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Supplementary information

Lab free protein-based moisture electric generators with high electric output

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Supplementary Information and Methods

1. MEG fabrication

Hydrochloric acid (HCl), poly(ethylene glycol) (PEG, Mn 2050), potassium hydroxide (KOH), sodium hydroxide (NaOH), potassium chloride (KCl), Ag paste, carbon nanotube (CNT), fluorine-doped tin oxide (FTO) and polyethylene terephthalate (PET) were purchased from Sigma. The protein powders were purchased from Whey Protein Isolate (WPI), which is cheap and widely used in industry. The dispersion or solution was fabricated by dispersing or dissolving chemicals in the distilled water with a sonication for 30 min. The 10 wt.% protein dispersion and 10 wt.% PEG solution were mixed with a weight ratio of 2:1. The acid and basic solution were used to adjust pH of mixed protein dispersion. The protein films were fabricated on the FTO by drop-casting of protein dispersion at 50 °C (air) for 24 h. The CNT paste was spread on the surface of protein film to form 0.5×0.2 cm² top electrode (Supplementary Fig. 30). In MEG array, CNT was used as bottom electrode by drying 30 % CNT dispersion on the PET substrate at 50 °C (air) for 24 h. Ag paste was used to connect different units in series. The plasma treatment was carried out in PlasmaFlo (Harrick Plasma) with O₂ pressure of 550 mTorr.

2. Electrical measurement

To control the relative humidity (RH) in the sample chamber, compressed N_2 flows through distilled water to carry the moisture and increase RH of sample chamber, while dry N_2 flows into the sample chamber directly to decrease RH (Supplementary Fig. 31). The room humidity of laboratory was about 40% in the measurement. The top/bottom electrodes were connected to the Keysight B2902A precision source/measure unit directly for output signals.

3. Material characterization

The morphological images of protein films were observed by scanning electron microscopy (FEI Nova NanoSEM 450). The surface hydrophilicity was analyzed using Contact Angle Goniometer from Ossila U.K. The elemental compositions were employed by X-ray photoelectron spectroscopy (XPS, ESCALAB250Xi spectrometer). Kelvin probe force microscopy was adopted to analyze surface potential of protein films on a Bruker Dimension Icon machine at room humidity of 40%. Fourier-transform infrared spectroscopy (FTIR, PerkinElmer, Spectrum 100) was used to investigate functional groups of protein film.

Electrochemical impedance spectroscopy (EIS) of devices was carried out in a two-electrode system at room humidity (RH=40%) using Autolab PGSTAT302N electrochemical workstation. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using commercial (Invitrogen) gradient gels (4–12%) purchased from Thermo Fisher Scientific. Optical images of the protein films were taken using an Olympus PMG3 Microscope.

4. Molecular dynamics simulation

A 10 ns molecule-dynamics (MD) simulation of β-Lactoglobulin was carried out with the GROMACS 2022 package^{1,2} using the CHARMM27 force field.³ The initial structure of β -Lactoglobulin was obtained from the protein data bank (PDB ID: 1BSO), containing five cysteine residues that one exists as a free thiol buried beneath the α -helix that lies alongside the β -barrel, whereas the other four form two disulfide bridges.⁴ The topology parameters of β-Lactoglobulin were created by GROMACS program. The protein was centered in the cubic box with ~ 1.0 nm from the box edge. Then water molecules were filled the cubic box to simulate the hydrated state and applied a generic equilibrated 3-point solvent model for the water molecules. The solvated systems were neutralized by adding sodium ions and the entire systems were composed of 9 Na⁺ counterions and 9851 solvent atoms. Before molecular dynamics simulation, the structure was relaxed through energy minimization process to ensure that the system has no steric clashes or inappropriate geometry, followed by an *NVT* equilibration MD simulation with the solute (β -Lactoglobulin and counterion) was fixed and the position-restrained at 300 K for 100 ps. Finally, the full system was MD simulated for 10 ns at 300 K temperature and 1 bar pressure. The atom coordinates were recorded every 0.2 ps during the simulation for analysis.

