.\[^{182}\] In these directions, the effects of an AC field applied onto *E. coli* cultures have been studied.\[^{183}\] AC electric fields applied to bacterial cultures have been shown to produce large-scale aggregates due to the strong attraction between bacteria. This transient phenomenon was reverted with

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**Figure 8.** Electrically induced reversible assembly of synthetic micro-/nanoparticles. a) Reversible assembly of silver nanoparticles into large nanocrystal superlattices. Scale bar: 10 µm. Reproduced with permission.\[^{169}\] Copyright 2017, ACS. b) Reversible structural color based on a reversible assembly of colloids on an ITO electrode induced by the application of a direct current electric field. Scale bar: 5 µm. Reproduced with permission.\[^{170}\] Copyright 2014, ACS. c) Assembly of metal–dielectric Janus colloids into programmable collective states by induced imbalanced polarizable interactions between the colloids. Scale bar: 30 µm. Reproduced with permission.\[^{171}\] Copyright 2016, Springer Nature. d) 3D hierarchical assembly of microrobots driven by the dielectrophoretic interactions between a structural frame and multiple microengines. Scale bar: 10 µm. Reproduced with permission.\[^{172}\] Copyright 2016, Wiley-VCH.
the decrease or the removal of the electric field. The clustering competes with the steric repulsion of bacteria aiming to swim away. Therefore, a critical AC value is required to force the clustering of the living organism and induces the external flow to modify the trajectories of the bacteria. This electric-field controlled fluid flow resulted in long-range interactions between the cells and served to transport cells in the far field.

The induced polarization in bacteria induces the formation of assemblies aligned to the direction of the applied field. *Micrococcus luteus* bacteria that commonly cluster into tetrad structures (group of four units) under normal incubation conditions, were assembled into 1D structures composed of single chains or double helices. The assemblies disintegrated upon the removal of the field. At high frequencies, the dipolar interaction is stronger, creating elongated structures (Figure 9a).[184]

The assembly of rod-shaped *Salmonella typhimurium* in the presence of an AC electric field was also studied. At different field strengths, the bacteria generated different dimensional structures. The use of low fields induced the formation of line assemblies, whereas at higher fields 2D reversible arrays were generated and extended to 3D columnar structures when high bacterial concentration were used.[187] Microarrayed electrodes were used to assemble pathogenic *Salmonella* and *E. coli* into predetermined patterns using dielectrophoresis-based assembly (Figure 9b).[185] In another case, the aggregation of *S. cerevisiae* cultures was studied under the presence of an electrical field. Interestingly, close-packed hexagonal 2D crystals were generated over time because of the lateral attraction between the formed chains when using four electrodes for applying an electric field in two perpendicular directions.[188] Interestingly, yeast cells (*S. cerevisiae*) formed chains using this format, but did not form 2D assemblies. Reversible disassembly was induced by turning off the applied electrical field. The use of functionalized micro-particles with Concanavalin A, a protein that binds to the polysaccharides at the yeast cell, was used to permanently bond the live cell structures.[189] This concept of dielectrophoresis was exploited for creating hybrid bioassemblies consisting of yeast bacteria, cells, and inorganic colloidal particles into 1D and 2D assemblies.[190] Applications of dielectrophoretic forces were used for assembling microalgae (*Chlamydomonas reinhardtii*) in 2D arrays. The response of the algae assemblies to diverse inorganic pollutants from solutions was used as a probe sensor.[191] *E. coli* was assembled over the surface of a dielectric micromotor, which functions as a motile electrode, under the presence of an electrical field. The disassembly of bacteria was achieved when the electric field was turned off. Moreover, targeted electroporation of the assembled bacterial was achieved with a pulsed signal, which could lead to programmable cell assemblies.

