Autonomous Treatment of Bacterial Infections in Vivo Using Antimicrobial Micro- and Nanomotors

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Cite This: ACS Nano 2022, 16, 7547−7558

ABSTRACT: The increasing resistance of bacteria to existing antibiotics constitutes a major public health threat globally. Most current antibiotic treatments are hindered by poor delivery to the infection site, leading to undesired off-target effects and drug resistance development and spread. Here, we describe micro- and nanomotors that effectively and autonomously deliver antibiotic payloads to the target area. The active motion and antimicrobial activity of the silica-based robots are driven by catalysis of the enzyme urease and antimicrobial peptides, respectively. These antimicrobial motors show micromolar bactericidal activity in vitro against different Gram-positive and Gram-negative pathogenic bacterial strains and act by rapidly depolarizing their membrane. Finally, they demonstrated autonomous anti-infective efficacy in vivo in a clinically relevant abscess infection mouse model. In summary, our motors combine navigation, catalytic conversion, and bactericidal capacity to deliver antimicrobial payloads to specific infection sites. This technology represents a much-needed tool to direct therapeutics to their target to help combat drug-resistant infections.

KEYWORDS: nanomotors, antimicrobial peptides, nanoparticles, self-propulsion, bacterial infection, autonomous treatment

INTRODUCTION

Bacterial infections are predicted to kill 10 million people by 2050, which corresponds to one death every three seconds. Today, they are the fourth leading cause of death in hospitals in the U.S. and cause millions of deaths worldwide, constituting a major public health threat.1 Indeed, bacteria are becoming increasingly resistant to broad-spectrum therapies, such as antibiotics, and approaches for countering recalcitrant infections are urgently needed.2−7 Currently available antibiotics are limited by their broad and deleterious off-target effects due to the lack of delivery methods that effectively release payloads at the infection site. Advances in nanomedicine have contributed to the development of antibiotic-loaded nanoparticles,8 but these delivery systems only counter bacterial biofilms at the material interface and can become inert over time due to protein adsorption.9 Despite these drastic interventions, infections often remain untreated and untreatable.10,11 Hence, approaches for effectively delivering antimicrobial payloads to the infection site are urgently needed.12

Micro- and nanoparticles can be designed to achieve self-propulsion by converting diverse energy sources into mechanical motion, yielding swimming micro- and nanomotors. Depending on their composition, particles with active motion can be designed for a variety of applications ranging from environmental roles13 to biomedicine.14,15 Biohybrid active motion has been extensively explored in the past decade in the form of micro- and nanosized bioactive motors for minimally invasive interventions, given their potential for active navigation to reach otherwise inaccessible areas.16−18 Recent breakthroughs have been reported utilizing these systems for biomedical applications, including targeted and enhanced drug delivery,19,20 cell manipulation,21,22 microsurgery,23,24 biochemical sensing,17,25,26 and diagnostics.27,28

Received: December 11, 2021
Accepted: April 6, 2022
Published: April 29, 2022
Bioactive micro- and nanomotors have also been applied to exploit their energy conversion to combat planktonic bacteria and biofilms. Indeed, the enhanced mixing and towing force of these moving colloids improves the efficiency of their interaction with bacteria, enabling more effective infection eradication than when using passive particles. Their navigation increases the probability of contact with the surrounding bacteria and also enhances their penetration capacity. However, the antibacterial efficacy of micro- and nanomotors has not yet been extensively reported in clinically relevant mouse models, an essential step to eventually translate these applications into the clinic.

In this work, we present bioactive micro- and nanomotors that deliver antimicrobial peptide (AMP) payloads to the infection site through the combination of active motion, catalytic conversion, and bactericidal capabilities. AMPs have emerged as promising antimicrobials whose amphipathic character enables their interaction with, and subsequent disruption of, bacterial membranes. Clinical translation of numerous antimicrobials, including AMPs, is currently restricted by their limited bioavailability, susceptibility to enzymatic degradation, and low penetrability toward the target infection. Thus, efficient delivery methods are required for these molecules to more readily reach their target area. Delivery vehicles such as unbound liposomes and nanoparticles have been used to encapsulate peptides for the treatment of microbial biofilms and cancer; however, these methods rely on passive interactions with the infected area and passive movement through the infection site, e.g., an infected wound.

Here, we used the enzyme urease (a biocompatible source of active motion) to deliver linear, and thus susceptible to proteolytic degradation, cationic AMPs (LL-37 and K7-Pol) on silica micro- and nanoparticles that actively navigate in liquid toward the infection site (Figure 1). LL-37 is an amphipathic α-helical and cationic peptide with a 37-amino acid long sequence (LLGDDFFRKSKEKIGKEFKRIVQRK-DHFLRNVPRTES). This natural peptide is widely known for its antimicrobial, wound healing, and immunomodulatory properties, making it a promising candidate for testing in animal models. K7-Pol (ILGTILKLLSKL-NH2) is a potent synthetic α-helical and cationic peptide with an amidated C-terminus. This naturally emerged as promising antimicrobials whose amphipathic catalytic conversion, and bactericidal capabilities. AMPs have emerged as promising antimicrobials whose amphipathic character enables their interaction with, and subsequent disruption of, bacterial membranes. Clinical translation of numerous antimicrobials, including AMPs, is currently restricted by their limited bioavailability, susceptibility to enzymatic degradation, and low penetrability toward the target infection. Thus, efficient delivery methods are required for these molecules to more readily reach their target area. Delivery vehicles such as unbound liposomes and nanoparticles have been used to encapsulate peptides for the treatment of microbial biofilms and cancer; however, these methods rely on passive interactions with the infected area and passive movement through the infection site, e.g., an infected wound.

