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## Summary

Over the course of the past five years we have studied evaporation driven energy conversion in bacterial spores. This work was divided into three thrusts: (1) to understand the nanoscale processes that govern energy conversion in spores and the limits of force and power generation, (2) develop proof of concept devices that demonstrate technical scalability of the energy conversion process to large open water surfaces where evaporation takes places, and (3) understand the limits imposed to the energy conversion process by the atmospheric conditions in the vicinity of spore-based energy converting materials.

## Investigation of energy conversion by bacterial spores from changes in relative humidity

Spores respond to changes in relative humidity by absorbing and releasing water vapor. The expansion and contraction accompanying this process allows the spore function like muscle that can be controlled with humidity. Prior to this project, we predicted that the spores can be highly effective muscles, in that they can potentially generate a significant amount of mechanical work relative to their size or volume. At the initial phase of this project, we probed the limits of this energy conversion process by bacterial spores. Specifically, we measured the amount of mechanical work that can be

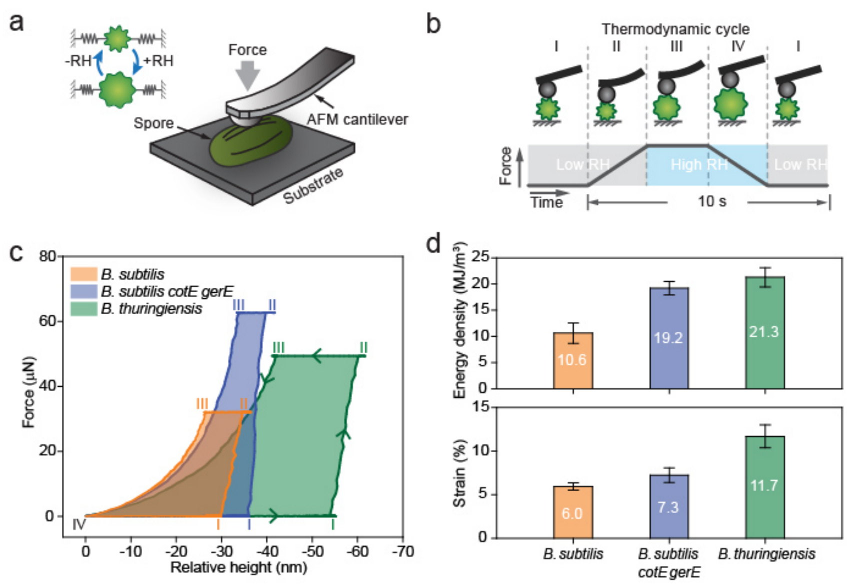


**Figure 1| Probing energy conversion in bacterial spores a**, Basic indentation experiment for quantifying energy density of the spores in response to humidity cycling. **b,** humidity ramp for energy density experiment where the area inscribed in the plots in **c** reveal the work generated. **d,** Quantification of the energy densities (work to volume ratio) top, and strain, bottom.

generated by individual spores as the relative humidity of the air is cyclically modulated from low to high. For this goal, we developed an atomic force microscope (AFM) based experimental setup, in which the expanding spores push the force sensing cantilever of the AFM away thereby generate mechanical work. Recording force and distance allows determining the total amount of work that is generated in one cycle of changing relative humidity. (Fig 1) [1].

The effectiveness of synthetic stimuli-responsive materials has generally been limited with respect to mechanical actuators, so our observations of energy densities greater than two orders of magnitude than those of synthetic water-responsive materials are intriguing. Importantly, we found that genetic alterations that cause spores to loose majority of their coat layer result in nearly double the amount of work density. This finding indicates that the layer responsible for energy conversion is the cortex layer.

