Electronic Supplementary Material (ESI) for Energy & Environmental Science.

Electronic Supplementary Information for:

Nitrogenase bioelectrocatalysis: heterogeneous ammonia and hydrogen production by MoFe protein

by
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Supplementary Figures and Tables

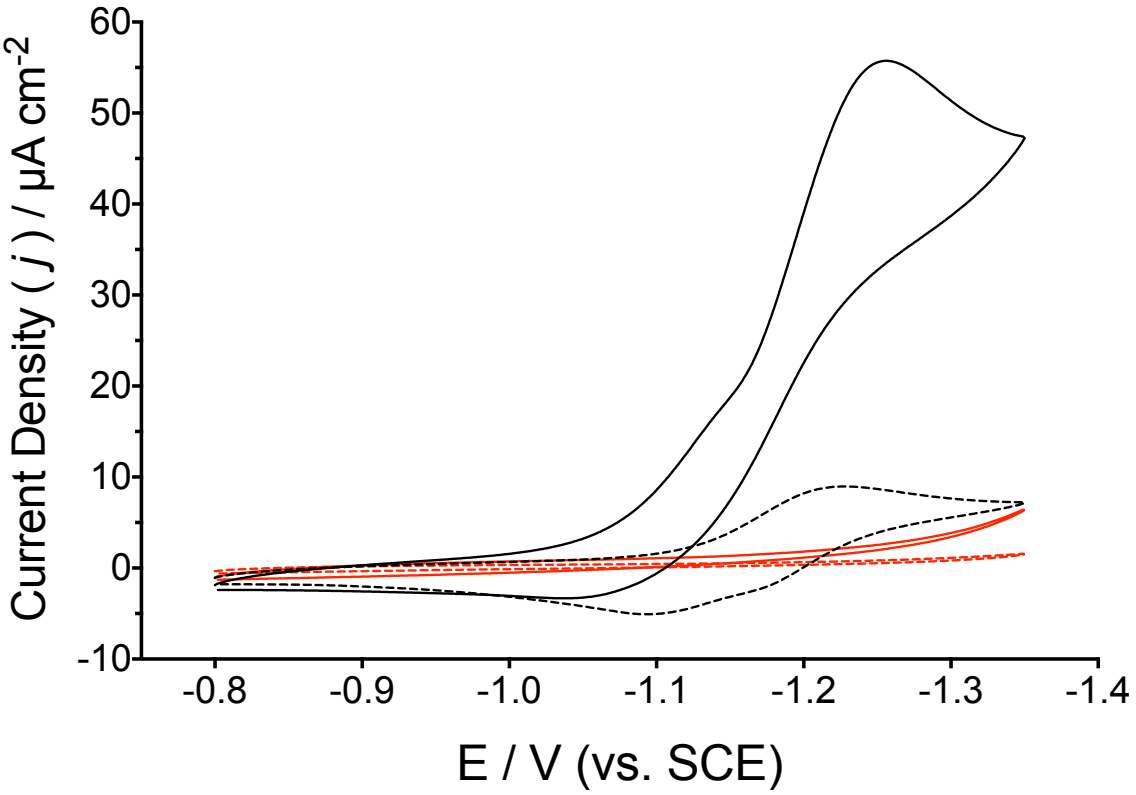

Figure S1 – Cyclic voltammetry of a BSA control bioelectrode in the absence (dashed line) and presence (solid line) of 50 mM NO_2^-, evaluated at a scan rate of 2 mv s^{-1} in HEPES buffer (pH 7.4, 250 mM) in the absence (red lines) or presence (black lines) of 200 µM Cc^+.

