Supporting Information:

Nitrogenase Bioelectrocatalysis: ATP-Independent Ammonia Production using Redox Polymer/MoFe Protein System

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1. Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless specified otherwise. Poly(vinylamine) and ethylene glycol diglycidyl ether (EGDGE) were purchased from PolySciences, Inc. Saturated calomel (SCE) reference and glassy carbon working electrodes were purchased from CH Instruments, Inc. The cobaltocene functionalized poly(allylamine) was synthesized as previously-reported.[1]

2. Growth of *Azotobacter vinelandii* and Purification of Nitrogenase

Mo-dependent nitrogenase from *A. vinelandii* was expressed as described previously.[2, 3] Briefly, the wild-type cells were cultivated in 18 L modified Burk medium supplemented with 10 mM ammonium (NH_4^+). Then, the cells were collected via centrifugation and resuspended into NH_4^+ free growth media to enhance nitrogenase expression. The cells were regrown until the optical density at 600 nm reached 1.5. All of the following steps were carried out under strictly anaerobic conditions. The cells were lysed by sonication in the presence of 2 mM sodium dithionite (DT). The crude extract was separated by anion exchange chromatography (Q-Sepharose, GE Healthcare) over a linear gradient of sodium chloride (NaCl) from 200 mM to 640 mM to separate the MoFe and FeP proteins. His-tagged MoFe was purified using HisTrap HP column (GE

Healthcare) and was desalted using a Hitrap desalting column equilibrated with 100 mM MOPS and 2 mM DT at pH = 7.0. The purified protein was concentrated to ≥ 20 mg mL^{-1} and was shock-frozen in liquid N_2 until further use. The purity of the MoFe proteins was greater than 95% based on the SDS-PAGE analysis using Coomassie blue staining. The purity of the protein was verified by SDS-PAGE gel (Figure S1). Pure protein was concentrated to ~20 mg/mL.

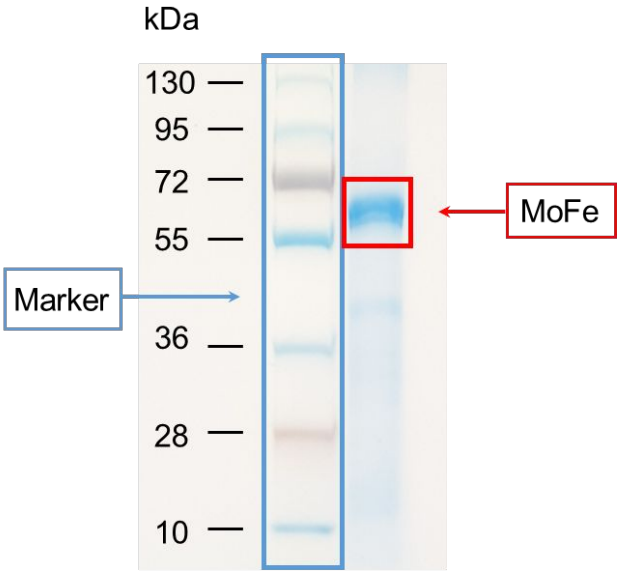

Figure S1. SDS-PAGE analysis of purified MoFe protein.

3. Bioelectrode Fabrication of Control bioelectrodes

Control bioelectrodes

MoFe protein was denatured by exposure to air for 1day. Denatured MoFe solution (5 µL, 25 mg mL^{-1} in a 100 mM MOPS buffer, pH 7.0) was mixed *via* vortex for 10 seconds with 10 µL of Cc-PAA solution (10 mg mL^{-1} in H_2O). To the polymer/protein mixture, a solution of EGDGE (1 µL, 2.7% by volume in H_2O) was vortexed in for 10 s. Non-wet-proofed carbon paper (AvCarb MGL 190) was cut into 3 cm x 0.5 cm strips. This strip was dipped into melted paraffin wax to obtain a 0.5 cm x 0.5 cm square remained exposed at one end. The denatured MoFe/Cc-PAA/EGDGE mixture (10 µL) was then drop-coated onto a carbon paper electrode and then dried

under an atmosphere of Ar/H_2 (3.2%) at room temperature for 10 hours. The BSA/Cc-PAA electrode was made by the same manner with the equivalent loading of BSA in place of the MoFe. Blank controls were made by loading the 10 μL of Cc-PAA solution (10 mg mL^{-1} in H_2O) then dried under an atmosphere of Ar/H_2 (3.2%) at room temperature for 10 hours.

