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Membrane-Mimetic Dendrimersomes Engulf Living Bacteria via Endocytosis

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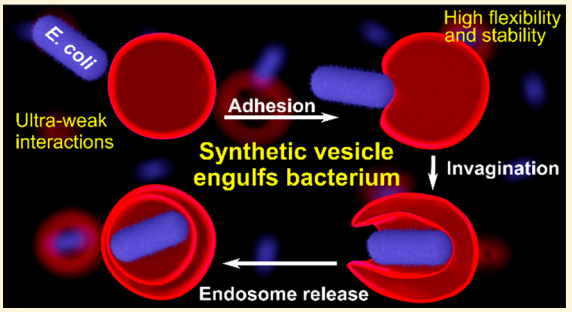
Supporting Information

ABSTRACT: There is much interest in developing vesicular microcompartments from natural and synthetic amphiphiles, enabling programmable interactions with living matter. Of particular interest is the development of vesicles capable of endocytosis of living bacteria. Despite the complexity of this process, theoretical studies predict that the endocytosis of prolate micro-objects is possible without the need of active cell machinery if the energy released upon bacterial adhesion to the membrane surpasses the energy required to bend the membrane. Nonetheless, natural liposomes and synthetic polymersomes fail to sufficiently recapitulate membrane properties to perform this advanced function. Here we report the engulfment of living bacteria into endosomes by cell-like dendrimersomes assembled from Janus dendrimers. Full engulfment occurred in less than a minute after contact. The process is driven by the adhesion of the bacterium to the dendrimersome's membrane by ultraweak interactions, comparable to those utilized by nature. The key to success relies on the combination of high flexibility and stability of the dendrimersomes. The key properties of the dendrimersomes are programmed into the molecular structures of their building blocks. The ability to support endocytosis highlights opportunities for the design and programming of dendrimersomes in biomedical research.



KEYWORDS: *Synthetic vesicles, artificial endocytosis of bacteria, Janus dendrimer vesicles, synthetic cells, cell membrane mimic*

Bacterial and viral invasion into host cells depends critically on their engulfment by the plasma membrane. In functioning living organisms, this process, termed endocytosis, is usually active, i.e., involving components of the cell machinery.[1−3] Typically, endocytosis begins with the binding of specialized receptors on the cell membrane to cognate ligands on the target bacteria or virus.[1−4] In the case of bacteria, receptor−ligand engagement triggers a series of ATP- and GTP-driven processes that ultimately curve the membrane culminating in the engulfment into an endosome that is released to the cytoplasm.[1,2,5−7] Theoretical work over the past two decades suggested that purely passive endocytosis may occur relying solely on weak interactions between nanoscale objects and the enveloping membrane.[7−17] In a liposome model, the bacteria−lipid interaction was sufficient to drive the wrapping of the membrane around a bacterium but not enough to accomplish fission and release of the endosome.[18] In this work, we undertake the challenge of building a synthetic vesicle capable of a rudimentary form of endocytosis of bacteria to address the question: what are the minimal requirements necessary for endocytosis of a living bacterium?

A simple model of endocytosis considers the adhesion of a particle to the flexible membrane of a unilamellar vesicle (Figure 1).[14] Much of the theoretical research has focused on understanding the physics of nanoparticle wrapping by a homogeneous membrane.[7,8,10−17,19,20] Passive endocytosis can occur spontaneously if it results in lowering the free energy of the system. If the energy gained upon adhesion ($\Delta G^{adhesion}$) of

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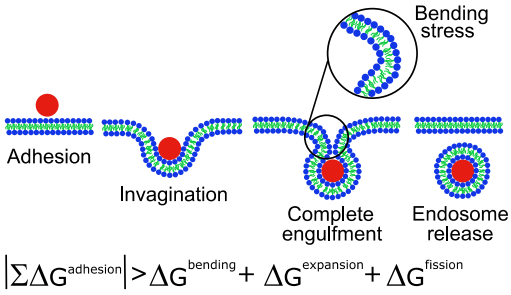