Results and Discussion

Synthesis and Characterization of Bioactive Micromotors. Bioactive micromotors were fabricated as previously reported through a modified Stöber method (Figure S1).

Figure 1. Bioactive micro- and nanomotors coated with antimicrobial peptides for the autonomous treatment of infections. Schematic of the AMP-coating process of the urease micro- and nanomotors and their autonomous propulsion to target pathogenic infections both in vitro and in vivo. Briefly, AMP–urease motors will encounter and hydrolyze urea (yellow spheres) in solution. The hydrolysis reaction will propel the motors, and when exposed to bacterial membranes, the AMPs onto their surface will act as antimicrobials and lyse bacterial cells in controlled (e.g. in vitro assays) and complex (e.g., infected wound) biological environments.

(see Methods section for details). Briefly, commercial spherical beads made of polystyrene (PS) with a diameter of 2 μm were used to grow silicon dioxide (SiO2) on their surface through a combination of tetraethylorthosilicate (TEOS) and 3-aminopropyltriethoxysilane (APTES) as silica precursors. Later, N,N-dimethylformamide (DMF) was used to dissolve the PS core and obtain hollow silica microparticles (HSMPs). We characterized the shape, size, and morphology of the microparticles by scanning electron microscopy (SEM) (Figure 2a) and transmission electron microscopy (TEM) (inset Figure 2a), revealing an average microparticle diameter of 1.87 ± 0.01 μm (n = 91, mean ± standard error of the mean). We used TEM to measure the silica particle shell as 48 ± 1 nm (n = 120, mean ± standard error of the mean).

To obtain bioactive micromotors, urease was attached to silica with a glutaraldehyde (GA) linker (Figure 1) on the amino groups of the bare silica particle, which were confirmed by dynamic light scattering (DLS), revealing a zeta potential of 2.64 ± 0.40 mV. For motion experiments, urease micromotors were recorded at 25 FPS for 20–25 s. The videos were analyzed using a custom-designed Python-based software in order to extract the trajectories of the micromotors, the mean squared displacement (MSD), and the speed (see Methods section for details). The active motion of urease microparticles was studied as a function of urea (substrate of urease) in water. When the urea concentration increased, the micromotors showed higher MSD (Figure S2) and speed (Figure 2b), which increased in a similar fashion to the Michaelis–Menten saturation curve, as observed in previous publications of urease-powered motion. The self-propulsion capacity reached a plateau with a maximum speed of 3.3 ± 0.3 μm s⁻¹ when adding 500 mmol L⁻¹ urea. The increment in
After ensuring the attachment of peptides onto the urease micromotors, we tested their motion using 200 mmol L\(^{-1}\) of urea in water, as this constituted the lowest urea concentration needed for maximum speed (Figure 2b). For LL-37-modified micromotors, no significant differences were detected in the urease micromotors’ speed (3.1 ± 0.3 μm s\(^{-1}\)) when the AMP concentration used was below 62.5 μg mL\(^{-1}\) (Figure 2c). Nonetheless, higher doses of AMP (250 μg mL\(^{-1}\)) caused a significant boost in self-propulsion, increasing the speed by 38.7% and reaching 4.3 ± 0.5 μm s\(^{-1}\). This increase in directional motion was directly observable by looking at the tracking trajectories extracted from the videos (Video 2). In the case of K7-Pol-modified micromotors, self-propulsion was also observed when adding K7-Pol, which increased the speed by 32.2% for higher concentrations of peptide, as indicated by the trajectory tracking experiments (Video 3). In this case, the maximum speed reached was 4.1 ± 0.4 μm s\(^{-1}\) at 250 μg mL\(^{-1}\) of K7-Pol (Figure 2d).

While the underlying motion mechanism powered by urease has still not been resolved in the literature, it has recently been pointed out that the electric field generation by the release of ionic products could play a significant role.\(^ {34,60} \) In this regard, the fact that higher concentrations of peptide led to higher propulsion capabilities could be explained by an increase in the surface net charge of the particle, leading to higher conductivity and enhanced electric field generation.

**Synthesis and Characterization of Bioactive Nanomotors.** Mobil Composition of Matter No. 41 (MCM-41) mesoporous silica nanoparticles (MSNPs) were chosen as a base material for the bioactive nanomotors because of their biocompatibility and ease of surface modification,\(^ {61,62} \) as well as their extensive use for biomedical applications.\(^ {63} \) Urease nanomotors were fabricated as recently reported\(^ {36,64} \) using a modified Stöber method\(^ {65} \) (Figure S3) (see Methods section for details). The hydrolysis and condensation of TEOS were used to synthesize silica nanoparticles in aqueous media using triethanolamine (TEOA) as a basifier and hexadecyltrimethylammonium bromide (CTAB) as the structure-directing agent. Next, to obtain the mesoporous silica nanoparticles, the CTAB surfactant was removed by acidifying the solution in methanol. The generated MSNP were then treated with APTES to modify the silica surface with amine groups. The morphology of the nanoparticles was characterized by both SEM and TEM (Figure 3a), displaying an average diameter of 694.698 ± 0.003 nm (n = 63, average size ± standard error of the mean). A clear radial mesoporosity was observed when increasing the magnification of TEM micrographs (Figure S4). The amino groups on the surface of the bare MSNP were later used to attach the urease enzyme through the GA linker to yield urease nanomotors, which were first confirmed through DLS, revealing a zeta potential of 16.22 ± 3.94 mV.

