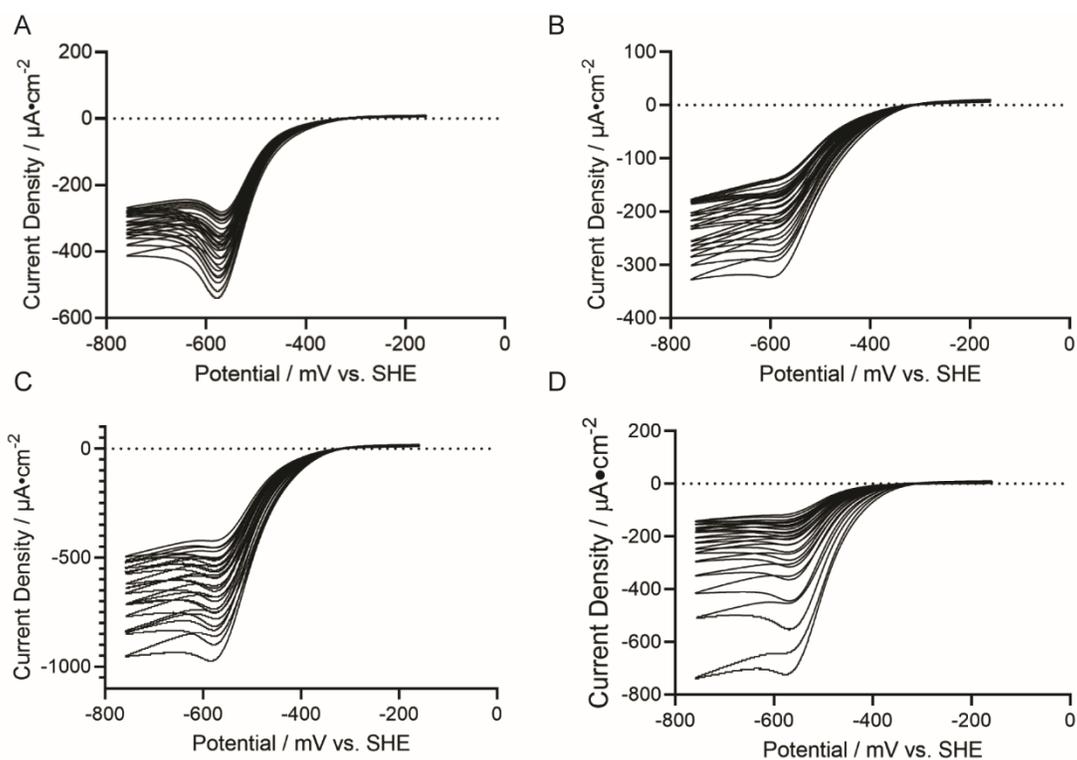


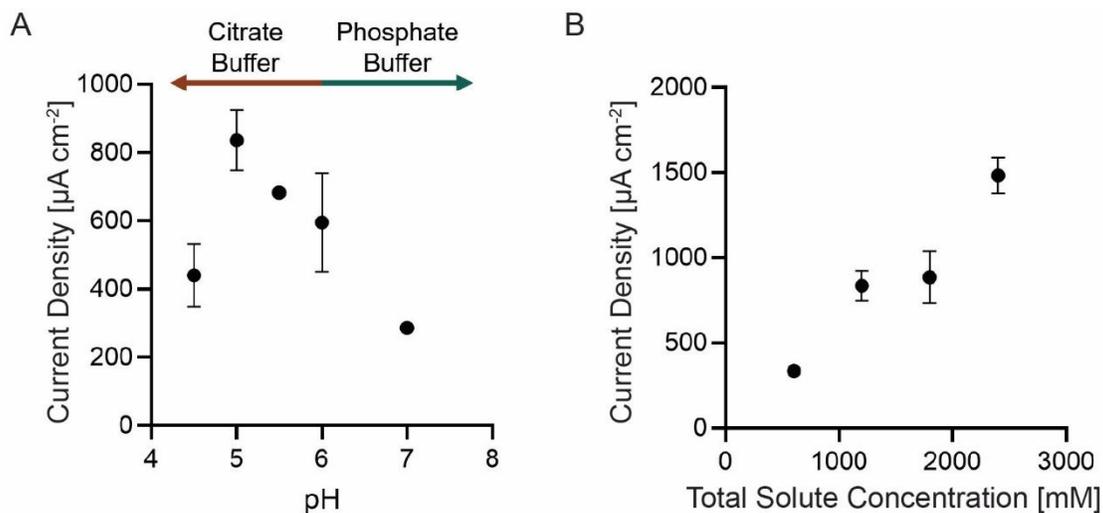
# Supplemental Information: Enzymatic Hydrogen Electrosynthesis at Enhanced Current Density Using a Redox Polymer