**Nanoscale water transport kinetics in bacterial spores**

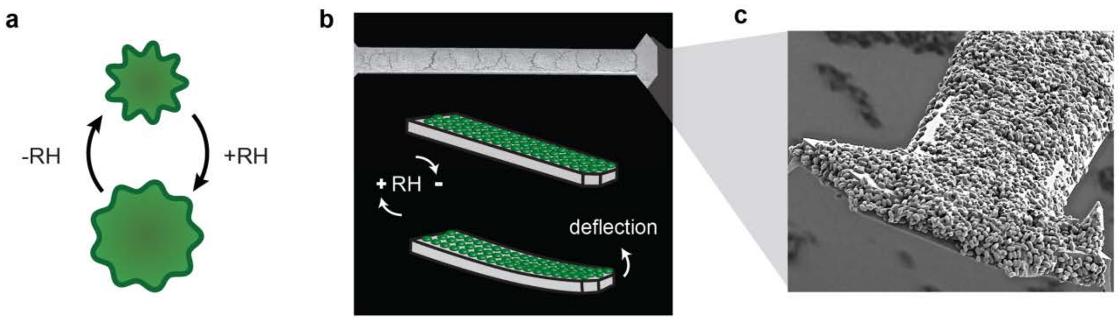


**Figure 2| Microcantilever design for studying spore water transport. a**, spores change shape rapidly when equilibrating in the presence of humidity gradients. **b**, AFM cantilevers are unilaminally coated with spores and their physical size change at the nanoscale results in deflection at the microscale (cantilever is ~200 µm in length). **c**, Cantilever tip is shown coated with spores through scanning electron microscopy (SEM).

We have built an experimental setup to probe nanoscale water dynamics in bacterial spores with millisecond time resolution [2]. The setup is based on the principal of bilayer actuation where a spore's expansion due to water-uptake is bends a microcantilever (Fig 2). We exploited this principle to systematically observe re-equilibration kinetics. We applied photothermal pulses with a light emitting diode near the cantilever, thus driving evaporation and causing cantilever deflection (Fig 3). We found that the method allows estimating the kinetics of individual spores, and not the entire layer of spores, as we found changing spore quantity on cantilevers did not affect the response time while genetic changes to the spore's architecture, including coat deletion did drastically speed the water transport. The result is a highly effective platform to probe nanoscale water transport kinetics in complex biological materials.

We found that hydration-dehydration speed depends strongly on the relative humidity and temperature of the environment, and appear unique to the spore when compared against other hygroscopic materials. We have determined that the spore behaves as a poroelastic material and that the speed of hydration re-equilibration is inversely related to the gap space within the hygroscopic spore layers. The confinement gap is directly tunable with relative humidity (RH), making the system applicable to examination of a range of confinement lengths. This is because the spore expands in proportion to humidity.

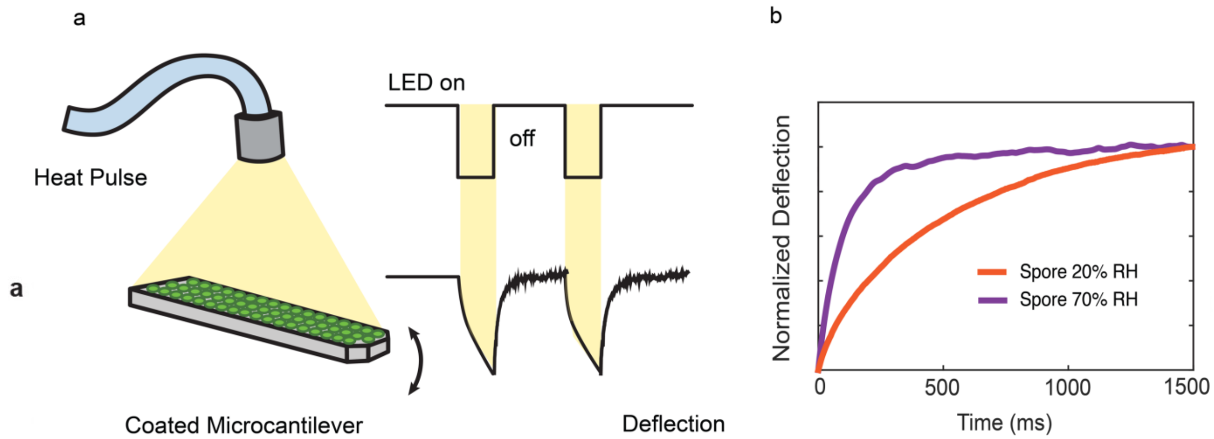


**Figure 3| Nanoscale water transport experiment a,** Heat pulse by LED results in local evaporation of spore water on spore-coated cantilevers **b,** Deflection trace from AFM tracks re-absorption of water following evaporative heat-pulse and shows that the water transport speed is dependent on the RH state.