For the motion experiments, we studied the electrophoretic mobility of urease nanomotors through DLS to obtain both their diffusion coefficient and apparent hydrodynamic radii (see Methods section for details). The active motion of urease nanomotors was studied as a function of urea concentration in phosphate-buffered saline (PBS), and for all conditions a sharp population peak was observed (Figure S5), confirming monodispersity of the sample. The diffusion of nanomotors was enhanced when increasing the urea concentration (Figure 3b), from a base Brownian diffusion of 0.54 ± 0.01 μm\(^2\) s\(^{-1}\) when no substrate was present to a maximum enhanced diffusion of 0.73 ± 0.03 μm\(^2\) s\(^{-1}\) at 200 mmol L\(^{-1}\) urea. This

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Figure 2. Effect of antimicrobial peptides on the active motion of bioactive micromotors. (a) SEM micrograph of the hollow silica microcapsules. Inset: TEM micrograph of the hollow silica microparticles. (b) Average speed of urease micromotors for different concentrations of urea. Inset: Representative 15 s trajectories for different concentrations of urea. (c) Average speed and zeta potential of urease micromotors for different concentrations of LL-37 peptide used to functionalize the silica surface. (d) Average speed and zeta potential of urease micromotors for different concentrations of K7-Pol peptide used to functionalize the silica surface. All results are shown as the mean ± standard error of the mean.

self-propulsion was clearly distinguished by tracking the trajectories of the individual urease micromotors (Figure 2b, inset, and Video 1).

**Preparation of AMP-Coated Bioactive Micromotors.** Once the motion behavior of urease micromotors was thoroughly studied and characterized, we incorporated human cathelicidin LL-37 and peptide K7-Pol onto the surface of the silica microparticles (Figure 1). In the last functionalization step to yield the peptide-modified bioactive micromotors, we added both urease and AMP to be anchored to the silica surface by the GA linker (Figure S1) (see Methods section for details). Different concentrations of each peptide were added (Figure 2c and d), indicating that the positively charged AMPs were properly coated onto the micro- and nanomotors. For concentrations of LL-37 higher than 125 μg mL\(^{-1}\), the microparticle zeta potential changed to positive, reaching a maximum of 35.1 ± 1.4 mV when adding 500 μg mL\(^{-1}\) of LL-37 (Figure 2c). In the case of K7-Pol, the zeta potential also increased with higher peptide loads, up to ~12.6 ± 0.2 when adding 500 μg mL\(^{-1}\) of K7-Pol, but never reached a net positive charge (Figure 2d). The presence of multiple positively charged amino acids in LL-37’s amphiphatic sequence (pI = 11.13) (i.e., five lysine (K) and five arginine (R) residues) explains the drastic change toward a positive zeta potential. In the case of the K7-Pol sequence (pI = 10.6), the presence of two lysine (K) residues and the amidated C-terminus also caused an increase in zeta potential but are insufficient to reach a net positive value.
decreased accordingly to match the di-
surface (see Methods section for details). A range of AMP
binding of urease and the AMP to the GA linker onto the silica
AMP to the solution containing nanoparticles, to enable
the fabrication process was modi-
Upon determining the motion behavior of urease nanomotors,

nanoparticles (Figure 1).

cathelicidin peptide LL-37 and the potent synthetic anti-
microbial peptide K7-Pol onto the surface of the silica
S5) (see Methods section for details).

minimum of 329.2 nm at 200 mmol L^{-1} of K7-Pol, which was saturated for higher
AMP concentrations. Hence, the enhanced diffusion decrease when
attaching AMPs is likely due to direct competition of the
peptides with urease for the available functional groups on the
silica surface, causing a decrease in both enzyme attachment
and active motion. However, for the purpose of fabricating
active antimicrobial tools, it is important to note that the
peptide–urease nanomotors still presented active motion for
all the different peptide concentrations tested.

In Vitro Antimicrobial Activity of Micro- and Nano-
motors against Pathogenic Bacteria. The AMP-modified
bioactive micro- and nanomotors were tested against bacteria
from the ESKAPE pathogen list declared by the World Health
Organization as critical threats for humans: Acinetobacter baumannii AB177, Escherichia coli ATCC11775, Klebsiella pneumoniae ATCC13883, Pseudomonas aeruginosa PAO1, and Staphylococcus aureus ATCC12600. Minimum inhibitory concentration (MIC) values were determined as the lowest concentration of our motors tested in the presence of 200 mmol L^{-1} urea that inhibited 100% of bacterial growth. The MIC values for each condition were assessed by determining the optical density of the solution at 600 nm and ranged from 7.8 to 125 μg mL^{-1} depending on the bacterial strain (Figure 3 and Table S1). Both micro- and nanomotors exhibited 4- to 16-fold enhanced antimicrobial activity when the AMPs were incorporated (Figure 3). Interestingly, AMP-modified motors displayed activity against K. pneumoniae and S. aureus, whereas neither the bare micro- nor the nanomotors presented activity toward S. aureus, and the bare nanomotors were inactive against K. pneumoniae.