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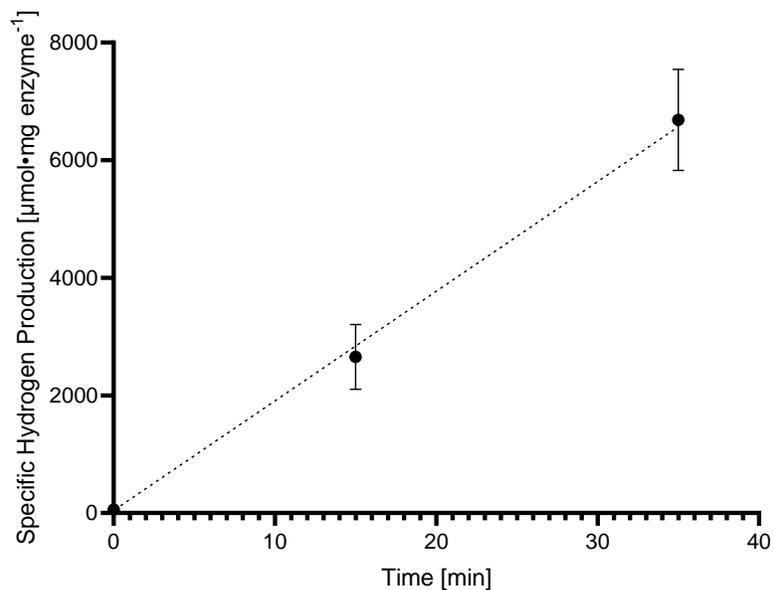
## SUPPLEMENTAL FIGURES



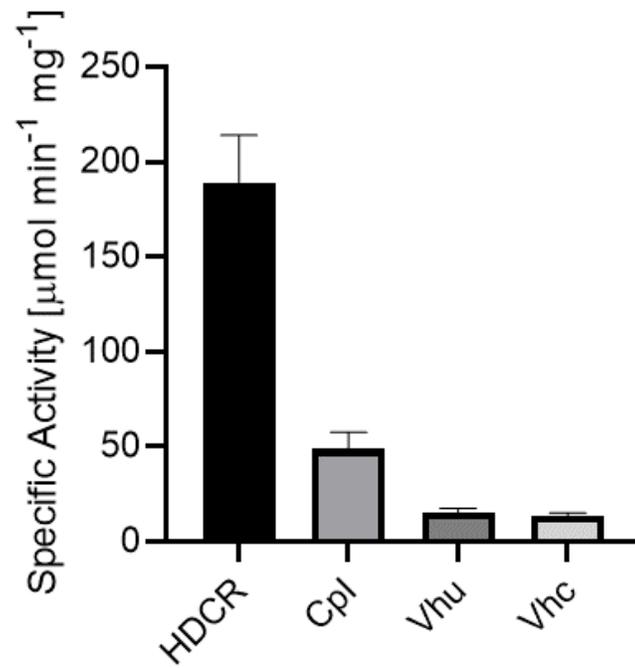
**Figure S1.** Activity comparison of HDCR activity in Cc-PAA at different temperatures. A: 25°C, B: 30°C, C: 40°C, D: 60°C. Tests at 40°C showed high activity, but with less activity loss over time compared to higher temperatures. Phosphate buffer, pH 6.