Figure 1. Interaction of a rigid particle with the flexible membrane of a giant unilamellar vesicle and the steps leading to endocytosis of the particle. The inequality below the figure gives the thermodynamic conditions necessary for endocytosis.

the particle to the membrane surpasses the bending ($\Delta G^{bending}$) and areal expansion ($\Delta G^{expansion}$) energies, the membrane invaginates the particle (Figure 1).[7−9,12−16,20] This leads to complete engulfment and the formation of a closed neck. The stability of the neck depends on the spontaneous curvature, flexibility of the membrane, $\Delta G^{adhesion}$, particle size, and constriction forces.[21] Full membrane crossing of the particle requires fission ($\Delta G^{fission}$) at the neck, leading to endosome release (Figure 1).[15] Experimentally, the interaction of nanoparticles with synthetic cell membrane models has been extensively explored using unilamellar vesicles.[19,22−24] Liposomes allow the internalization of nanoparticles into the endosomes.[19,22,24] Nonetheless, they lack long-term stability at environmental conditions severely limiting their use for advanced functions.[25,26] Polymersomes from amphiphilic block copolymers have sufficient stability, but the thickness of their membrane is three to several times higher than of natural cells.[27−31] The increase in thickness is accompanied by an increase in bending rigidity (usually more than 10 times higher than in liposomes).[28,29] Engulfment with such rigid membranes requires much higher adhesion energy.[23,24] Such strong forces will rupture the bacterial membrane before engulfment.[32] Importantly, nature utilizes adhesion energies as low as 8−80 k_BT coupled with lateral and perpendicular fluctuation (aiding cooperativity in binding), which demands membranes that are extremely flexible yet stable.[7,8,13,33] These seemingly antagonistic properties cannot be achieved simultaneously with either liposomes or polymersomes.[34] Recently,