Mechanism of Action Studies. Mechanism of action (MoA) studies were carried out to explore whether AMP-modified micro- and nanomotors killed bacterial cells by permeabilizing their outer membrane or, instead, acted by depolarizing their cytoplasmic membrane at their MIC. To assess if the AMP-modified bioactive motors were able to permeabilize the outer membrane of A. baumannii and K. pneumoniae cells, we used the fluorescent probe NPN [1-(N-
phenylamino)naphthalene]. NPN exhibits weak fluorescence emission in aqueous environments and can permeate the bacterial outer membrane when damaged. The probe interacts with the lipidic environment of permeable outer membranes and displays an increased intensity of fluorescence emission (Figure S6). As a positive control, we used the FDA-approved and widely used peptide antibiotic polymyxin B (PMB), a well-

known membrane permeabilizer. When we exposed A.
baumannii or K. pneumoniae cells to the AMP-modified micro- and nanomotors and PMB, only PMB was able to permeabilize the outer membrane (Figure S6).

To evaluate whether the AMP–urease micro- and nanomotors were able to depolarize the cytoplasmic membrane of A. baumannii and K. pneumoniae cells, we used the probe DiSC3-5 (3,3′-dipropylthiadicarbocyanine iodide). DiSC3-5, a potentiometric probe, works by accumulating in the cytoplasmic membrane and aggregating at high concentrations, causing fluorescence quenching. When the cytoplasmic membrane is depolarized, DiSC3-5 migrates to the cytoplasm, leading to increased fluorescence emission intensity (Figure S5a). The micro- and nanomotors functionalized with antimicrobial peptides, depolarize bacterial membranes more efficiently than defensins (e.g., LL-37). All experiments were performed in the presence of 200 mmol L\(^{-1}\) urea.

**Anti-infective Activity against A. baumannii in a Skin Abscess Animal Model.** All the combinations of micro- and nanomotors and peptides and their bare urease micro- and nanomotors counterparts were tested in vivo against A. baumannii at 2-fold their MIC, 15.6 and 31.2 \(\mu\)g mL\(^{-1}\), respectively (Figure 6a). Skin infection was induced by administering an A. baumannii solution at 10\(^7\) CFU mL\(^{-1}\) on the back of mice previously scratched with a needle. The treated groups were administered with a single dose of free AMPs and micro- or nanoparticles (with and without AMP) 2 h postinfection. The samples were added to one extremity of the infected wound, and 100 \(\mu\)L of a 200 mmol L\(^{-1}\) urea solution was spread over the entire length of the wound (Figure 6b). Four days after a single treatment dose, the tissue was homogenized and the CFU bacterial load was quantified, as this quantitative method accurately accounts for the number of bacterial cells in the infected area. The most active systems were LL-37–urease micromotors and K7-Pol–urease nanomotors, significantly reducing the bacterial load by 2 and 3 orders of magnitude, respectively (Figure 6c), to levels that can be cleared up by the immune response (<10\(^3\) CFU mL\(^{-1}\)).

On the contrary, treatment with AMPs free in solution (Figure 6b) that were administered in one of the extremities of the wound displayed anti-infective activity only locally where the peptides were administered (Figure 6d). More specifically, peptide treatment reduced CFU counts by 2–3 orders of magnitude in the administration site (~1/3 of the whole extent of the wound), whereas infected areas farther away from the administration site remained infected at levels similar to the untreated controls. Interestingly, for LL-37, which presents immunomodulatory and wound-healing properties, we also observed partial wound healing in the area where the peptides were added (Figure S7). To test the effect of the motion in the complex in vivo environment, we treated mice with all the systems in the absence of urea and compared them to an untreated control (Figure S8a). All peptide-loaded micro- and nanomotor systems were active against bacteria in the administration site (Figure S8b). Stand-alone treatment with the urease micromotors and urease nanomotors did not decrease bacterial load significantly (Figure S8b). Peptides free in solution and peptide-loaded micro- and nanomotors reduced CFU counts by 3 orders of magnitude. On the other hand, none of the systems tested in the absence of urea were able to significantly reduce CFU counts far from the administration site (Figure S8b). This contrasts with peptide-loaded micro- and nanomotors in the presence of urea, which led to anti-infective activity in vivo at a distance from the administration site (Figure S8). No side effects (e.g., itchiness, redness, swelling) or in vivo toxicity was observed under any of the conditions tested (Figure S8c). To assess whether the peptides free in solution and the AMP-modified bioactive micro- and nanomotors were toxic to the animals, the weight of the mice was monitored throughout the experiment. Variations of up to 20% in weight are a widely used proxy of distress, morbidity, and overall toxicity. No side effects (e.g., itchiness, redness, swelling) or in vivo toxicity was observed under any of the conditions tested (Figures 6e,f and S8c). The LL-37–urease nanomotors and K7-Pol–urease nanomotors. This was not entirely unexpected, since mastoparans, such as K7-Pol and all polybia-CP-derived peptides, depolarize bacterial membranes more efficiently than defensins (e.g., LL-37). All experiments were performed in the presence of 200 mmol L\(^{-1}\) urea.
micromotors and the motors without peptide presented bacteriostatic effects (Figure 4).