In order to better understand the physical state of the water at the various structure-states within the spore we investigated the temperature dependence of water transport. We relied on an Arrhenius type relationship, in which viscosity scales with $e^{(Q/RT)}$, where Q is the activation energy of water transport, R is the molar gas constant, and T is the absolute temperature. When we consider the change in Q across a range of humidity levels, we see that in the dried spore, water behaves as in bulk, despite its transport speed being limited by geometric confinement. Above about 25% RH, we observe higher activation energy, suggesting that a transition occurs at this humidity level. Nanoconfined water, with this degree of viscosity, has been predicted in various theoretical models when confining surfaces exhibit crystallinity, but has not been observed directly in a complex biological structure.

### Scaling up nanoscale energy conversion

The strong response of spores to changes in relative humidity suggests that they can be potentially useful in converting energy from naturally occurring evaporation. We developed methods to scale up (in size) the energy conversion process of individual spores [3]. To explore mechanisms that enhance water transport kinetics in materials with macroscopic dimensions, we have followed a hierarchical design strategy. We developed hygroscopy driven artificial muscles (HYDRAs) that exhibit fast speed and high strain actuation in response to changing humidity. These HYDRAs, prepared by periodically coating alternating sides of polyimide tapes with bacterial spores, can quadruple their length and lift 50 times their own weight while exchanging less than 5% water by weight. We validated the functionality these materials by demonstrating evaporation driven engines that generate sustained linear oscillatory motion (piston-like) in the presence of evaporation. These engines are able to start autonomously when placed at an air-water interface. In addition, we have demonstrated an electricity generator that rests on water, harvesting evaporation, to power a light source as the water evaporates.

We have fabricated these HYDRAs using *cotE gerE Bacillus subtilis* spore mutants, which eliminate most of the outer protein protective layers of the spore coat (Fig 4,5). Longer HYDRAs were created and assembled in parallel. The overall length of the resulting wavy-shaped HYDRAs change dramatically in humid and dry conditions.

The actuation behavior of individual HYDRA strips was characterized by placing them inside transparent plastic tubes and flowing air with changing relative humidity. We found HYDRAs are able to elongate over 400 % of their dry length as the relative humidity changes from below 30% to above 80%. In this process, measured from differences in strip weights at low and high relative humidity, the amount of moisture absorbed by the HYDRAs is less than 5% by weight. Calculated from the overall lengths of strips, the average curvature of individual arcs varies from 0.7 mm to 2.8 mm. To test the actuation stability of HYDRAs, we performed a long-term dynamic cycle experiment. We found that extension length of strips reduces only slightly even after 1 million cycles and 80 days, and they can still respond within approximately 3 seconds.

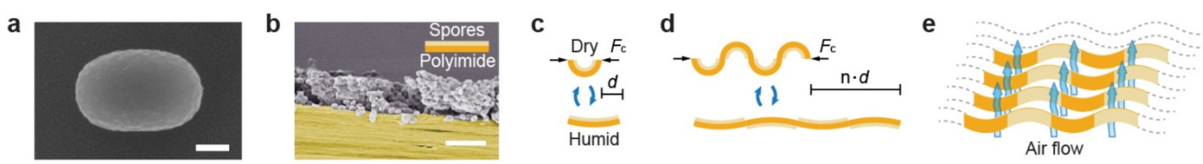


**Figure 4| Hygroscopy driven artificial muscles (HYDRA) a,** A scanning electron microscopy (SEM) image of a *B. subtilis cotE gerE* spore. **b**, A false-colored SEM image of spores deposited on a polyimide tape. **c**, The spore-coated films change its curvature in response to changing relative humidity. **d**, Linearly expanding and contracting structures are created by patterning equally spaced spore layers on both sides of the plastic tape. **e**, Assembling the tapes in (**d**) with air gaps between them results in a material that can be scaled in two dimensions without affecting hydration/dehydration kinetics. Scale bars: (**a**) 200 nm, (**b**) 5 μm.