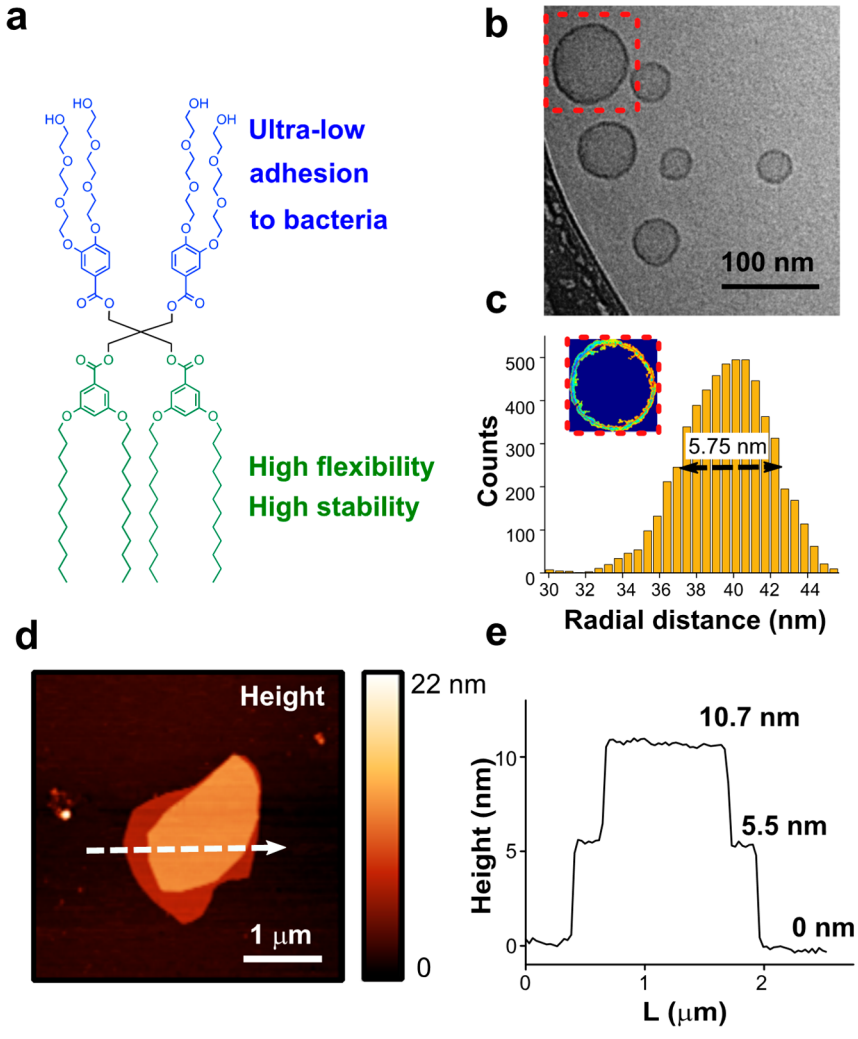

Figure 2. Assembly of JD into DSs. (a) The chemical structure of JD. (b) Cryo-TEM of JD assembled into DSs. (c) Distance distribution of pixels within the membrane of individual DSs from cryo-TEM in b. The thickness of the membrane of the DSs was calculated by statistically analyzing the distribution of radii on multiple DSs (Supporting Information). (d) AFM height image of a broken DS on mica. (e) Height profile from the section indicated by the arrow in d.

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amphiphilic Janus dendrimers (JDs), and their sugar-presenting analogues, Janus glycodendrimers (JGDs), have been reported to self-assemble in water into cell membrane-mimics, named dendrimersomes (DSs) and glycodendrimersomes (GDSs).[34−37] They display superior mechanical stability compared to liposomes with energies at break closer to polymersomes, while maintaining the flexibility (bending rigidity; κ_b = 30−180 k_BT) close to that of natural cell membranes.[34]

In this work, JDs were designed and synthesized to self-assemble into unilamellar vesicles with a biomimetic thickness, high flexibility, and low adhesion to bacteria (Figure 2a, Scheme S1).[34] Hydrophilic and hydrophobic dendrons were linked in an accelerated iterative modular approach in a symmetric way to the a symmetric pentaerythritol core to enable the cylindrical shape of the amphiphile (vesicle forming).[25] The hydrophilic tri(ethylene oxide) was selected to ensure ultraweak adhesion to bacteria.[38,39] The hydrophobic dendrons were synthesized via direct etherification of 3,5-dihydroxybenzoate with 1-bromododecane (Scheme S1). The 3,5-substitution pattern allows for interdigitation of hydrophobic chains, which increases stability, while the dodecane hydrophobic chains provided biomimetic thickness (t = 5.5 nm; Figure 2b−e, Figure S2) as evidenced by cryogenic transmission electron microscopy (cryo-TEM) of a vesicle dispersion and atomic force microscopy (AFM) of vesicles deposited on mica.[37,40] Besides the low phase transition temperature of dodecane chains ensures soft undulating membranes as observed in confocal laser scanning microscopy (CLSM, Videos S2−4). Addition of 60 pmol of α-hemolysin, a pore-forming peptide, to a DS dispersion resulted in the formation of functional pores (Figure S3). The ability of the DSs to accommodate the insertion of α-hemolysin and its self-assembly into channels demonstrates the biomimetic thickness (no hydrophobic mismatch) and lateral mobility of DSs membranes, while having sufficient stability.

Engulfment of Nanoparticles Driven by Ion-Dipole Interactions. The engulfment of fluorescently labeled silica nanoparticles (SNs, average size = 50 nm) was studied as a preliminary step toward the engulfment of living bacteria led by ultraweak interactions. Here the adhesion energy is mostly based on ion-dipole interactions between the surface of SNs and the tri(ethyelene oxide) periphery of the DSs. Such interactions are stronger than those with bacteria. However, they are still weaker than those of previous reports of cationic polymersomes and liposomes with SNs, where ionic interactions were exploited to drive engulfment.[23,24] SNs were added to a DSs suspension, and their interaction was visualized in real time using CLSM and cryo-TEM. After a few minutes, the vast majority of SNs were observed inside the DSs in large numbers (Figure 3a,b). The 3D reconstruction of confocal z-scans of the bottom half of a DS shows green and red fluorescence in its lumen. The green and red fluorescent stem from the SNs and the Nile-red-labeled DS's membrane forming the endosomes (Figure 3b). Cryo-TEM images revealed that the mechanism of SNs trafficking through the DSs' membrane followed the proposed model: invagination of adhered particle (Figure S4a), complete engulfment with a closed membrane neck (Figure S4b (vesicle on the left)) and endosome release (Figure S4b (vesicle on the right) and Figure S4).