Supplementary Figures



Fig. S1 Protein powder used in this work. The product is the ordinary protein consumption, which is cheap $(0.08 \text{ AUD} \cdot \text{g}^{-1})$ and readily accessible.



Fig. S2 Schematic illustration of film fabrication. Protein is dispersed in distilled water with PEG and HCl/KOH to improve film uniformity and surface charge. Plasma treatment is applied to improve surface hydrophilicity of protein film.



Fig. S3 Electric output of protein-based MEG. (a) Photo of MEG voltage with source meter at outdoor field. (b) Voltage output and current output of MEG in open circuit and short circuit, respectively, at ambient humidity of 25 °C and 40 % RH.

Functional material	Electrode	RH (%)	Output type	Electric output	Refs.
Protein	Au/Au	50	Continuous	0.5 V, 17 μA·cm ⁻²	5
TiO ₂	Al/ITO	85	Transient	0.5 V, 10 μA·cm ⁻²	6
Cellulose paper	Au/ITO	70	Transient	0.25 V, 10 nA·cm ⁻²	7
GO	Au/Ag	80	Transient	1.5 V, 27.2 nA·cm ⁻²	8
GO/sodium polyacrylate	Au/Ag	80	Continuous	$0.6 \text{ V}, >1 \mu \text{A} \cdot \text{cm}^{-2}$	9
Cellulosic nanofibrils	Pt/Pt	99	Transient	115 mV, 43 nA·cm ⁻²	10
Reduced GO/GO	Au/Au	85	Continuous	0.45 V, 0.9 μA·cm ⁻²	11

Table S1 Summary and o	comparison of recent MEGs
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	$2 \mu \pi cm$ 12
Protein Ag/FTO 40 Continuous 1.4.	V, 113 This
μ.	A·cm ⁻² work



Fig. S4 Voltage cycle of protein-based MEG. The MEG is placed in the room humidity of 40% to reach V_{max} , followed by moisture removal with dry N_2 (RH is about 0%).



Fig. S5 The cross-section morphology of protein film. The protein film is porous with micronsize pores and a thickness of $\sim 292 \ \mu m$.



Fig. S6 Protein films with and without PEG. (a) Photo of protein surface with and without PEG. (b) Voltage retention for 28 h in room humidity of 40%. The protein films are fabricated by drop-casting of protein dispersion with a pH of 14 by KOH solution.



Fig. S7 Protein mixed with NaOH and KCl. (a) Voltage output of protein film fabricated by adjusting pH of protein solution to 14 with NaOH. Voltage output of protein film fabricated by adding KCl with same concentration into the protein dispersion without KOH. The protein was mixed with PEG with a weight ratio of 2:1. (b) Zeta potential of 1 wt.% protein dispersion with a pH of 14 by NaOH and 1 wt.% protein dispersion with a pH of 7 by KCl.



Fig. S8 Whey protein with different common protein brands. Zeta potential is measured with 1 wt.% protein dispersion. The voltage is recorded with highest output in room humidity of 40%. The protein ratio is 89 wt.% (BioTechUSA), 79 wt.% (Optimum Nutrition) and 93 wt.% (Vitalstrength), respectively.



Fig. S9 Surface topography and contact potential difference (CPD) by Kelvin probe force microscope (KPFM). Topography (a) and CPD (b) of protein surface fabricated from protein solution with pH=0. Topography (c) and CPD (d) of protein surface fabricated from protein solution with pH=14.



Fig. S10 Schematic illustration of electricity generation. Charge separation of acid protein film (a) and basic protein film (b) exposed to the moisture. The hydrophilic protein films absorb water from the moisture, which diffuses along the micro-nano channels to form a vertical water gradient and ion gradient for electricity generation.



Fig. S11 Voltage output of MEGs with different configuration. The voltage output between two top electrodes is recorded to compare with voltage output between top and bottom electrode in room humidity of 40% RH.



Fig. S12 Electric outputs of devices in room moisture and water drop. The distilled water was dropped on the surface of protein film with 0.1 cm² CNT electrode to measure the electric output with water drop. After dropping water on the MEGs, the voltage output decreases, while current output increases.



