Supporting Information

Catalytic Activity, Stability, and Loading Trends of Alcohol Dehydrogenase Enzyme Encapsulated in a Metal–Organic Framework

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Contents

1. Materials

All Starting materials and solvents were used as received without further purification from commercial suppliers. Materials and suppliers were as follows: $AlCl_3$ (Alfa Aesar), CrO_3 (Alfa Aesar), Acetic Anhydride (Bean Town Chemical), Glacial Acetic acid (VWR), Sulfuric acid (95-98%)(VWR), anhydrous $FeCl_3$ (III) (Alfa Aesar), 2,4,6-Trichloro-1,3,5-triazine (Acros Organics), Nicotinamide adenine dinucleotide hydrate (NADH)(VWR), and Tris-HCl (VWR). Solvents used included acetonitrile (ACN), acetone, *N,N*-dimethylformamide (DMF), CH_2Cl_2 (DCM), dimethylsulfoxide (DMSO), and tetrahydrofuran (THF) each obtained from VWR.

GC-MS studies were run on a Shimadzu TQ8050 triple quadrupole gas chromatograph mass spectrometer with a Restek Rtx-5ms column. The headspace vials were heated at 50 °C for 10 minutes and 300 μL of headspace was injected into the instrument. It was an isothermal run at 30 °C for 3-5 minutes.

All ^{1}H NMR spectra were recorded on a Bruker® 400 MHz NMR spectrometer at room temperature in $CDCl_3$ or DMSO-d_6 solution.

2. Enzyme Loading Tables

Table S1: Table representation of the loading of ADH into PCN-333 while receiving infrequent agitation (vortexed twice every other day) during the stability test. Loading solutions contained 0.5 mg/mL ADH, 0.5 mg/mL PPCN-333, and 100 mM Tris-HCl buffer.

Enzyme Loaded (mg)			
Condition	**pH 7 (4 °C)**	**pH 7 (25 °C)**	**pH 8 (4 °C)**
Day 0	0.00457	0.00490	0.00500
Day 1	0.00517	0.00562	0.00629
Day 3	0.00518	0.00590	0.00896
Day 5	0.00478	0.00237	0.00374
Day 7	0.00664	0.00477	0.00572

Table S2: Table representation of the loading of ADH into PCN-333 while receiving frequent agitation. Loading solutions were vortexed to resuspend components several times each day. Loading solutions contained 0.5 mg/mL ADH, 0.5 mg/mL PCN-333, and either 10, 33, or 100 mM pH 7 Tris-HCl buffer.

Enzyme Loaded (mg)				
Condition	**pH 7 10 mM Buffer (25 °C)**	**pH 7, 33 mM Buffer (25 °C)**	**pH 7, 100mM Buffer (25 °C)**	**pH 7, 100 mM Buffer (4 °C)**
Day 1	0.00160	0.00354	0.00429	
Day 3	0.00560	0.00594	0.00713	
Day 7	0.00473	0.00665	0.00899	0.00592