The efficiency and dynamics of the engulfment process were accessed by dynamic light scattering (DLS). A pure dispersion

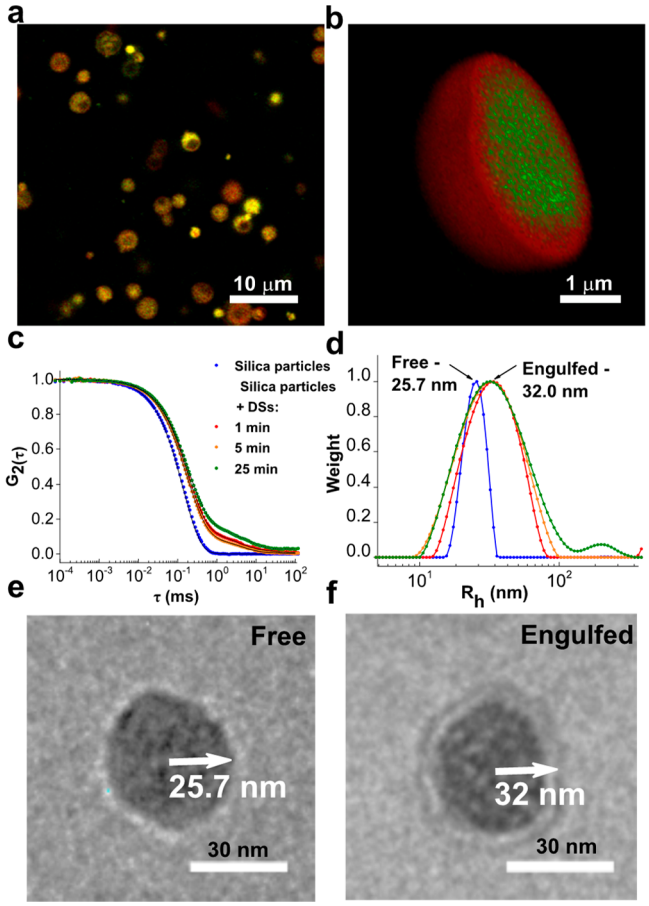

Figure 3. Engulfment of SNs by DSs. (a, b) CLSM image of DSs (red) and SNs (green) observed 5 min after mixing. (b) 3D reconstruction of 80 confocal scans of DS half with engulfed SNs. (c) Normalized DLS-ALV correlation functions and (d) size distribution of silica particles (blue), the mixture of SNs with DSs measured 1 min (red), after 5 min (orange) and 25 min (green). (e, f) cryo-TEM micrographs shows (e) free SN (R_h = 25.7 nm) and (f) engulfed SN into an endosome (R_h = 32.0 nm). Scale bar in parts e and f is 30 nm.

of SNs in water showed a monoexponential relaxation corresponding to the simple diffusion of particles with an average radius of 25.7 nm (Figure 3c,d (blue line)). Immediately after addition of DSs to the dispersion of SNs, the correlation functions showed a slower relaxation, indicating the formation of bigger objects. This was accompanied by an increase in the hydrodynamic radius of 6.3 nm even after only 1 min. This corresponds with the tight wrapping of a DS bilayer around the SN. In the same vein, cryo-TEM demonstrated that all of the internalized SNs were tightly surrounded by a DS' bilayer (Figure 3e,f and Figure S4), suggesting rather strong interactions of the DSs with the silica surface as predicted. These results prove that SNs are internalized into DSs by an endocytic process and not by passive diffusion across the membrane. This is in agreement with previous observations in liposomes and polymersomes.[22,24,41] Moreover, the autocorrelation functions increasingly deviate from the monoexponential relaxation, and a second relaxation mode is observed. This second relaxation mode is associated with a restriction in the diffusion of SNs after being engulfed into DSs. This results in the appearance of a second population in the size distribution function after 25 min of incubation that corresponds to larger objects