Relative humidity in typical environmental conditions changes on daily and seasonal timescales, which is too slow to generate enough power for practical applications. However, spatial gradients in relative humidity near evaporating water surfaces provide an opportunity to alternate local humidity. For example, the relative humidity experienced by the spores would change rapidly in a cyclical fashion, if a small

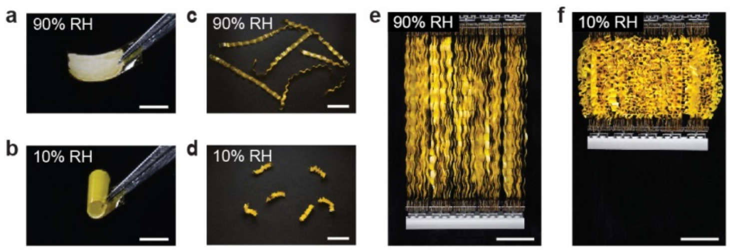


**Figure 5| Photos of HYDRAs in humid and dry conditions. a**, **b** Photos of individual spore-coated polyimide tapes at low and high relative humidity. **c**, **d,** Linearly expanding and contracting muscles are created by patterning equally spaced spore layers on both sides of a plastic tape. **e**, **f**, HYDRA strips can be packaged in parallel to lift weights. Scale bars: (**a**, **b**) 2 mm, (**c**-**f**) 2 cm.

portion of the power generated by these spores could be used to control the evaporation rate. Figure 6a illustrates this strategy, which exposes HYDRAs to rapidly changing relative humidity, to control the evaporation rate using a shutter system with feedback by using the power generated by HYDRAs. A fully assembled prototype, shown in Figure 6b, exhibits oscillations and is able to continuously extract energy from evaporation when placed above the water-air interface.

In our system, HYDRAs are horizontally placed above the water surface and coupled to a buckling beam that is compressed beyond its buckling limit to achieve mechanical bistability. A buckled beam controls a shutter mechanism that allows or blocks passage of moist air (feedback). There are four key stages this device exhibits oscillations: (Stage I) When the shutters are closed, the relative humidity of the chamber increases, causing the HYDRAs to expand. (Stage II) As the HYDRAs expand towards the right, they trigger the buckled beam to switch positions. (Stage III) Opening the shutters results in the relative humidity of the chamber recede, leading to HYDRAs contraction. The cycle is completed when contracting HYDRAs pull the buckled beam, triggering it to switch back to the left configuration (Stage IV), which then closes the shutters and brings the system back to Stage I. This oscillator is self-starting and generates piston like oscillatory motion.

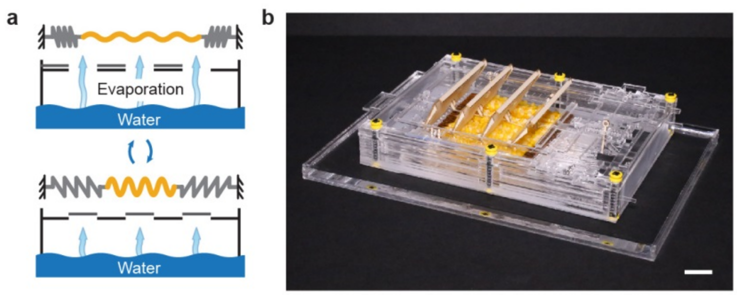


**Figure 6| Evaporation-driven engine a,** A shutter mechanism can change the relative humidity surrounding HYDRAs. **b**, Photo of a device that exhibit self-started oscillatory movement when placed above water. Scale bars: 2 cm.

To demonstrate that this rapid piston-like motion of the oscillator allows it to act as an engine and supply power to external system, we coupled the oscillatory engine to a generator and provided electricity to light emitting diodes (LEDs). When water temperature was maintained at 30°C, two oppositely connected LEDs gave light repeatedly in alternating order depended on the direction of oscillatory motion. The electrical power was measured by replacing the LED pair with a resistor of 100 kΩ. The corresponding power showed that energy was supplied in bursts at power levels reaching 60 μW. Waiting periods, which separate these bursts and bring the average power to 1.8 μW, could potentially be reduced with more compact designs so that humidity levels change more rapidly. Nevertheless, considering the small area of water covered by HYDRAs (9.6 cm by 7.6 cm), the ability to generate light with LEDs and the microwatt scale power measured with a load resistor is still significant. Our demonstration also shows that despite the slow natural variations in relative humidity, it is possible to create rapid motions by actively modulating evaporation rates at surfaces and generate a useful amount of work for particle applications.