To shed light on the underlying mechanisms associated with the differences in antimicrobial activity observed between our in vitro and in vivo experiments, we investigated the speed of each of the motors. No significant differences were observed when comparing the speed of both AMP-modified micro-motors nor when comparing the diffusion coefficient of both AMP-modified urease nanomachines (Figure S9). Thus, we hypothesize that LL-37–urease micromotors and K7-Pol–urease nanomotors presented activity in the animal model because less peptide is needed to obtain more positively charged systems for those combinations. LL-37–urease micromotors presented zeta potential values of $-10.3 \pm 1.9$, while K7-Pol–urease micromotors showed $-36.6 \pm 0.1$ at the concentration used in the animal model ($15.6 \mu g \text{ mL}^{-1}$). On the other hand, K7-Pol–urease nanomotors presented zeta potential values of $1.5 \pm 0.2$, whereas LL-37–urease nanomotors exhibit a zeta potential value of $0.4 \pm 0.2$ at the concentration used in vivo ($31.2 \mu g \text{ mL}^{-1}$) (Figures 2 and 3). These values indicate that the initial electrostatic interactions between the negatively charged bacterial membranes and the AMP–urease motors are likely crucial to trigger depolarization and subsequent bacterial death.

CONCLUSION

We report the fabrication and detailed characterization of urease micro- and nanomotors that actively deliver peptides to combat clinically relevant infections. The motion dynamics of each particle was studied under different concentrations of urea, and active motion was confirmed upon functionalization with the antimicrobial peptides. The antimicrobial micro- and nanomotors displayed broad-spectrum bactericidal activity in vitro against both Gram-negative ($A. baumannii$, $E. coli$, $K. pneumoniae$, $P. aeruginosa$) and Gram-positive ($S. aureus$) bacterial pathogens. An in-depth mechanistic study confirmed...
infected area. On the other hand, peptides by themselves exhibited antimicrobial activity only within the area they were administered and did not clear the infection at a distance. Briefly, after the infection was established, urea was spread over the entire length of the wound (1 cm). Next, the micro- and nanomotors coated with peptides and the peptides alone were inoculated to one of the extremities of the infected wound. (c) Four days postinfection, 1 cm² of the infected area was excised and the ability of the micro- and nanomotors to travel throughout the wound alone and when functionalized with peptides was assessed. (d) Treatment with peptides alone decreased bacterial counts only in the extremity where they were administered (light yellow background), as revealed by similar bacterial counts detected in areas at a distance from the administration site (dark yellow background) and those of untreated control groups. (e) Mouse weight was monitored throughout the experiments, serving as a proxy to assess the toxicity of both micro- and nanomotors and (f) peptides in solution. None of the treatment groups led to toxicity in mice. Eight animals were used per group. This figure was created with BioRender.com.

**METHODS**

**Chemicals.** Two micrometer microparticles based on polystyrene (Sigma-Aldrich cat. no. 78452), ethanol 99% (PanReac AppliChem cat. no. 131086-1214), ammonium hydroxide solution 28–30% (Sigma-Aldrich cat. no. 221228), 3-aminopropyltriethoxysilane 99% (Sigma-Aldrich cat. no. 440140), triethanolamine 99% (Sigma-Aldrich cat. no. 90279), hexadecyltrimethylammonium bromide 99% (Sigma-Aldrich cat. no. 52365), methanol (MeOH) 99% (PanReac AppliChem cat. no. 361091-1611), hydrochloric acid (HCl) 37% (PanReac AppliChem cat. no. 211020-1611), tetraethylorthosilicate 99% (Sigma-Aldrich cat. no. 86578), N,N-dimethylformamide 99% (Acros Organics cat. no. 423640010), 1× phosphate-buffered saline (Thermo Fisher Scientific cat. no. 70011-036), glutaraldehyde (25 wt %) (Sigma-Aldrich cat. no. G6257), urease from *Canavalia ensiformis* (Jack bean) (Sigma-Aldrich cat. no. U4002), urea (Sigma-Aldrich cat. no. US128) were used.

**Instruments.** SEM images were captured by a FEI NOVA NanoSEM 230. TEM images were captured by a Zeiss EM 912. The zeta potential (ζ potential) measurements were performed with a Zetasizer Nano S from Malvern Panalytical. The hydrodynamic radius and diffusion coefficient measurements were performed using a Möbius from Wyatt Technology. The optical videos of urease micromotors were recorded using the camera (Hamamatsu digital camera C11440) of an inverted optical microscope (Leica DMi8). The optical density (OD) of the antibacterial assays was measured at 600 nm in a Thermo Scientific Varioskan LUX fluorescence spectrophotometer.
Antimicrobial Peptide Synthesis. The peptides LL-37 and K7-Pol were purchased from Aapptec (Kentucky, USA). They were purified by high-performance liquid chromatography (HPLC). The peptide purity used in all assays was higher than 95%.