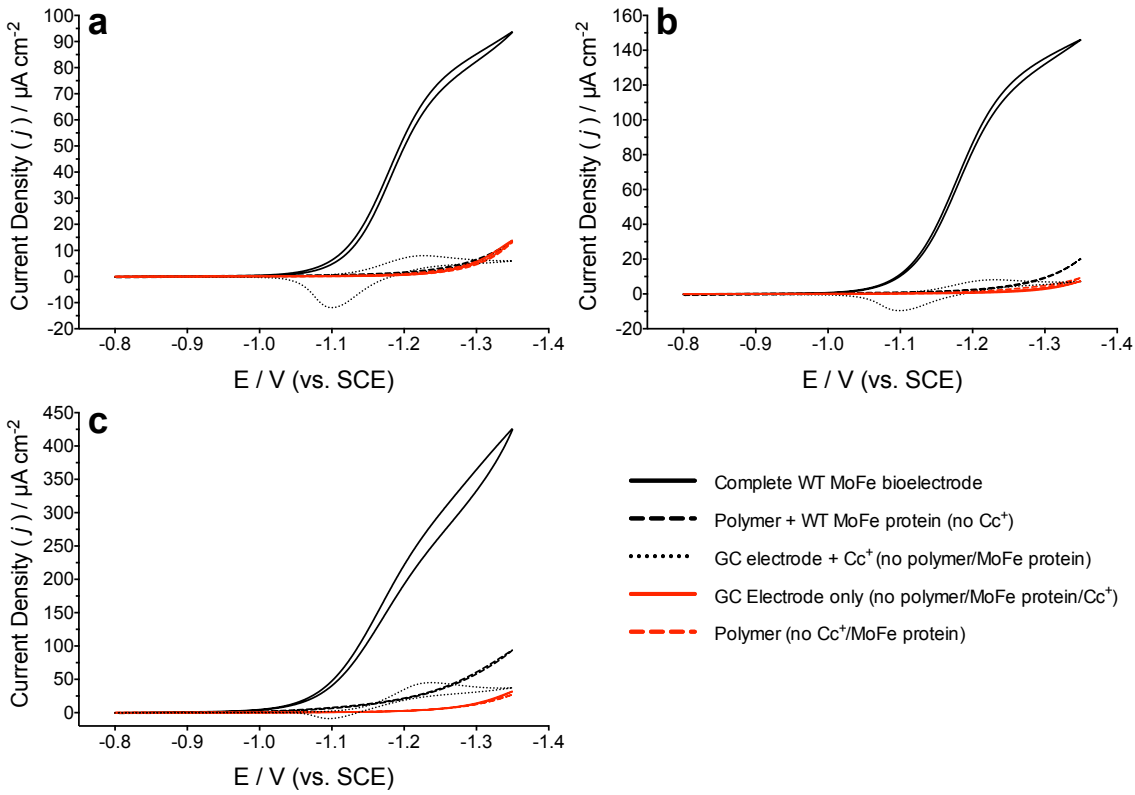

Figure S2 - Cyclic voltammetry of additional control bioelectrodes in the (a) absence or presence of 50 mM (b) N_3^- or (c) NO_2^-, evaluated at a scan rate of 2 mv s^{-1} in HEPES buffer (pH 7.4, 250 mM). Complete MoFe protein bioelectrodes (prepared with WT MoFe protein) are compared against bioelectrodes without Cc^+/Cc (black dashed line), without polymer and MoFe protein (black dotted line), as blank glassy carbon (GC) electrodes only (red solid line) or without Cc^+/Cc and MoFe protein (red dashed line).