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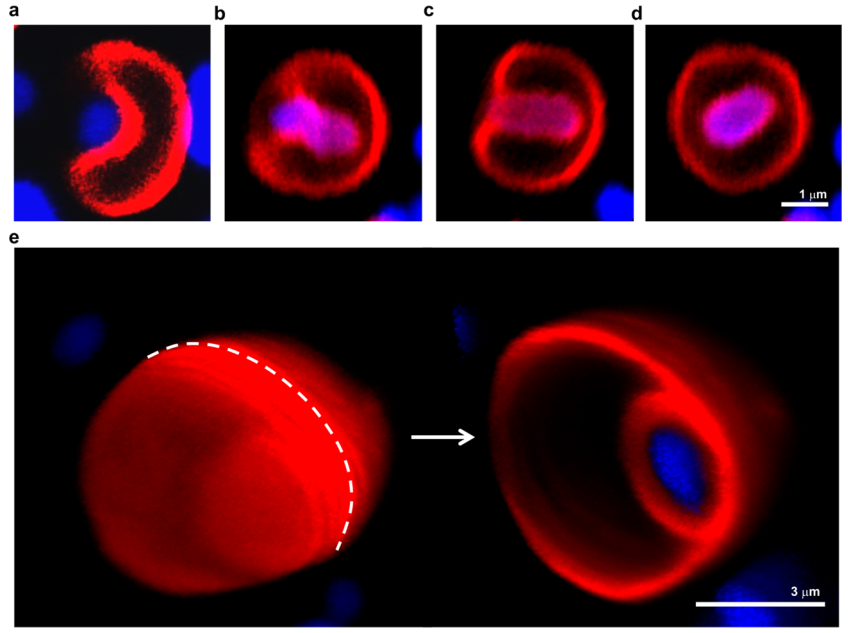

Figure 4. CLSM images show the process of engulfment of bacteria (blue) by DSs (red). (a) Adhesion of *E. coli* to the DS membrane. (b, c) Invagination of *E. coli* into the interior of the DS. (d) Formed endosome with living bacteria inside. (e) 3D reconstruction of 150 confocal scans for whole (left) and 80 confocal scans for half (right) of the DS with engulfed *E. coli*. The white dashed line on a whole DS indicates the place of intersection for the presentation of half of the DS to show the interior of an endosome with engulfed bacteria.