Synthesis of Hollow Silica Microparticles. The HSMPs were fabricated by mixing 500 μL of PBS beads with a diameter of 2 μm (Sigma-Aldrich cat. no. 78452) with 1 mL of ethanol 99% (Panreac AppliChem cat. no. 131086-1214) and 0.8 mL of ultrapure water. Next, 50 μL of ammonium hydroxide 28–30% (Sigma-Aldrich cat. no. 221228) was added, and the solution was magnetically stirred for 5 min. Then, 5 μL of APTES 99% (Sigma-Aldrich cat. no. 440140) was added, and the reaction was stirred for 6 h. After, 15 μL of TEOs ≥99% (Sigma-Aldrich cat. no. 86578) was added, and the solution was allowed to react overnight (17 h) under continuous mixing. Next, the PS particles covered with silica were washed three times with ethanol (centrifugation of microparticles at 1503 rcf during 4 min). The PS was then dissolved from inside the silica shell with four washes of DMF ≥99.8% (Acros Organics cat. no. 423640010), with a 15 min mixing step in each wash. Afterward, three washes of ethanol 99% were performed to the HSMP solution, and the final samples were stored at room temperature.

Functionalization of HSMPs with Urease and AMPs. The HSMPs were washed three times with ultrapure water and one time with 1X PBS (pH = 7.4) (Thermo Fischer Scientific cat. no. 70011-036). Then, the particles were suspended in a 2.5 wt % GA solution (Sigma-Aldrich cat. no. G6257) in 1X PBS and kept mixing at room temperature for 3 h. Next, the silica microparticles with GA were washed three times with 1X PBS (pH = 7.4) and resuspended again in 1X PBS (pH = 7.4) with 3 mg mL\(^{-1}\) of urease powder from Canavalia ensiformis (Jack bean) (Sigma-Aldrich cat. no. U4002). When fabricating micromotors with peptides attached, different concentrations of AMP (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, and 0 μg mL\(^{-1}\)) were added simultaneously with urease. The solution was kept mixing overnight (16 h) and then washed three times with 1X PBS (pH = 7.4). Then, the urease micromotors in a 1X PBS (pH = 7.4) solution was divided in aliquots and stored at 4°C for further use.

Optical Video Recording of Bioactive Micromotor Motion. To study the active motion of urease micromotors, optical videos were recorded using a digital camera (Hamamatsu digital camera C11440) coupled with an inverted optical microscope (Leica DMi8). The 63× water immersion objective was used to record the micromotors placed on a glass slide, which were thoroughly mixed with the urea aqueous solutions (enzymatic substrate) at different concentrations (500, 200, 50, 10, 1, and 0 mmol L\(^{-1}\)), specifically selected for the urease Michaelis–Menten kinetics saturation, as reported in BRENDA, the Comprehensive Enzyme Information System (https://www.brenda-enzymes.org/). For the motion experiments as a function of different AMP concentrations (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, and 0 μg mL\(^{-1}\)), 200 mM urea was used to ensure optimal catalytic rate and active motion. The micromotors solution was put on a glass slide covered with a coverslip, and videos of 25 FPS and 20–25 s were recorded for 3 min. For each condition studied of urea and AMP concentration, 10–14 individual urease micromotors were recorded.

Data Analysis of Bioactive Micromotor Motion. The videos were analyzed using custom-designed tracking Python software to obtain the tracking trajectories of the microparticle displacement. From the \(x\) and \(y\) axes values over time, the MSD was calculated using the following equation:

\[
\text{MSD}(\Delta t) = \left( \sum_i (r_i(t + \Delta t) - r_i(t))^2 \right)
\]

where \(t\) is the time, \(r_i(t)\) is the position of the particle in the coordinate \(i\) at time \(t\), \(n = 2\) are the dimensions of 2D analysis, and \(\sum\) denotes the ensemble and time average. The velocity \(v\) was then extracted from fitting the MSD to

\[
\text{MSD}(\Delta t) = 4Dt + v^2 t^2
\]

where \(D\) is the diffusion coefficient and \(v\) is the speed, since we analyze the propulsive regime when \(t < \tau\) with \(\tau\) being the rotational diffusion time and \(t\) the time the MSD represented.\(^{57,58}\) The results are presented as mean ± standard error of the mean.

Synthesis of Mesoporous silica nanoparticles. The MSNPs were synthesized through the sol–gel methodology.\(^{59}\) Briefly, 570 μg of CTAB (Sigma-Aldrich cat. no. 53265) and 35 g of TEOA (Sigma-Aldrich cat. no. 90279) were dissolved in 20 mL of ultrapure water and heated at 95 °C in a silicon oil bath. This solution was stirred for 30 min, after which 1.5 mL of TEOs (Sigma-Aldrich cat. no. 86578) was added dropwise. The solution was then stirred at 95 °C for 2 h stirring in a silicon oil bath. Then, the resulting particles were collected by centrifugation and washed three times with ethanol (centrifugation of nanoparticles at 1503 rcf for 5 min). To remove the CTAB from the MSNP pores, the particles were suspended in a solution composed by 30 mL of methanol (PanReac AppliChem cat. no. 361091-1611) and 1.8 mL of HCl (PanReac AppliChem cat. no. 211020-1611) and left in the reflux at 80 °C for 24 h. After collecting the particles by centrifugation, three washes of ethanol were applied with 10 min of sonication for each wash. Finally, three aliquots of 0.5 mL were taken from the resulting solution to centrifuge them and determine the concentration by measuring the weight before and after air-drying the solvent.

