Supporting Information

Assembly of F_oF_1-ATPase into solid state nanoporous membrane

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1. Preparation of nanoporous PET membrane

The nanoporous PET membrane was prepared with the well-known ion track-etching technique.[S1, S2] Before chemical etching, PET membrane (Hostaphan RN12 Hoechst, 12 μm thick, with ion track of $10^7/cm^2$) was exposed to the UV light for 1h from each side. Etching process was carried from one side with 9 M NaOH solution. On the other side, a stopping solution with 1 M KCl and 1M HCOOH was used to neutralize the NaOH from etching solution after the nanopores open. A voltage of 1V was used to observe the etching process. When the etching process stopped, the etching solution was taken place by stopping solution. Then the nanoporous membrane was washed by deionized water thoroughly, and immersed in water for next experiment. Fig. S1 is the SEM images of the prepared porous PET membrane. The large opening (base) of the pores was ~1.5 μm, and the small opening (tip) was ~50 nm (Fig. S1).

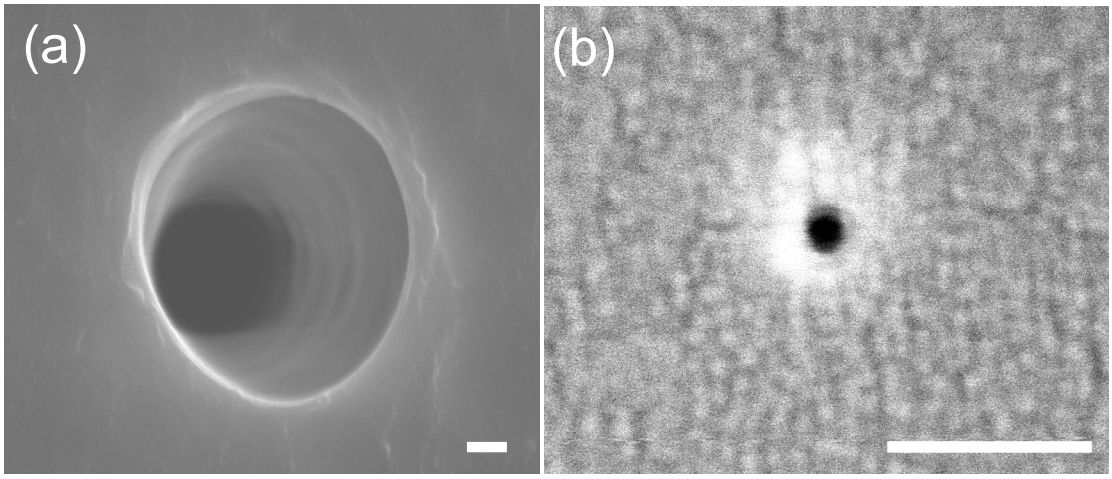

Fig. S1. SEM images of base (a) and tip (b) of nanoporous PET membrane. The scale bars are 200 nm.

2. Silanization of surface of nanopores

The as-prepared nanoporous PET membrane was immersed into a saturated ethanol solution of *(tridecafluoro-1, 1, 2, 2-tetrahydrooctyl)trichlorosilane* for 24 hours.[S3] After reaction, the membrane was washed with ethanol and deionized water thoroughly. Water contact angle (CA) measurements were taken on an OCA20 (DataPhysics, Germany) CA system. The volume of water drop was 2 μL. After modification, the CAs of nanoporous PET membrane changed from 71.0 ^{o}±2.3 ^{o} to 104.2 ^{o}±0.7 ^{o} (Fig. S2). It illustrates that the surface of the nanoporous membrane was changed to be hydrophobic, so the lipid bilayers can be formed across the nanopores easily.

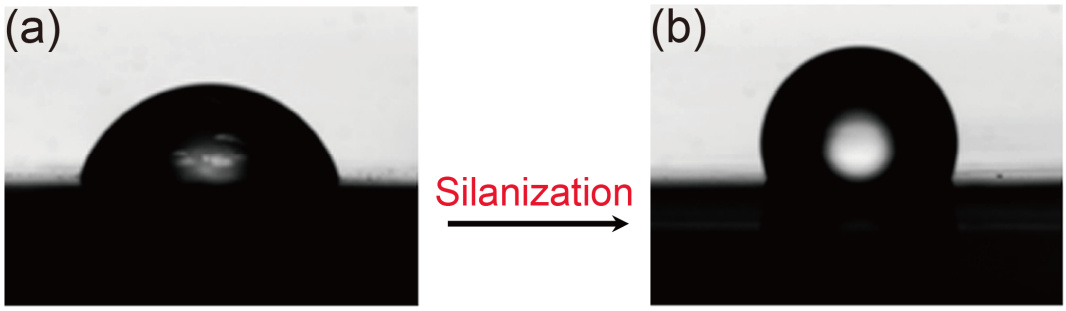

Fig. S2. The water contact angles before (a) and after (b) silanization of nanoporous PET membrane.

3. Formation of lipid bilayers across the nanopores

After the hydrophobic surface of the nanopores was obtained, liposome bilayers deposition was carried out using the well-known painting method.[S4] Briefly, 5 μL of decane solution of *1, 2-diphytanoylphosphatidylcholine* (DPhPC) with a concentration of 10 mg/mL was spread on the tip side of nanoporous PET membrane, and then its both sides were filled with 0.1 M KCl solution. The lipid bilayers formed in 10 minutes by self-assembling. Transmembrane current was measured by a Keithley 6487 picoammeter (Keithley Instruments, Cleveland, OH) to confirm the successful formation of the lipid bilayers. The sample was fixed between two half cells. Two Ag/AgCl electrodes were inserted into the two cells to apply voltage and to detect the current. Test results are shown in Fig. 2.