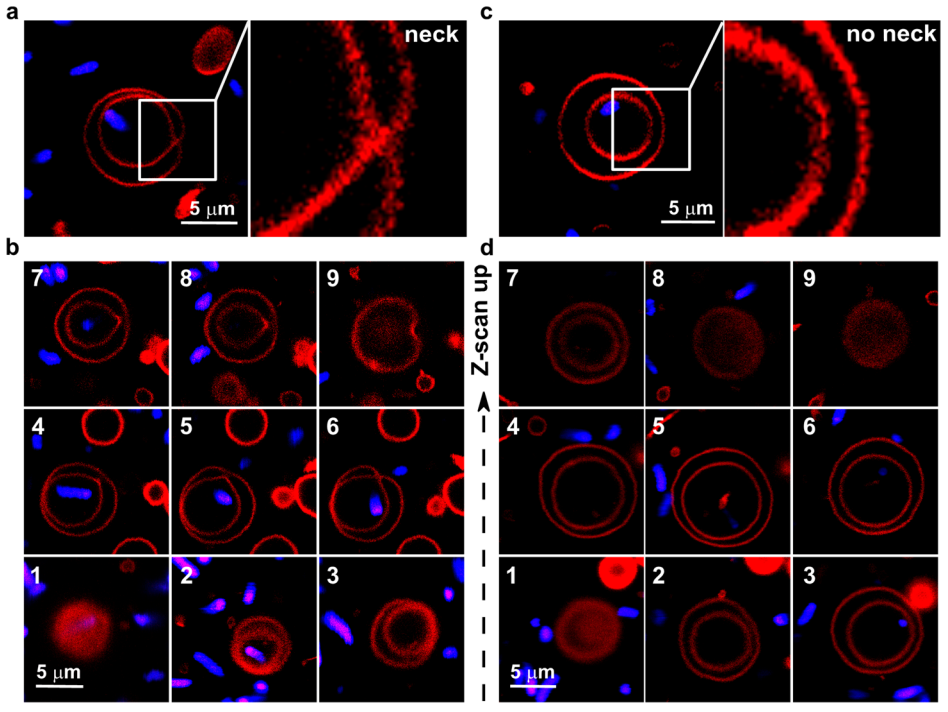

Figure 5. CLSM images of an engulfed bacterium into an endosome before (a and b) and after (c and d) fission of the neck. (a) Detailed observation of the neck connecting the endosome and the DS. (b) *Z*-axis scans from bottom to top (1→9) of the DS showing an engulfed bacterium with the presence of a closed neck. After 60−90 s the neck connecting the endosome to the membrane could no longer be observed (c). (d) *Z*-axis scans from bottom to top (1→9) of a DS that proves the fission of the neck.

(~200 nm) (Figure 3d (green line)). Presumably, the large number of engulfed SNs (CSLM, Figure 3b) causes their collective motion as if they were associated.

Endocytosis of Bacteria by Cell-Mimetic DSs. The engulfment of SNs proves the mechanism of particle trafficking, but is it possible to reproduce such mechanism with living animated bacteria relying on even weaker interactions; also, will an engulfed bacterium remain trapped into the endosome? In nature, various strains of bacteria bind to glycosphingolipids in the plasma membrane of macrophages to induce actin polymerization, which is responsible for bending of the membrane, leading to engulfment.[42,43] Experiments of liposomes containing the same glycosphingolipid and cholesterol interacting with *Pseudomonas aeruginosa*

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showed that the bacterial lectin-glycosphingolipid-mediated binding was only sufficient to induce membrane invagination, suggesting that actin polymerization was indispensable to surpass the bending rigidity for the full engulfment into an endosome.[18] However, studies by molecular dynamic simulations and theoretical models based on Helfrich energy, adhesion energy, and entropy indicated that engulfment of a prolate particle (a crude model for a bacterium) was a thermodynamically favored process and thus could be carried out without the active endocytic machinery.[8,13,15] The interaction of DSs with *E. coli* (BL21-Gold (DE3), expressing green fluorescent protein) was observed in buffer in real time using CLSM. The selected strain lacks pili and flagella (Figure S5),[44] where most macrophage-binding receptors are present. Thus, it represents a challenging model where only surface interactions between the outer membrane of the bacterium and the DS periphery are present. If the adhesion energy surpasses the bending energy, the engulfment of *E. coli* is thermodynamically favored. This occurs when the size of the bacterium is larger than a critical radius defined as[7,12]

$$R_w = \sqrt{\frac{2\kappa_b}{\Delta G^{adhesion}}} \quad (1)$$

where κ_b is the bending rigidity of DS (7.3×10^{-19} J)[34] and the $\Delta G^{adhesion}$ is the Gibbs free energy of *E. coli* binding to PEO surfaces estimated from the force distance curves measured by AFM (2.0×10^{-5} J m^{-2}), resulting in R_w = 270 nm.[45] The minimal dimension of the chosen *E. coli* is larger than this critical value. Thus, the engulfment is thermodynamically favored. It was found that about 10% of all of the added DSs contained one or more bacteria (Figure S6). CLSM confirmed the predicted mechanism of engulfment (Figure 4a–e and Video S1).