Amino Modification of the MSNP Surface. The silica surface of the MSNPs was modified with APTES to incorporate amino groups, by adjusting an already reported method.\(^{60}\) First, a solution of 2 mg mL\(^{-1}\) of MSNP was prepared in a round-bottom flask under magnetic stirring and with a stable temperature of 50 °C. Next, APTES was added to a stirring solution to obtain a final concentration of 5 mM, and it was left under reflux at 50 °C for 24 h. After this process, the resulting MSNPs-NH\(_2\) were washed three times with ultrapure water, and three aliquots of 0.5 mL were taken to determine the concentration by measuring the weight before and after air-drying the solvent.

Functionalization of MSNP-NH\(_2\) with Urease and AMPs. The resulting MSNP-NH\(_2\) were diluted in ultrapure water to obtain a 1 mL solution of 1 mg mL\(^{-1}\) of nanoparticles, then centrifuged, resuspended in 900 μL of PBS (pH = 7.4), and sonicated for 10 min. After this, 100 μL of GA at 25 wt % was added and left to mix for 2.5 h at room temperature. The MSNP-NH\(_2\) were then washed three times with PBS, applying a 10 min sonication for each wash. Next, the silica nanoparticles functionalized with GA were washed three times with 1X PBS (pH = 7.4) and resuspended again in 1X PBS (pH = 7.4) with 3 mg mL\(^{-1}\) of urease powder from Canavalia ensiformis (Jack bean). When fabricating silica nanomotors with attached peptides, different concentrations of AMP (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95, and 0 μg mL\(^{-1}\)) were added concomitantly with urease. The solution was kept mixing overnight (16 h) and then washed three times with 1X PBS (pH = 7.4). Afterward, the solution of urease nanomotors in 1X PBS (pH = 7.4) was divided in aliquots and stored at 4 °C to be used for further experiments.
Analysis of Bioactive Nanomotor Motion. The Möbius from Wyatt Technology was used to analyze the electrophoretic mobility through DLS and extract both the apparent hydrodynamic radius and the diffusion coefficient of the urease nanomotors. Active motion of urease nanomotors was studied as a function of different concentrations of urea (500, 200, 50, 10, 1, and 0 mmol L⁻¹) and AMPs (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95, and 0 μg mL⁻¹). The hydrodynamic radius is correlated with the diffusion coefficient according to the following Einstein–Stokes equation:

$$D = \frac{k_B T}{6\pi\eta R}$$  

where $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, $\eta$ is the solvent viscosity, and $R$ is the hydrodynamic radius of the diffusing particle. The electrophoretic mobility was studied using an acquisition time of 5 s, with a laser of 532 nm wavelength and a detector angle of 163.5°. For each condition, the diffusion coefficient was calculated for the average of 17–26 acquisitions obtained directly from the analysis of the scattering data on the Dynamics software. All the results are presented as mean ± standard error of the mean.

Zeta Potential Analysis of Bioactive Micro- and Nanomotors. The Zetasizer Nano S from Malvern Panalytical was used to study the electrophoretic mobility through DLS and obtain the zeta potential of the micro- and nanomotors. The zeta potential of AMP-modified urease micromotors was analyzed as a function of peptide concentration (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, and 0 μg mL⁻¹), and the zeta potential of AMP-modified urease nanomotors as a function of peptide concentration (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95, and 0 μg mL⁻¹). For each condition, the resulting value was the mean of three measurements (which in its turn were an average of a minimum of 10 acquisitions), detected using a scattering angle of 173° and calculated using the Henry equation. The results are presented as mean ± standard error of the mean. The isoelectric points (pI) of the AMP were calculated through the Web service Isoelectric Point Calculator (http://isoelectric.org/).

Bacterial Strains and Media. In this study, we used the following pathogenic strains: Escherichia coli ATCC11775, Acinetobacter baumannii AB177, Pseudomonas aeruginosa PA01, Staphylococcus aureus ATCC12600, and Klebsiella pneumoniae ATCC13883. All bacterial strains were grown and plated on Luria–Bertani (LB) plates, except for P. aeruginosa, which was grown in Pseudomonas Isolation agar plates. All the agar plates were incubated overnight at 37 °C. After the incubation period, we transferred one colony to 5 mL of LB broth and incubated it overnight at 37 °C (240 rpm). On the next day, we prepared inocula by diluting the bacterial overnight solutions 1:100 in 5 mL of LB broth and incubating at 37 °C until logarithmic phase (OD₆ₐ₀ = 0.3–0.5) was reached.

Antibacterial Assays. MICs of micro- and nanomotors were determined using the broth microdilution technique in LB with an initial inoculum of 5 × 10⁶ cells mL⁻¹ in untreated polystyrene microtiter plates (Corning, USA). The detailed methodology is described by Cesaro et al. Briefly, capsules were added to the plate as solutions in LB broth in concentrations ranging from 0 to 1000 μg mL⁻¹. The MIC value was considered as the lowest concentration of the antimicrobial system that inhibited the visible growth of bacteria. After 24 h of incubation at 37 °C, the plates were read in a spectrophotometer at 600 nm. All assays were done in three independent replicates.