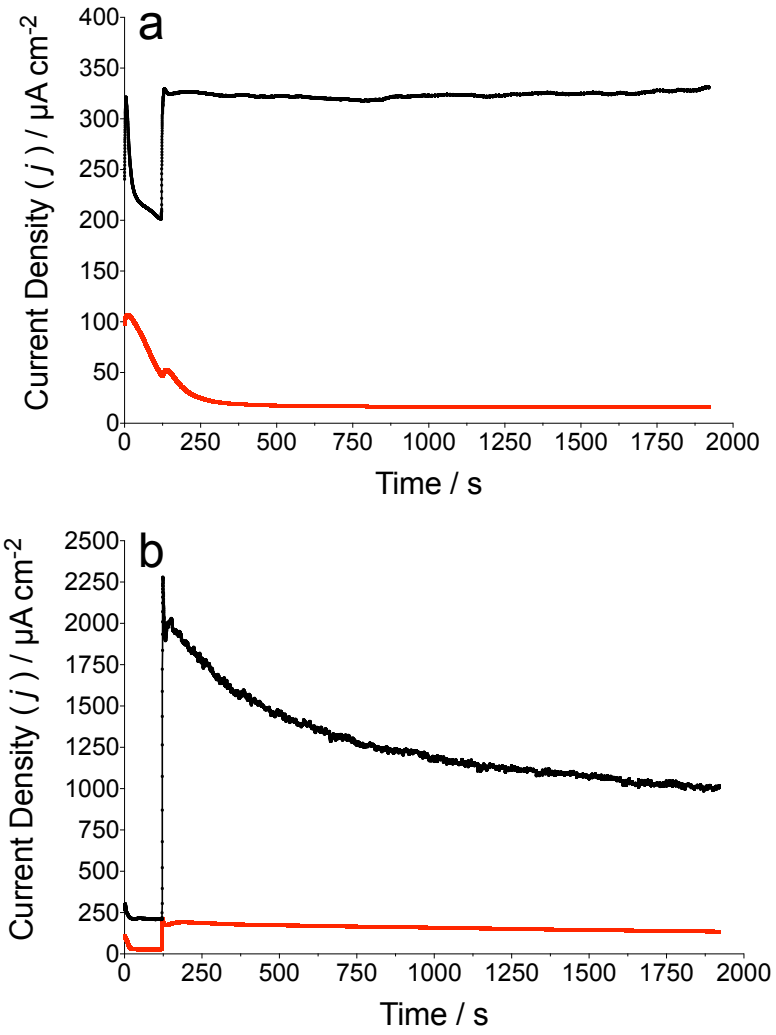

Figure S3 – Bulk bioelectrosynthetic reduction of 50 mM (a) N_3^- and (b) NO_2^- by β-98$^{Tyr\rightarrow His}$ MoFe protein bioelectrodes (black lines), operating in stirred HEPES buffer (pH 7.4, 250 mM) containing 200 µM Cc^+ ($E_{applied}$ = -1.25 V *vs.* SCE). Control bioelectrodes (red lines) were prepared using apo-MoFe protein (red lines). Substrates were injected at 120 s.

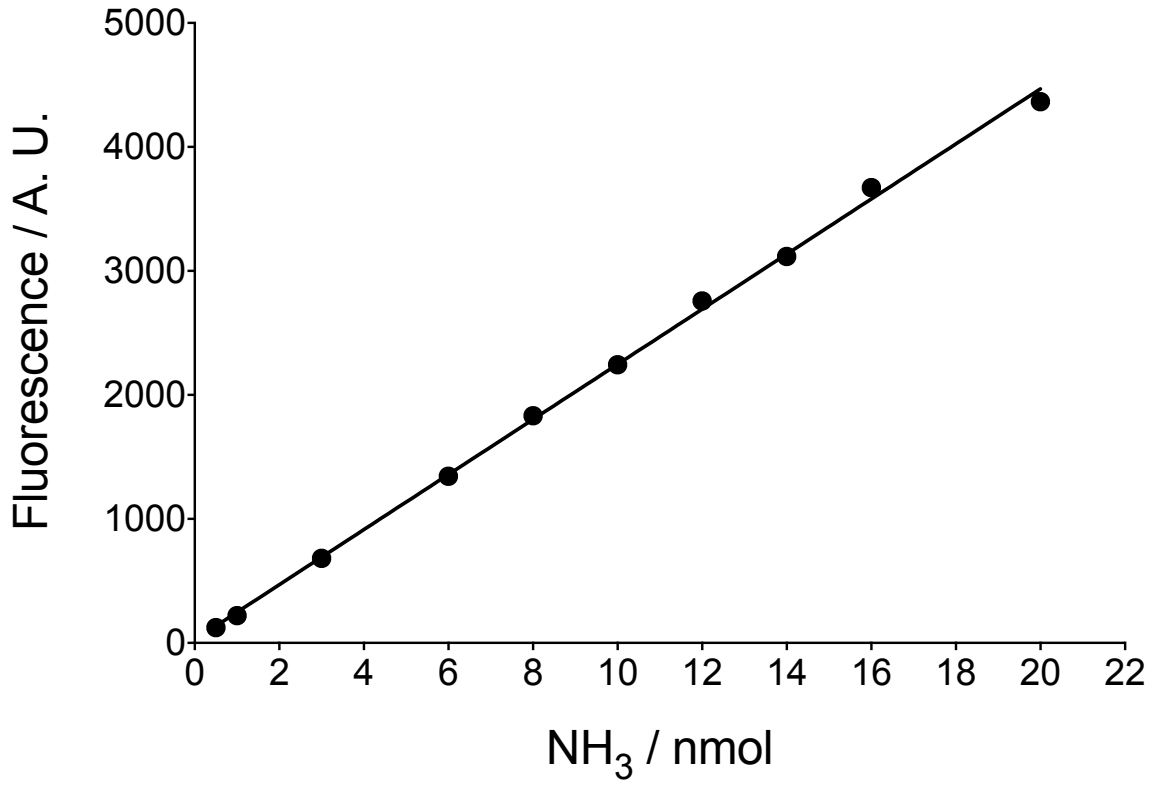

Figure S4 – Calibration standards for NH_3 using *ortho*-phthalaldehyde (detailed below, *n* = 3).