4. Electrochemical Experiments of Control Bioelectrodes

Cyclic Voltammetry

BSA/Cc-PAA and blank electrode were prepared according to the above section. The electrode was submerged into MOPS buffer (pH 6, 4 mL, 100 mM) solution in the presence of nitrite (100 mM) and azide (100 mM) respectively. The blank, Cc-PAA, shows that the reductive peak of the cobaltocene/cobaltocenium (Cc/Cc^+) redox couple appeared at −0.792 ± 0.001 V *vs*. SCE with the respective current density of 39.6 ± 2.1 μA cm^{-2}.[4] Likewise, other control bioelectrode (BSA/Cc-PAA) showed similar CV profile with blank electrode under the same turnover condition.

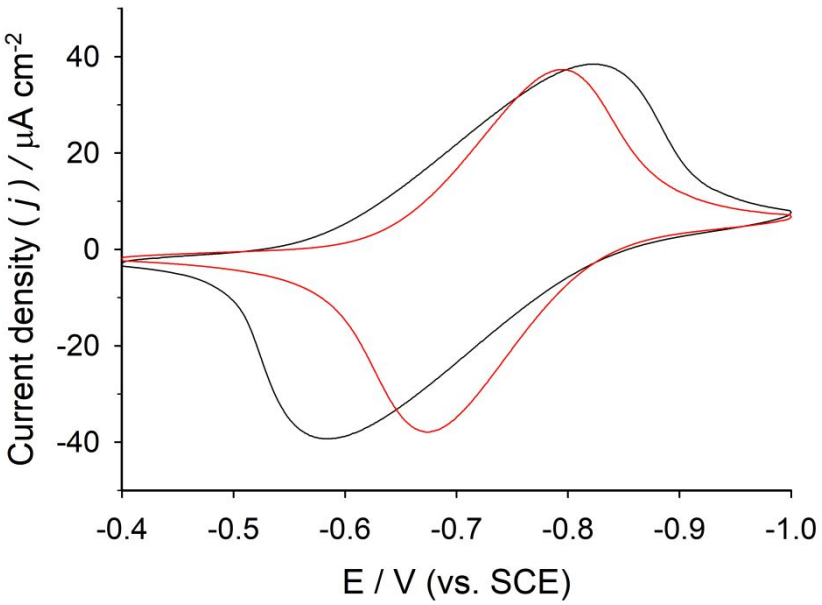

Figure S2. Cyclic voltammetry of BSA/Cc-PAA electrode (red line) and Cc-PAA electrode (black line) evaluated at a scan rate of 5 $mV \cdot s^{-1}$ in 100 mM MOPS buffer (pH 6) in the absence of any additional substrates. The voltammetric experiments were performed under anaerobic conditions where the O_2 concentration was continuously <1 ppm at room temperature.

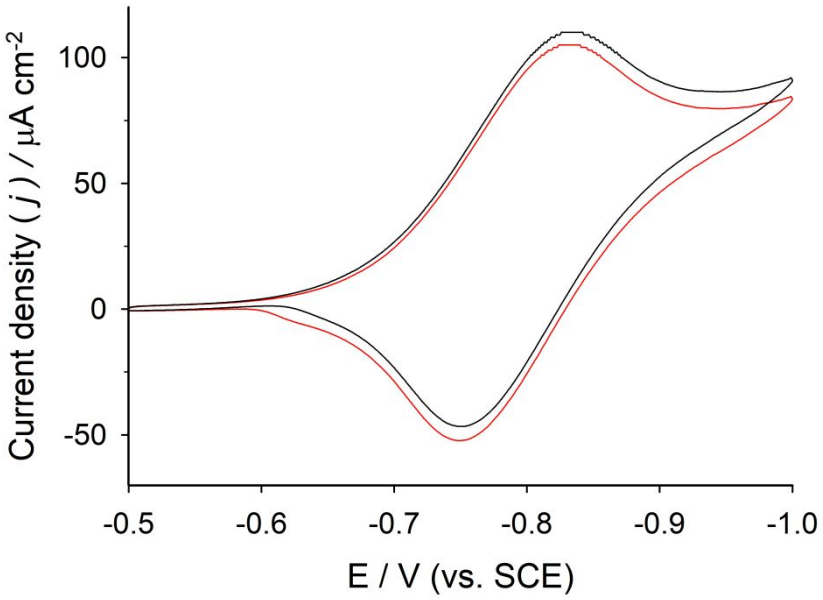

Figure S3. Cyclic voltammetry of BSA/Cc-PAA electrode (red line) and Cc-PAA electrode(black line) evaluated at a scan rate of 5 $mV \cdot s^{-1}$ in 100 mM MOPS buffer (pH 6) in the presence of 100 mM N_3^-. The voltammetric experiments were performed under anaerobic conditions where the O_2 concentration was continuously <1 ppm at room temperature.