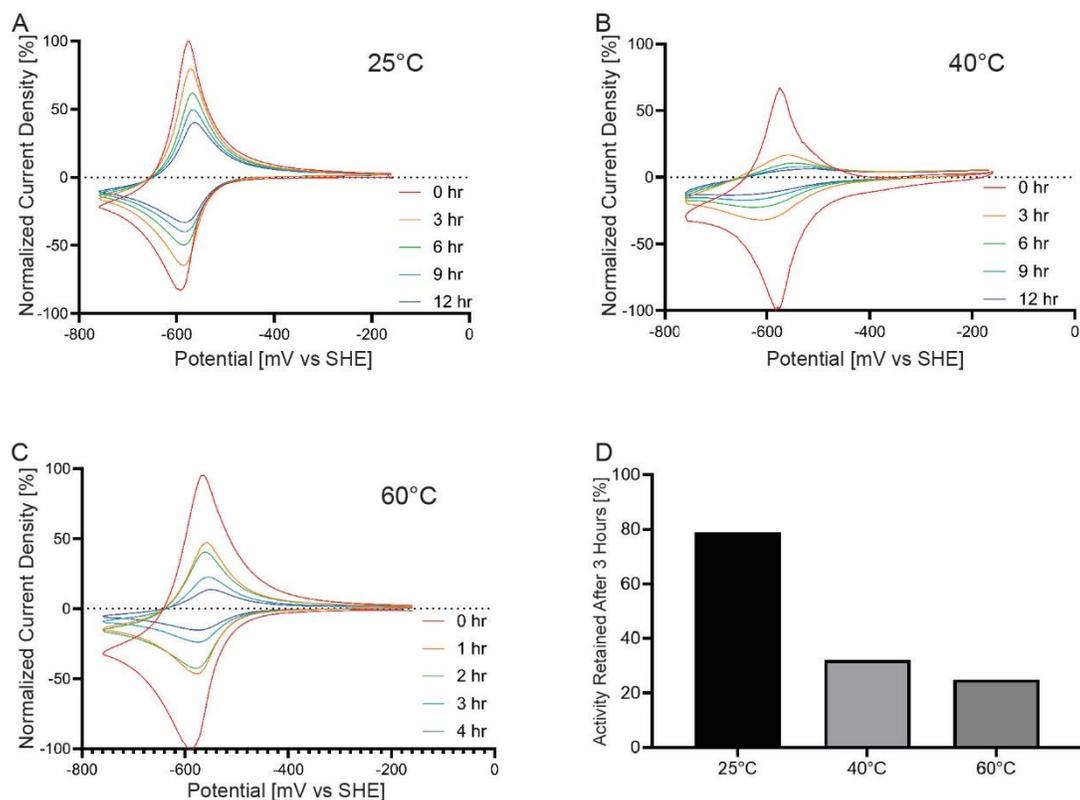
**Figure S2.** pH and buffer concentration optimizations revealed an optimal pH of 5.0 and higher activities at increasing buffer concentrations. **A)** Comparison of the magnitude of the reductive wave for electrochemical hydrogen evolution in buffers of different pH. Citrate buffer was used for pH 6 and below, and phosphate buffer for pH 6 and above, with negligible difference between the buffers at pH 6. **B)** Citrate buffer had a molar ratio of 5:1 sodium citrate:KCl, and this ratio was maintained across all solute concentrations. Activity increased with increasing solute concentration up to the solubility limit of the buffer – a similar trend to that observed in previous FeFe hydrogenase, cobaltocene-mediated systems [1]. Rotation rate: 2000 RPM, scan rate: 10 mV/s. Replicates: N=3.



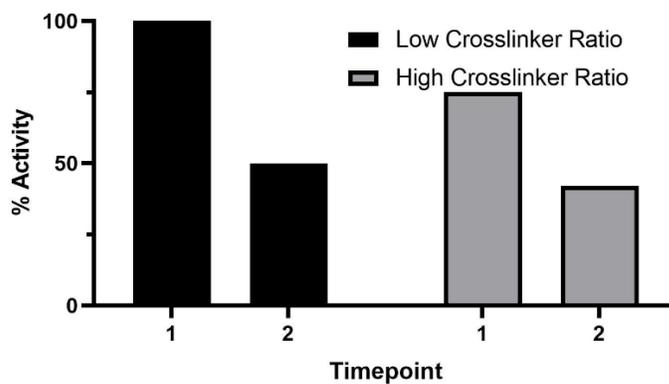
**Figure S3.** Specific hydrogen production by HDCR in 0.2 M sodium citrate and 40 mM KCl, pH 5.0, when mediated by methyl viologen with Ti(III) citrate as electron donor. Similar trends at different rates were observed in phosphate buffer and HEPES buffer, both at pH 6 with dithionite as the electron donor. Test completed at 40°C. Replicates: N=3.