Membrane Depolarization Assays. The cytoplasmic membrane depolarization activity of the AMP–urease motors was determined by measurements of fluorescence of the membrane-potential-sensitive dye DiSC3(5). Briefly, A. baumannii AB177 and K. pneumoniae ATCC13883 were grown at 37 °C with agitation until they reached mid log phase (OD₆₀₀ = 0.5). The cells were then centrifuged and washed twice with HEPES buffer (5 mmol L⁻¹) with 20 mM L⁻¹ glucose at pH 7.2 and diluted 1:10 in the same buffer with KC1 (0.1 mmol L⁻¹). The cells (100 μL) were then incubated for 15 min with 20 mmol L⁻¹ of DiSC3(5) until fluorescence emission values were stable, indicating the incorporation of the dye into the bacterial cytoplasmic membrane. Membrane depolarization was tracked over 60 min by the change in the fluorescence emission intensity of DiSC3(5) ($λ_{em} = 622$ nm, $λ_{ex} = 670$ nm), after the addition of the micro- and nanomotors (100 μL solution at MIC values).

Membrane Permeabilization Assay. The membrane permeability of the AMP–urease motors was determined by using the NPN uptake assay. A. baumannii AB177 and K. pneumoniae ATCC13883 were grown to an OD₆₀₀ of 0.4, centrifuged (10 000 rpm at 4 °C for 10 min), and washed and resuspended in HEPES (5 mmol L⁻¹) buffer with 5 mmol L⁻¹ glucose at pH 7.4. NPN solution (4 μL at 0.5 mmol L⁻¹) was added to the bacterial solution (100 μL) in a white 96-well plate. The background fluorescence was recorded at $λ_{em} = 350$ nm and $λ_{ex} = 420$ nm. Micro- and nanomotors functionalized with urease and AMPs in water (100 μL solution at their MIC values) were added to the 96-well plate, and fluorescence was recorded for 45 min.

Skin Abscess Infection Mouse Model. A. baumannii AB177 cells were grown in tryptic soy broth (TSB) medium. Subsequently, cells were washed twice with sterile PBS (pH 7.4, 13 000 rpm for 1 min) and resuspended to a final concentration of $10^8$ CFU/20 μL. Female 6-week-old CD-1 mice, anesthetized with isoflurane, had the fur on their backs removed followed by a 1-cm-long superficial linear skin abrasion with a needle. The wound was made to damage only the stratum corneum and upper layer of the epidermis. A single aliquot of 20 μL containing the bacterial load was inoculated over the wound area. Four hours after the infection, free peptides and micro- and nanomotors functionalized with urease and AMPs at their MIC value were administered to the infected area in the presence (100 μL of a 200 mmol L⁻¹ solution) and absence of urea. Animals were euthanized, and the wound area was excised 4 days postinfection. The tissue was then homogenized for 20 min (25 Hz), and the resulting solution with suspended bacteria in PBS was 10-fold serially diluted for CFU quantification. Mannitol salt agar plates were used for easy differentiation of A. baumannii cells present in the homogenized tissue. Two independent experiments were performed with 8 mice per group in each condition.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsnano.1c11013.

Schematic representation of the synthesis of AMP-coated micro- and nanomotors; mean square displacement of urease micromotors; TEM micrographs of the silica nanoparticles; hydrodynamic radius of urease nanomotors in the presence of different urea concentrations; membrane permeabilization experiments with urease micro- and nanomotors functionalized with peptides; images of the infection site on the back of mice used for the animal model; comparison of the active motion of micro- and nanomotors with different antimicrobial peptide coating (PDF)

Video 1: Tracking trajectory of urease micromotors for different urea concentrations (0, 1, 10, 50, 200, 500 mM) over 20 seconds (AVI)

Video 2: Tracking trajectory of urease micromotors functionalized with different LL-37 peptide concentrations (0, 7.81, 31.25, 125, 500 μg mL⁻¹) with 200 mM urea and the control without urea over 20 seconds (AVI)

Video 3: Tracking trajectory of urease micromotors functionalized with different K7-Pol peptide concentrations (0, 7.81, 31.25, 125, 500 μg mL⁻¹) with 200 mM urea and the control without urea over 20 seconds (AVI)
Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
This work was supported by the Spanish MINECO (project RTI2018-095622-B-I00), the Catalan AGAUR (project 2017 SGR 238), and the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (grant agreement no. 866348: iNanoSwarms). It was also funded by the grant RTI2018-098164-B-I00 from the MICIN/AEI/10.13039/501100011033 and the “FEDER Una manera de hacer Europa” (BOTSinFluids project), the CERCA program by the Generalitat de Catalunya, and the “Centro de Excelencia Severo Ochoa”, funded by Agencia Estatal de Investigación (CEX2018-000789-S). X.A. thanks the Spanish MINECO for the Severo Ochoa program (SEV-2014-0425) for the Ph.D. fellowship (PRE2018-083712). C.F.-N. holds a Presidential Professorship at the University of Pennsylvania, is a recipient of the Langer Prize by the AIChE Foundation, and acknowledges funding from the Institute for Diabetes, Obesity, and Metabolism, the Penn Mental Health AIDS Research Center at the University of Pennsylvania, the Nemirovsky Prize, the Dean’s Innovation Fund from the Perelman School of Medicine at the University of Pennsylvania, the National Institute of General Medical Sciences of the National Institutes of Health under award number R35GM138201, and the Defense Threat Reduction Agency (DTRA; HDTRA11810041 and HDTRA1-21-1-0014). A.B. acknowledges funding from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2016/10585-4 and 2019/15871-3). The authors acknowledge the support of Ana Hortelão with the mesoporous silica particle synthesis and the TEM/SEM investigations. Some of the figures were created with BioRender.com.

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