3. GC-MS Analysis: Activity comparison between free and encapsulated ADH

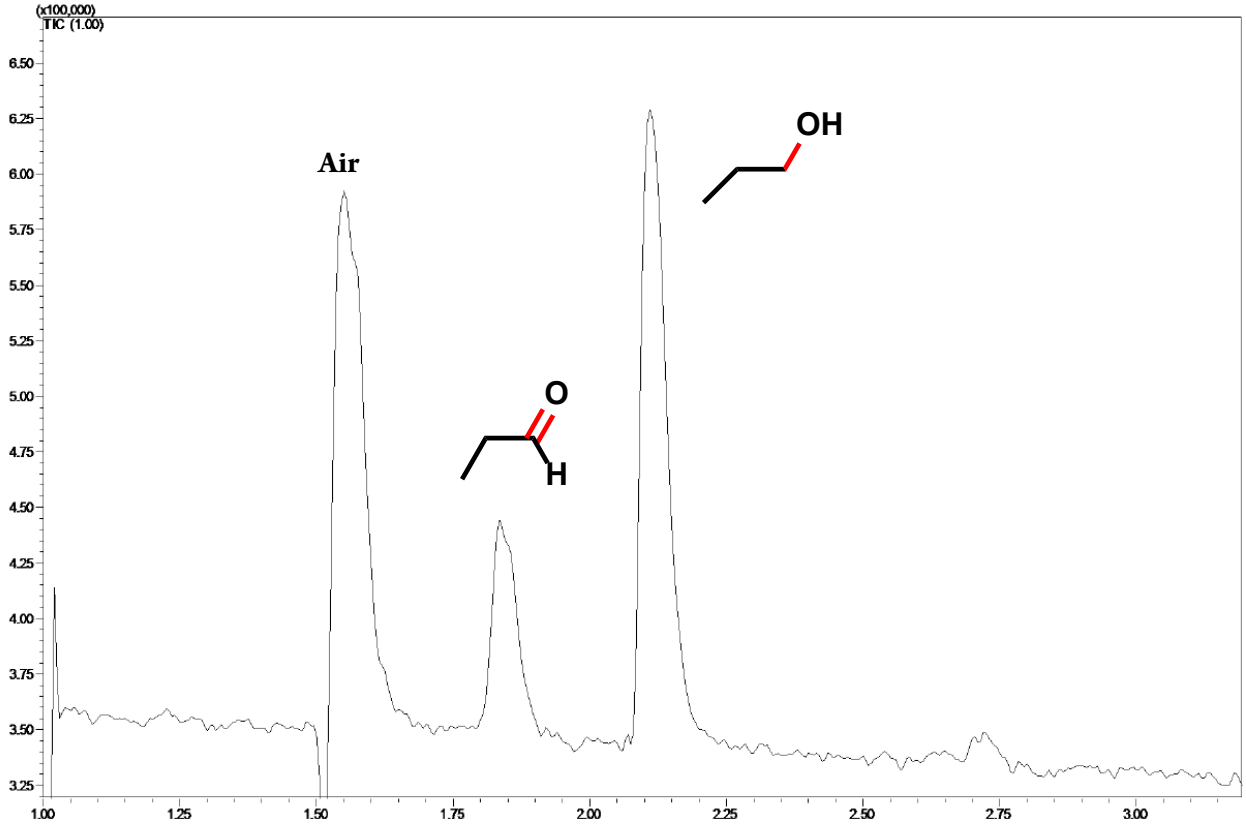

Figure S1: GC-MS of product from reaction of 2 µL propionaldehyde, 10 mM pH 8 Tris-HCl Buffer, 118.8 µL of 200 mM NADH, and 13.04 µg ADH encapsulated in PCN-333 isolated from a 50 µL aliquot of ADH@PCN-333 stock solution after 3 hours of loading. after a 30-minute reaction at 37 °C.

Yield: 72.6 mM 1-Propanol at 30 min (119.9% compared to free ADH).

