Supporting Information

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ATP biosynthesis catalyzed by the F\textsubscript{o}F\textsubscript{1}-ATP synthase assembled in polymer microcapsules

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**Experimental Section**

Hollow capsules were fabricated by alternating absorption of 2 mg/mL PAA (M\textsubscript{w} 5100, Sigma-Aldrich) and PAH (M\textsubscript{w} 70000, Sigma-Aldrich) in 0.2 M NaCl solution onto 3.93 μm MF core particles\textsuperscript{[14]} using the centrifugation method. After each absorption step the samples were centrifuged in an Eppendorf rotor and washed three times in 0.2 M NaCl solution. Addition of a bilayer of sodium poly(styrene sulfonate) (PSS) (M\textsubscript{w} 70000, Sigma-Aldrich) and poly(allylamine hydrochloride) (PAH) with the same concentration of 1 mg/mL in 0.2 M NaCl was adsorbed on the outer surface of PAA/PAH particles. The cores were dissolved in 0.1 M HCl (pH 1.0) solution for three times, followed by three washing cycles in deionized water. The outer layer is positively charged PAH.

Liposomes were prepared from a mixture of phosphatidyl choline from egg yolk (Sigma) and phosphatidic acid from egg yolk (Sigma) (9:1 by mass). The reconstitution of CF\textsubscript{o}F\textsubscript{1} into liposomes was performed by adding liposomes to Triton X-100-solubilized CF\textsubscript{o}F\textsubscript{1}-ATPase solution and then slowly removing Triton X-100 with Bio-beads SM-2 (Bio-Rad).\textsuperscript{[4]} The final
concentration of CF₀F₁ and lipid were 200 nM and 5 mg/mL, respectively. The bulk aqueous phase containing 20 mM Tricine, 40 mM NaCl and 5 mM MgCl₂ was adjusted to pH 8.0 with 1mM NaOH solution. Negatively charged proteoliposomes containing CF₀F₁-ATPase were mixed with a capsule dispersion and incubated for 30 min, then centrifuged at 4 °C and washed three times with the reconstitution buffer to remove any remaining liposomes from the bulk solution.¹³

Atomic force microscopy (AFM) images were recorded using a Nanoscope IIIa, Di. Silicon cantilever tips (TESP) with a resonance of approximately 300 KHz and a spring constant of about 40 N/m were used. The radius of the tip was ca. 3-5 nm.

Confocal laser scanning microscopy (CLSM) images were taken with an Olympus FV 500 IX81 confocal system (Olympus, Japan). 5% NBD-DPPE (Sigma) (w/w) were used as a fluorescent label for lipid layer visualization.

Fluorescence measurements were carried out on an F-4500 fluorescence spectrophotometer (HITACHI, Japan).

The luminescence measurements were carried out in an Ultra-Small luminescence analyzer connected to a chart recorder.