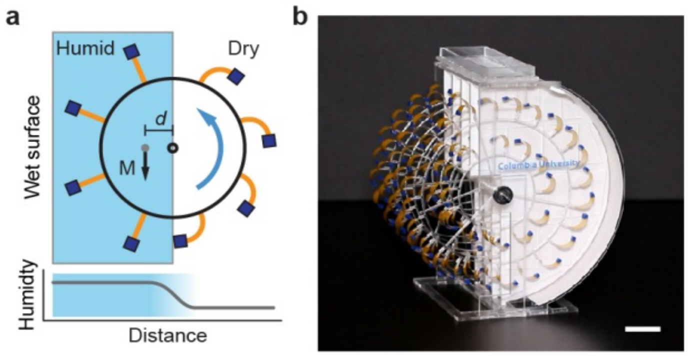


**Figure 7| The hygroscopic rotary engine. a,** rotary motion can result in cyclical changes of relative humidity experienced by the spores. The increased curvature of strips on the dry side shifts the center of mass of the entire structure away from the axis of rotation and creates torque. **b,** Photo of a rotary engine that can continuously rotate powered by evaporation from the wet paper within the device. Scale bar: 2 cm.

An alternative strategy to change local humidity is by moving spores in and out of a humid zone, which can be achieved by coupling hydration-induced movements into a rotational motion. Gradients in relative humidity near the evaporating surface can induce different degrees of curvature in spore-coated films assembled around a freely rotating disk. This horizontal shift in the center of mass of the entire structure creates torque that maintains the rotational motion. One such rotary engine, which exhibits continuous rotation when the paper lining the inner surfaces of the device is wetted, is shown in Figure 7.

**Predicting power output of evaporation driven energy harvesters**

Work in the previous sections focused on the nanoscale water transport and the associated energy conversion in bacterial spores, and then scaling this behavior into potentially useful devices for energy conversion and harvesting applications. An important factor that can affect this energy conversion process is the limits of heat and mass transfer in the environment. Therefore, we sought to understand these limits by modeling how evaporation in natural environment will be affected by the energy conversion process and what the resulting power output would be [4, 5].

In nature, evaporation is governed by the surface energy balance between net radiation and heat losses due to turbulent convection and evaporation (Fig 8). Combining this energy balance with equations of heat and mass transfer can predict the evaporation rate over a saturated water surface from meteorological data (i.e., net solar radiation, relative humidity, air temperature, and wind speed). For this part of our project, we used similar principles to construct a model of natural evaporation through an evaporation driven engine. For example, a bacterial spore based evaporation-driven engine placed just above the water surface absorbs water vapor at a high chemical potential, $\mu_s$, and releases it at a lower chemical potential, $\mu_e$, to the atmosphere, $\mu_a$ ($\mu_s > \mu_e > \mu_a$). For a reversible and isothermal conditions, the chemical potential drop of water vapor travelling through the spores is exactly $w = \mu_s - \mu_e$.

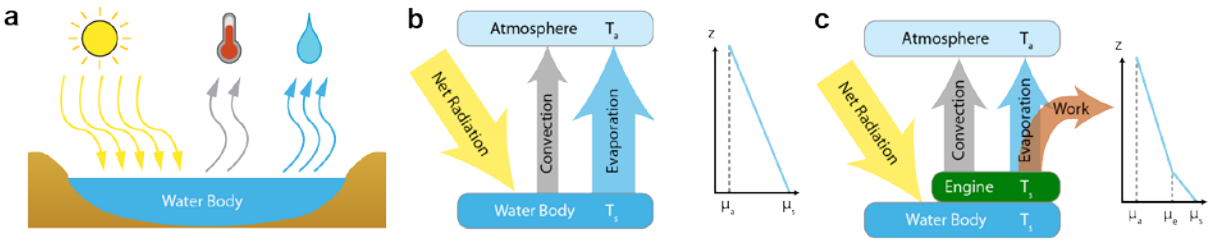


**Figure 8| a**, The net radiative energy into a water body is balanced by convection and evaporation. **b**, The flows between the water body and the atmosphere occur along a thermal gradient ($T_s$ to $T_a$) for convection and along a chemical gradient ($\mu_s$ to $\mu_a$) for evaporation. **c**, The new energy balance can be illustrated between net incoming radiation, convection, evaporation, and work extracted between $\mu_s$ and $\mu_e$.