Figure 9. Electrically induced reversible assembly of living micro-organism. a) Use of electrical induces assembly of multiple micro-organisms into microwells. Scale bar: 60 μm. Reproduced with permission.[184] Copyright 2020, ACS. b) Alternative current-induced reversible assembly of bacterial tetrads, chain assembly, and helix structures. Scale bar: 4 μm. Reproduced with permission.[185] Copyright 2016, Elsevier. c) Reversible assembly of bacteria over the surface of polarizable dielectric micromotors via dielectrophoresis. Reproduced with permission.[186] Copyright 2020, AAAS.
of acoustic waves that generate prede
netophoresis or magnetic levitation of subunits. The acoustically
govern the response to an external stimulus. For instance, mag-

living cells. The unique material properties of the building blocks
assemblies at small scales during the past decade. In this direc-
Tremendous progress has been made in engineering reversible
assembly methods. For instance, i) offering a high degree of design
flexibility, enabling to reconfigure the assembly in real time. ii) Localizing and reusing the subunits with a high packing density, thus reducing material consumption. iii) Giving dual functionality to a single type of building block, as shown in the reversible for-
mation of reflective/transparent surfaces by assembling nanopar-
ticles under an external stimulus. iv) Tuning the formation rate to
increase the resolution of the final structure; a feature that would
be hard to control using instantaneous assembly methods. v) Enabling the assembly and guidance of large groups of micro-
motors as a single swarm, whereas retaining the individual micro-
motor ability to propel autonomously after removal of the external
field. Nevertheless, despite such great progress, there are still
many challenges to overcome. Thus, we lay out a set of key chal-
lenges to set the foundation of future research. 1) How to scale up
the fabrication toward macroscale assemblies? 2) How to direct
the assembly using heterogeneous materials of different scales? 3)
What applications can these assemblies uniquely enable in
medicine and engineering? 4) How to interface externally driven
assembly and self-assembly to obtain different degrees of
reversibility?

To advance the use of reversible assembly protocols from lab-
ory settings to practical applications, new developments will
require interdisciplinary convergence between very different
backgrounds such as biology, physics, computer science, elec-
tronics, and nanotechnology. The combinatorial use of external
fields could expand the capabilities to fabricate more complex
structures, as each assembly method has unique advantages
for specific applications and types of subunits. Combining mul-
tiple external fields has enabled to generate more complex and
tunable assemblies. For instance, in previous sections, we cov-
ered how the combination of acoustic fields with magnetic[147] and optical fields[148,149] enabled dynamic reorganization of an
assembly in real time. This was also shown for combinations of optical and electrical fields.

Moreover, testing the response of novel shapes and materials to
diverse external fields could result in an ever-expanding library of
potential building blocks. As the field moves toward increasingly
complex assemblies at small scales, it will become important to
embrace machine learning and artificial intelligence toward
advancing parallel object tracking, feedback loops, and real-time
remote control. The development of novel methods of assembly
could entail a new technological revolution, enabling fabrication
of materials, where intelligence is embedded in the relationship
between the subunits, in a similar fashion to a brain being com-
posed of millions of neurons working together for intelligence and
consciousness. The design of reversible assemblies by external
fields permits to fabricate constructs that would not otherwise
be possible to fabricate. For example, the use of external stimuli
to drive the formation of assemblies does not rely on gravity and
has low material consumption due to their ability to preconcent-
trate subunits in closed-packed structures. Thus, they are applicable
for constructing assemblies in space.[99] Reprogramming tissue
engineering in real time inside a patient is another field of great
potential for reversible assembly. Instead of relying on invasive
surgery to implant tissue scaffolds or requiring a large quantity
of cell to overcome the low targeting efficacy of injections of indi-
vidual stem cells, externally guided cell assemblies could be recon-
figured to fit and secure fixation at a target site.

Considering the great progress made in reversible assembly,
we encourage researchers to look for unmet needs and applica-
tions by proposing problem orientated engineered structures or
methodologies to streamline the adoption of externally driven
fabrication methods. The methods may broaden their application
areas in a similar way that 3D printing has become ubiquitous in
rapid prototyping. We envision that the key challenges we
pointed out can be gradually addressed, thus expanding the hori-
zon and applications of guided bottom-up fabrication. The ability
to finely tune and modulate the assembly of complex structures
could result in diverse practical applications ranging from sens-
ing, 3D biofabrication, tissue engineering, bioprinting, microma-
ufacturing, patterning, and sample manipulation.

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Conflict of Interest
The authors declare no conflict of interest.

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