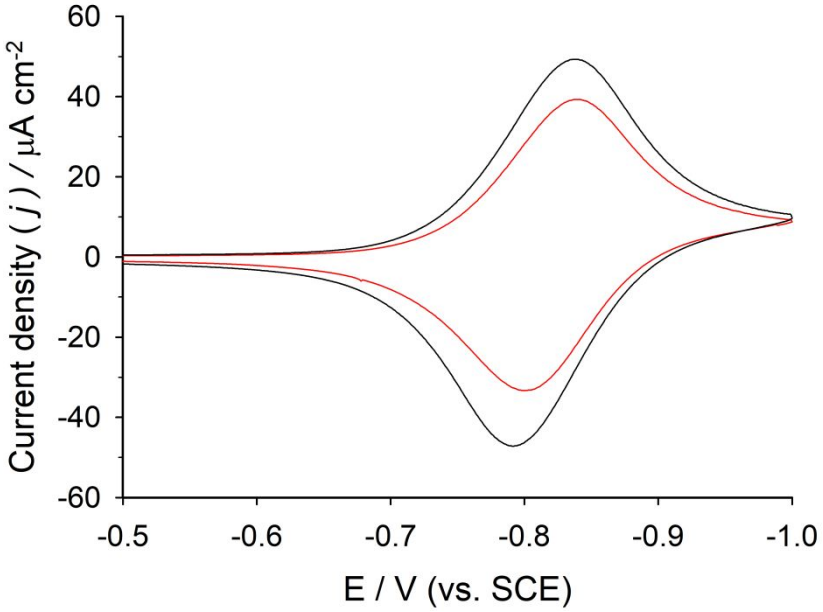

Figure S4. Cyclic voltammetry of BSA/Cc-PAA electrode (red line) and Cc-PAA electrode (black line) evaluated at a scan rate of 5 $mV \cdot s^{-1}$ in 100 mM MOPS buffer (pH 6) in the presence of 100

mM NO_2^-. The voltammetric experiments were performed under anaerobic conditions where the O_2 concentration was continuously <1 ppm at room temperature.

Bulk electrolysis

The Faradaic efficiency (FE) for the generation for the formation of NH_3 was calculated as follow:

$$\mathrm{FE} = \frac{aFn}{\int_0^t Idt} \times 100\% \ \#(\mathrm{S1})$$

where a is the number of electrons transferred for the formation of 1 molecule ammonia (In case of eqn (2), 2 electrons for the formation of 1 molecule NH_3; eqn (3), 8 electrons for the formation of 3 molecule NH_3; eqn (4), 2 electrons for the formation of 1 molecule NH_3), *F* is the Faraday constant (96485 C/mol), *n* is the moles of generated ammonia ammonia from the spectroscopic ammonia assay, *I* is the circuit current, and t is the reaction time.

References

1. Yuan, M., Sahin, S., Cai, R., Abdellaoui, S., Hickey, D. P., Minteer, S. D., Milton, R. D. Creating a Low-Potential Redox Polymer for Efficient Electroenzymatic CO_2 Reduction. *Angew. Chem.* **2018**, 130, 6692−6696.
2. Hickey, D. P., Cai, R., Yang, Z. Y., Grunau, K., Einsle, O., Seefeldt, L. C., Minteer, S. D., Establishing a Thermodynamic Landscape for the Active Site of Mo-Dependent Nitrogenase. *J. Am. Chem. Soc.* **2019**, 141, 17150−17157.
3. Milton, R. D.; Cai, R.; Sahin, S.; Abdellaoui, S.; Alkotaini, B.; Leech, D.; Minteer, S. D., The In Vivo Potential-Regulated Protective Protein of Nitrogenase in *Azotobacter vinelandii* Supports Aerobic Bioelectrochemical Dinitrogen Reduction In Vitro. *J. Am. Chem. Soc.* **2017**, *139* (26), 9044-9052.

4. Yuan, M., Matthew J. Kummer, Ross D. Milton, Timothy Quah, and Minteer, S. D., Efficient NADH Regeneration by a Redox Polymer-Immobilized Enzymatic System. *ACS Catal.* **2019**, 9, 5486-5495.