Adhesion of the bacterium to DS (Figure 4a and Figure S6) resulted in the formation of an invagination (Figure 4b) and complete engulfment with a closed neck (Figure 4c). 3D reconstructions of confocal planes showed the complete release of an endosome (Figure 4e and Figure S7). The release of the endosome was mediated by the neck fission as shown in Figure 5 and Figure S8. After fission, the local negative curvature associated with the neck relaxed to the spontaneous curvature of the vesicle (Figures 4e and 5c,d). Furthermore, we introduced a simple geometric model to analyze the effects of the neck in the minimal distance between the membranes of a DS and its endosome after engulfment of a bacterium. In this model, the shape of the membranes was fitted and the minimal distance between them was calculated from image analysis of the confocal planes (Supporting Information). The distance between the DS and endosome was only 80 nm when the neck was still present (Figure S8). Such distance is below the positional resolution; thus, the two membranes cannot be well resolved. Conversely, this average minimal separation increased to 450 nm after fission of the neck and the release of the endosome (Figure S8).

The low efficiency in *E. coli* engulfment can be ascribed to the extremely low adhesion of this bacterium to oligo(ethylene oxide) interfaces.[38,39] This is further evidenced by the loose nature of the endosome membrane around bacteria (Figure 6). Engulfment with such low adhesion energies can only be possible due to the extremely high flexibility of the DS membrane and has so far not been achieved with other synthetic vesicles. Engulfed bacteria were monitored individually for a period of 2 h. During the observation period, the living and rapidly moving bacterium could not escape from the endosome (Figure 6, Videos S2–4).

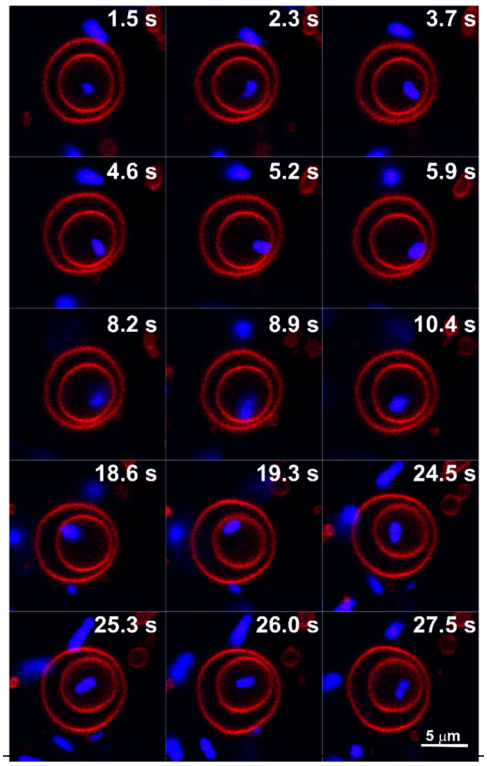

Figure 6. CLSM images show the stability of an endosome containing a rapidly moving bacterium during a 28 s observation time.

Conclusions. In summary, this work demonstrates endocytosis of *E. coli* by monocomponent synthetic vesicles. The mechanism of engulfment included the following steps: adhesion, invagination, complete engulfment with a closed neck, and release of an endosome. No active machinery was necessary to fulfill the engulfment as previously predicted by theory and molecular simulation. The process was solely mediated by the extremely low adhesion energy, which surpassed the bending energy opposing engulfment. This was possible due to the unique flexibility and stability of the DSs compared to polymersomes. Thus, this work lays the foundation for the development of protocells capable of complex and preprogrammed interactions with living matter.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.9b02349.

Materials and methods, details of DSs characterization by cryo-TEM, ion permeability of DSs, cryo-TEM micrographs showing engulfment of SNs, characterization of *E. coli* BL-21 by scanning electron microscopy, CLSM images showing DSs with attached or engulfed bacteria and the corresponding image analysis (PDF)

Video S1 (AVI)

Video S2 (AVI)

Video S3 (AVI)

Video S4 (AVI)

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Notes

The authors declare no competing financial interest.

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