Fig. S13 Voltage output of protein films with different thickness. The protein-based devices with CNT, protein, and FTO as top electrode, functional layer, and bottom electrode, respectively, were tested in room humidity of 40%.



Fig. S14 MEGs with different configuration. (a) Voltage of MEGs with different electrodes. The MEGs with different configuration (top electrode- bottom electrode: Ag-FTO, CNT-FTO and Ag-CNT) were tested in ambient environment (T=25 °C and RH=40%). (b) Cyclic voltammetry of MEG with different electrodes in ambient environment.



Fig. S15 Molecular modelling of interaction of water and protein. (a) Root-mean-square deviations of atomic positions relative to the crystal structure and equilibrated system. (b) The radius of gyration along the time change. (c) Solvent accessible surface area per residue.



Fig. S16 Voltage of protein films with different plasma treatment time. The protein films were fabricated from whey protein of other brands and tested in room humidity of 40%.



Fig. S17 Voltage output of protein films with post-treatment. The protein films are treated with vacuum drying and vacuum plasma for 4 h, respectively.



Fig. S18 O/C ratio of protein films with and without plasma treatment. O/C ratio is calculated from XPS spectra of protein surface with and without plasma treatment.



Fig. S19 FTIR of protein films with and without plasma treatment. The protein films with and without plasma treatment show similar spectra, indicating no changes of functional groups after plasma treatment. The 1644 cm⁻¹ and 1562 cm⁻¹ are attributed to amide and amid, respectively. The 1446 cm⁻¹, 1351 cm⁻¹ and 1103 cm⁻¹ are attributed to -C-O, -C-C and -C-OH, respectively.



Fig. S20 Bending test of free-standing protein film. Voltage output (a) and bending platform (b) of MEGs in room humidity of 40% after different bending cycles with a bending angle of 90°.



Fig. S21 Photograph of a calculator powered by a MEG array. The wearable MEG array operated with 3 units in series at room humidity of 40% and could be attached to the hand or cloth easily.



Fig. S22 Electric output (a) and power (b) of load resistors. The resistors with different resistance were connected to the MEGs directly in room humidity.



Fig. S23 Charge-discharge cycles of protein-based MEGs. The MEGs with an electrode size of 0.1 cm² were charged by placing MEGs in the room humidity of 40% and discharged with a current of 10 μ A.



Fig. S24 The open-circuit voltage and short-circuit current of MEGs. The MEGs were tested with different numbers of units in series and parallel.



Fig. S25 Self-healing process of protein film. Optical image (a) and schematical illustration (b) of protein film on glass substrate in a self-healing cycle. The protein film was first scratched on the surface by knife, and then water was dropped on the surface, followed by drying the film at 50 °C for self-healing process.



Fig. S26 The voltage of commercial capacitors charged by MEGs. The MEGs were fabricated with CNT as bottom electrode, protein film as functional layer and Ag as top electrode, respectively. The MEG units were connected in series in the room humidity of 40%.



Fig. S27 Photograph of wireless humidity/temperature sensors. The sensors were powered by 0.01 F capacitor with 3.5 V, charged by 16 MEG units in room humidity.



Fig. S28 Electric output of lab-free MEGs. The MEG unit was stacked with Cu foil, protein film and Al foil and tested at room humidity of 40%.



Fig. S29 Current output of lab-free MEG. (a) Current output of MEG with different test temperature at RH=40%. (b) Current output of MEG with different RH at T=25 °C.



Fig. S30 The average relative humidity distribution in Australia. The data was collected in January at 3pm (1976–2005) by Bureau of Meteorology in Australia.¹³



Fig. S31 Illustration of MEG fabrication and connection. (a) Fabrication process of single MEG unit. (b) Four MEG units connected in series for voltage output measurement. (c) Four MEG units connected in parallel for current output measurement.



Fig. S32 Photograph of experimental setup. Moisture is introduced by flowing N_2 through distilled water. RH is controlled by adjusting gas flow with flowmeter.

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