Substrate	Corrected Current Densities (μA cm^{-2})	
	Wild-type MoFe	ß-98$^{Tyr \rightarrow His}$ MoFe
H^+	61 ± 16	136 ± 20
N_3^-	128 ± 21	252 ± 31
NO_2^-	210 ± 57	503 ± 161

Table S1 – Catalytic current densities extracted from cyclic voltammograms of respective MoFe protein bioelectrodes (-1.35 V *vs.* SCE) operating at 2 mV s^{-1} in HEPES buffer (pH 7.4, 0.2 M) containing 200 μM Cc^+ (*n* = 3, mean ± SD). Catalytic current densities are reported corrected to corresponding current densities obtained from apo-MoFe protein control bioelectrodes (errors propagated).

Materials and Methods

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.

Electrochemical analysis

All electrochemical analyses were performed anaerobically (<1 ppm O_2) to avoid competitive O_2 reduction at the working electrode and to preserve nitrogenase MoFe protein activity. HEPES buffer (pH 7.4) was used as the supporting electrolyte at a concentration of 250 mM to avoid migration issues associated with N_3^- and NO_2^-. Electrochemical measurements were performed using a CH Instruments (Inc.) potentiostat (1230b), utilizing a large Pt counter electrode in a traditional 3-electrode configuration.

Cell growth and nitrogenase MoFe protein purification

Azotobacter vinelandii strains DJ995 (wild-type MoFe protein) and DJ1003 (apo-MoFe protein) were grown and polyhistidine-tagged proteins were purified as previously reported.[1] Similarly, strain DJ939 (β-98$^{Tyr\rightarrow His}$) was grown and the corresponding non-His tagged MoFe protein was purified as described. Protein manipulation was either performed under an argon atmosphere in septum-sealed vials (using a Schlenk line) or within an anaerobic tent (<1 ppm O_2, Coy Laboratory Products, MI, USA).

Nitrogenase MoFe proteins were assayed for H^+, C_2H_2 and N_2 activity.

	nmol/min/mg MoFe protein		
MoFe Protein	**H_2**	**C_2H_4**	**NH_3**
DJ995	2493	2349	606
DJ939	1100	600	220

MoFe protein bioelectrode preparation

Purified MoFe protein (20 mg mL^{-1}, 15 µL) was thawed anaerobically and immobilized at the surface of a glassy carbon electrode by mixing with poly(vinylamine) (10 mg mL^{-1}, 15 µL) and EGDGE (10% v/v, 2 µL); 5 µL of this mixture was applied to the surface of each glassy carbon electrode and dried under reduced humidity for 1 hour, prior to testing.

The ratio of polymer:crosslinker is of importance; too little crosslinker results in an unstable film, whereas excessive crosslinker also results in an unstable film whereby excessive EGDGE on the electrode surface results in a film that does not completely dry (EGDGE is a liquid at room temperature). Since EGDGE will crosslink between polymer-polymer, protein-protein and protein-polymer, the concentration of EGDGE also depends on the loading of protein per electrode. For the immobilization of nitrogenase MoFe protein within a poly(vinylamine) film, an approximate concentration of 23 mol%

EGDGE to poly(vinylamine) was utilized, resulting in approximately one epoxide functionality to every two primary amines present on the poly(vinylamine) backbone (where a 10 mg mL^{-1} solution of poly(vinylamine) approaches saturation). Previous work employing redox polymers and using EGDGE as the crosslinker indicate a concentration of between 20-30 mol% EGDGE to be optimal.[2, 3] Finally, a volume of 5 µL of the final enzyme/polymer/crosslinker mixture was applied to the surface of a 3 mm glassy carbon electrode; this volume ensure the complete yet non-excessive coverage of the surface of the working electrode.

Ammonia/ammonium detection assay

NH_3 was quantified as previously reported.[4, 5] An NH_3 detection reagent was prepared by mixing phosphate buffer (pH 7.3, 0.2 M, 100 mL), 2-mercaptoethanol (25 µL) and *ortho*-phthalaldehyde (270 mg dissolved in 5 mL of ethanol) and storing in the dark. Following bioelectrosynthetic experiments, 250 µL of the electrolyte solution (containing NH_3 produced by MoFe protein bioelectrodes) was mixed with 1000 µL of the NH_3 detection reagent and incubated for 30 min in the dark. NH_3 was quantified by exciting the solutions at 410 nm and measuring their emission readings at 472 nm. All samples were quantified as triplicates and corrected against background samples prepared with apo-MoFe protein bioelectrodes.

REFERENCES

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