4. Purification of F_oF_1-ATPase

Cells of *Thermomicrobium roseum* were harvested by centrifugation at 4000 rpm for 30 min and resuspended in 20 mM Tris-HCl buffer (pH 8.0) containing 100 mM NaCl, 1 mM DTT, 0.1 mM PMSF and 2 mM $MgCl_2$, followed by ultrasonic for 10 min on ice. The extract was centrifuged at 10000 rpm for 30 min to remove unbroken

cells. The cell-free supernatant was centrifuged at 40000 rpm for 1h to collect membrane vesicles. All steps above were performed at 4 ^{o}C.

Membrane from *Thermomicrobium roseum* cells was solublized with 20 mM Tris-HCl buffer (pH 8.0) containing 100 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT and 1% (w/v) n-Octyl-β-D-glucopyranoside. After 1 h of incubation at 4 ^{o}C with constant stirring, the nonsolubilized membrane was removed by ultracentrifugation at 40000 rpm for 1 h and then the supernantant was loaded onto a DEAE Sephrose Hiload 16/10 column, which had been pre-equilibrated with 5 column volumes of buffer A (pH 8.0, containing 20 mM Tris-HCl 100 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT and 0.1% (w/v) n-Octyl-β-D-glucopyranoside). The bound proteins were eluted with a linear of 0.1-0.5 M NaCl at pH 8.0. F_oF_1-ATPase activity assay was performed as described previously with minor modification.[S5] The fractions showing F_oF_1-ATPase activity were pooled and concentrated to 2 mL by 100 kDa cut-off amicon, then loaded onto a Superdex200 gel filtration column. The column was run with buffer A. It showed a main peak with some minor impurity (Fig. S3). Purified proteins were concentrated to about 10 mg/ml and used in 2 hours.

The purified enzyme was analyzed by SDS-PAGE.[S6] Polypeptide bands were visualized by staining with Coomassie brilliant blue.[S7] Eight main bands were observed in addition to minor contamination, which may contact the enzyme unspecificly (Fig. S4).

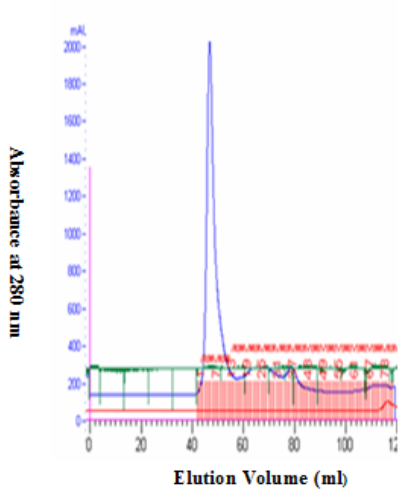

Fig. S3. Chromatography of partially purified F_oF_1-ATPase from Thermomicrobium roseum on Superdex 200 columns. The main peak contained purified F_oF_1-ATPase and the small subsequent shoulder contained low-molecular-mass impurities.

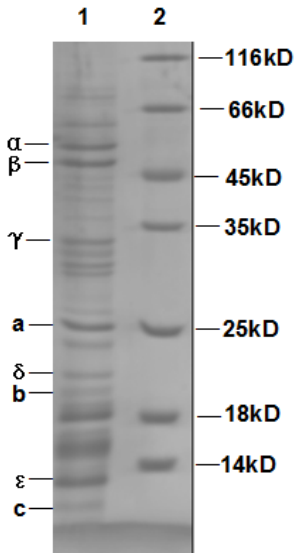

Fig. S4. SDS-PAGE of the purified F_oF_1-ATPase from *Thermomicrobium roseum* was run on 15% polyacrylamide gel. Lane 1: Purified F_oF_1-ATPase and the positions of the subunits are indicated on the left. Lane 2: Molecular weight markers.

5. Confirmation of reconstitution of FoF1-ATPase into the phospholipids bilayer on the PET membrane

Immunofluorescence assay was used to verify the reconstitution of F_oF_1-ATPase into the phospholipids bilayer on PET membrane. The PET membrane deposited with phospholipids bilayers but without F_oF_1-ATPase was used as negative control. Briefly, after blocking in PBS with 5% BSA for 1 h at room temperature, the samples were incubated with biotinylated βsubunit antibody (1:100) for 1 h at 37^{o}C, followed by washing with PBS thoroughly. Then they were incubated with streptavidin-FITC (40 μg/mL) in PBS for 1 h at room temperature. After washing with PBS thoroughly, the fluorescence images were obtained using Olympus IX71 fluorescence microscope and recorded with a digital CCD camera (iXon CCD, ANDOR Technology) （Excitation 492nm，Emission 520nm）. The results are shown in Fig. 2.

6. ATP synthesis with ANPMS

Two PBS buffers (pH 4.7 without ADP, and pH 8.3 with ADP) were inserted into the two half-cells of reaction system, respectively. And then the reaction system was set in a thermostatic oven of 37 ^{o}C.

References

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