We have derived as set of equations that can predict the power output and reduction in evaporation rates as a function of weather conditions and the energy converted by the evaporation-driven engine [4]. Figure 9 summarizes how power output depends on relative humidity, wind speed, and net radiation. Both the power output and evaporation reduction increase with increasing air temperature and net radiation. We also find that the potential water savings due to evaporation reduction increases with increasing wind speed and decreasing relative humidity. Interestingly, we find that the optimal power density varies weakly with wind speed and increases strongly with decreasing relative humidity.

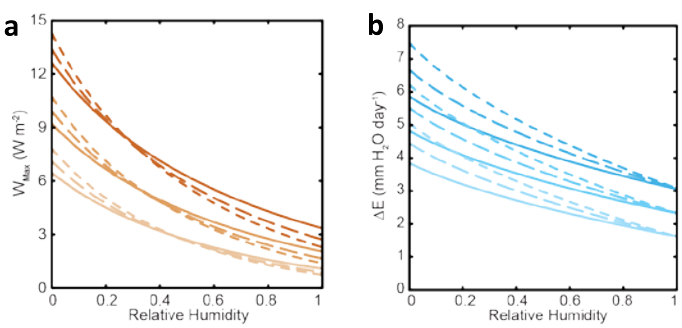


**Figure 9| a,** Maximum energy flux and **b,** water saved from evaporation as a function of RH at cool (pale, 12 $^oC$, 150 W $m^{-2}$), mild (neutral, 16 $^oC$, 200 W $m^{-2}$), and warm (dark, 20 $^oC$, 250 W $m^{-2}$) weather conditions and three wind speeds: 1.8 (4 mph, solid), 2.7 (6 mph, dashed), and 3.6 m $s^{-1}$ (8 mph, dotted).

**List of papers acknowledging DOE support:**

[1] Xi Chen, L Mahadevan, Adam Driks and Ozgur Sahin. Bacillus spores as building blocks for stimuli-responsive materials and nanogenerators. *Nature Nanotechnology* **9**, 137-141(2014).
[2] Michael DeLay, Xi Chen, Ahmet-Hamdi Cavusoglu, Jonathan Dworkin, and Ozgur Sahin. Ultraslow transport of nanoconfined water observed in a biological material. submitted.
[3] Xi Chen, Davis Goodnight, Zhenghan Gao, Ahmet Hamdi Cavusoglu. Nina Sabharwal, Michael DeLay, Adam Driks, and Ozgur Sahin. Scaling up nanoscale water-driven energy conversion into self-powered evaporation-driven engines. *Nature Communications* **6**:7346 (2015).
[4] Ahmet-Hamdi Cavusoglu, Xi Chen, Pierre Gentine, and Ozgur Sahin. Potential for natural evaporation from open-water surfaces as a low-intermittency and water-saving renewable energy resource. *Nature Communications* **8**: 617 (2017).
[5] Ahmet-Hamdi Cavusoglu, Xi Chen, Pierre Gentine, and Ozgur Sahin. Code and Data for Potential for natural evaporation from open-water surfaces as a low-intermittency and water-saving renewable energy resource. *Figshare* doi:10.6084/m9.figshare.4688512 (2017).

**List of people working on the project:**

Graduate students: Michael DeLay (50% support from DOE), Ahmet-Hamdi Cavusoglu (50% support from DOE), Zhenghan Gao (not supported by DOE), Juan F. Quijano (50% support from DOE)
Postdocs: Xi Chen (50% support from DOE), Onur Cakmak (12%)
Associate research scientist: Eran Schenker (25% support from DOE)
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