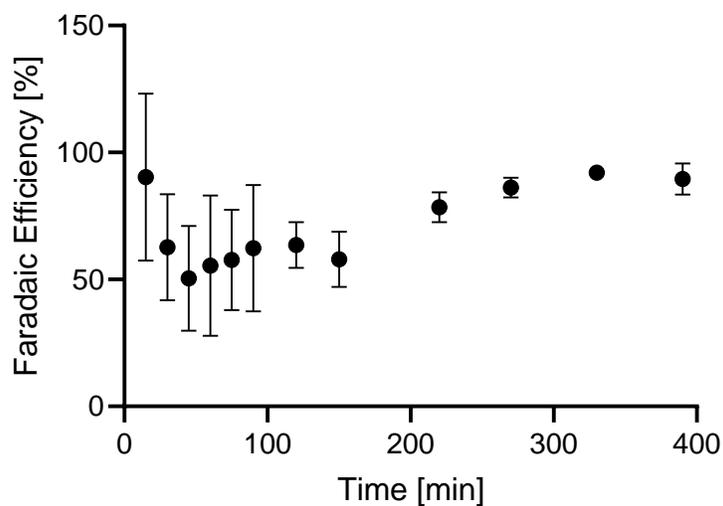
**Figure S4.** HDCR displayed high specific activities for methyl viologen mediated hydrogen evolution, compared to previously reported hydrogenases in their respective optimal electrochemistry buffers.[1] Replicates: N=3.



**Figure S5.** Cobaltocene redox peak magnitude is lost over time at increasing rates with increasing temperature. Of the tested temperatures, Cc-PAA shows the highest stability at 25°C (A), intermediate stability at 40°C (B), and lowest stability at 60°C (C). Note the different time scale between panels – 12 hours for A and B, 4 hours for C. Cycles are normalized for the maximum magnitude achieved at each temperature, respectively. Rotation rate: 2000 RPM, scan rate: 10 mV/s. pH 5.

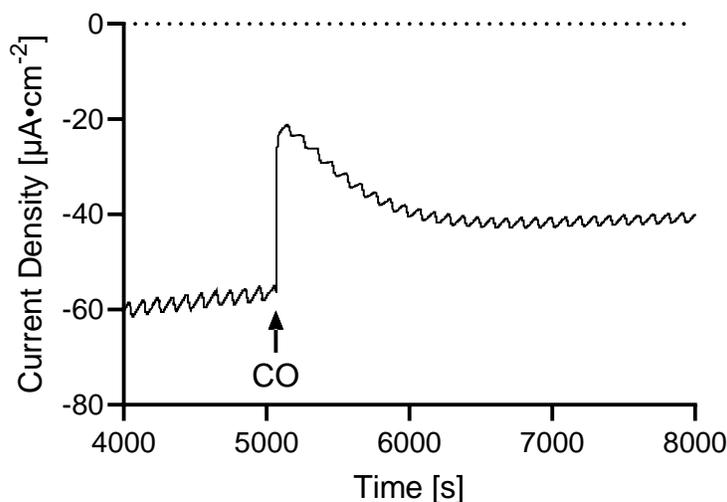


**Figure S6.** Increasing crosslinker ratio resulted in less activity loss over time, but with a lower initial activity. Data shown is the magnitude of the reductive wave during cyclic voltammetry of HDCR embedded in Cc-PAA with the usual crosslinker ratio as “low” and a 2x crosslinker concentration as “high”. Timepoint 1: 1 min; timepoint 2: 29 min. pH 5.



**Figure S7.** Faradaic efficiency of Cc-PAA-mediated HDCR approached 90% over extended testing periods. Large error bars at early timepoints are due to variation in measurements at very

low hydrogen concentrations. Error bars indicate one standard deviation. Replicates: N=2. pH 5, 40°C.



**Figure S8.** Carbon monoxide inactivation during reductive amperometric i-t of Cc-PAA-embedded HDCR displayed an initial strong decrease in activation before regaining activity to a stable value. Subsequent additional inactivation resulted in another initial loss of activity, followed by ca. 70% of activity being regained. Rotation rate: 2000 RPM, potential: -650 mV vs SHE. Citrate buffer, pH 5, 40°C.

## ABBREVIATIONS

HDCR, Hydrogen-Dependent  $\text{CO}_2$  Reductase; Cc-PAA, cobaltocene-functionalized poly(allylamine); BSA, bovine serum albumin; EGDGE, ethylene glycol diglycidyl ether; SHE, standard hydrogen electrode; RPM, rotations per minute.

## REFERENCES

1. Ruth, J.C.; Milton, R.D.; Gu, W.; Spormann, A.M. Enhanced Electrosynthetic Hydrogen Evolution by Hydrogenases Embedded in a Redox-Active Hydrogel. *Chem. - A Eur. J.*

2020, 26, 7323–7329, doi:10.1002/chem.202000750.