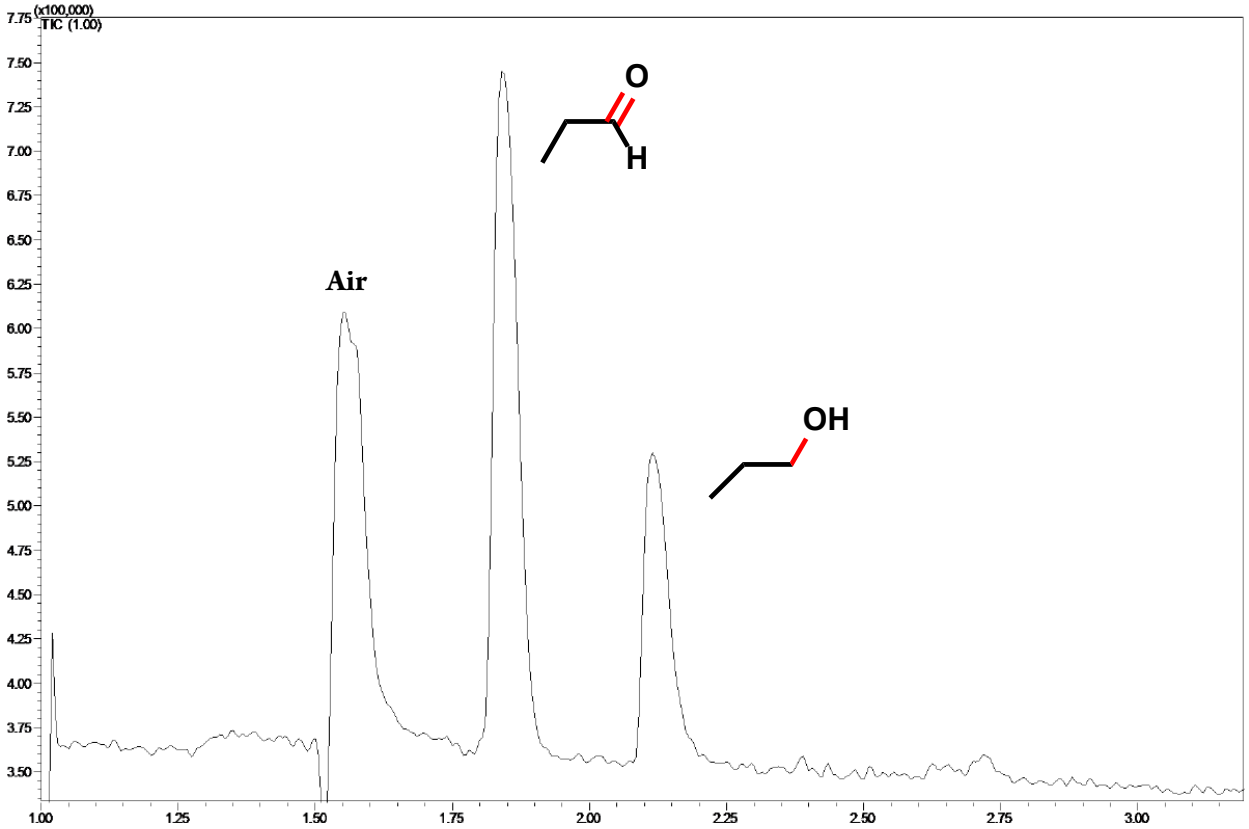

Figure S2: GC-MS of product from reaction of 2 µL propionaldehyde, 10 mM pH 8 Tris-HCl Buffer, 118.8 µL of 200 mM NADH, and 13.04 µg free ADH after a 30-minute reaction at 37 °C.

Yield: 60.6 mM 1-Propanol at 30 min.

4. ^{1}H NMR spectra of linker and precursor

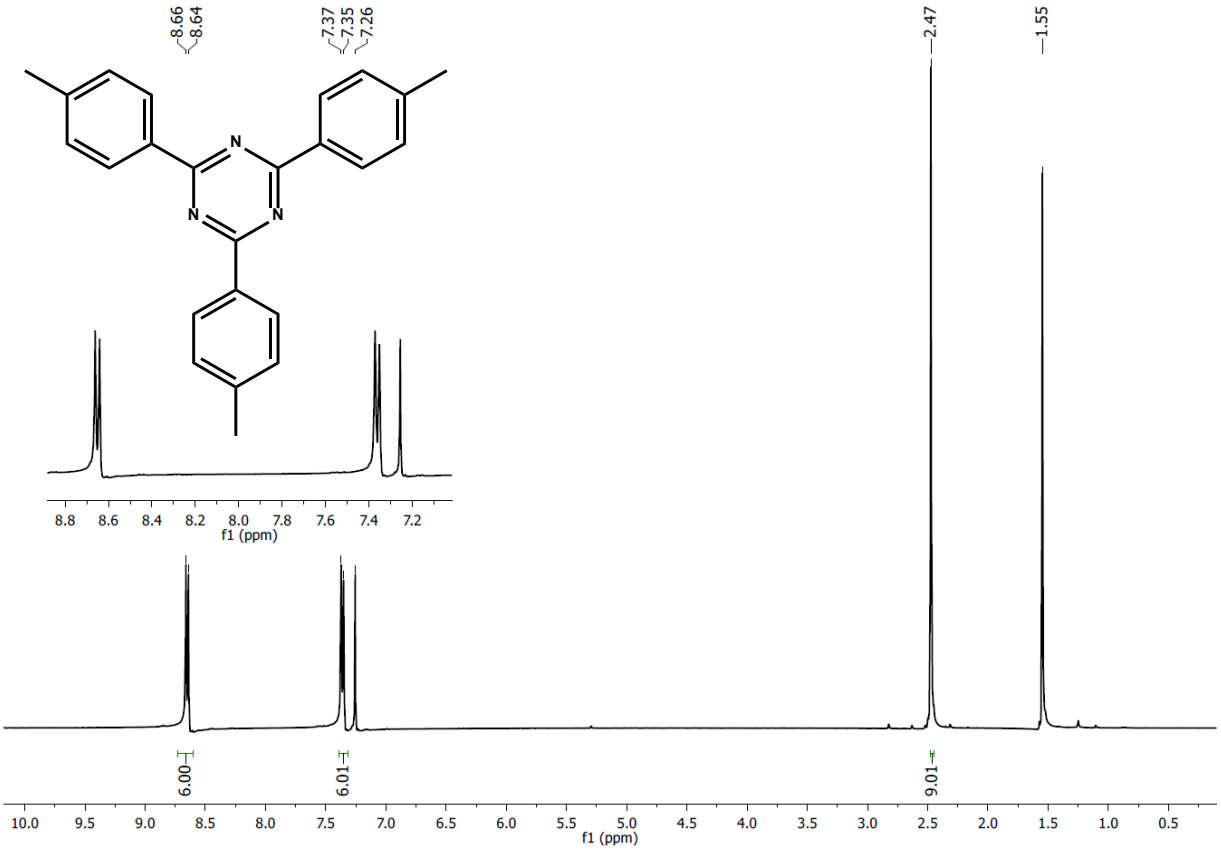

Figure S3: ^{1}H NMR spectra of 2,4,6-Tris(4-methylphenyl)-1,3,5-triazine product ($CDCl_3$). δ = 8.64 (d, 6H), 7.35 (d, 6H), 2.47 (s, 9H).[1]

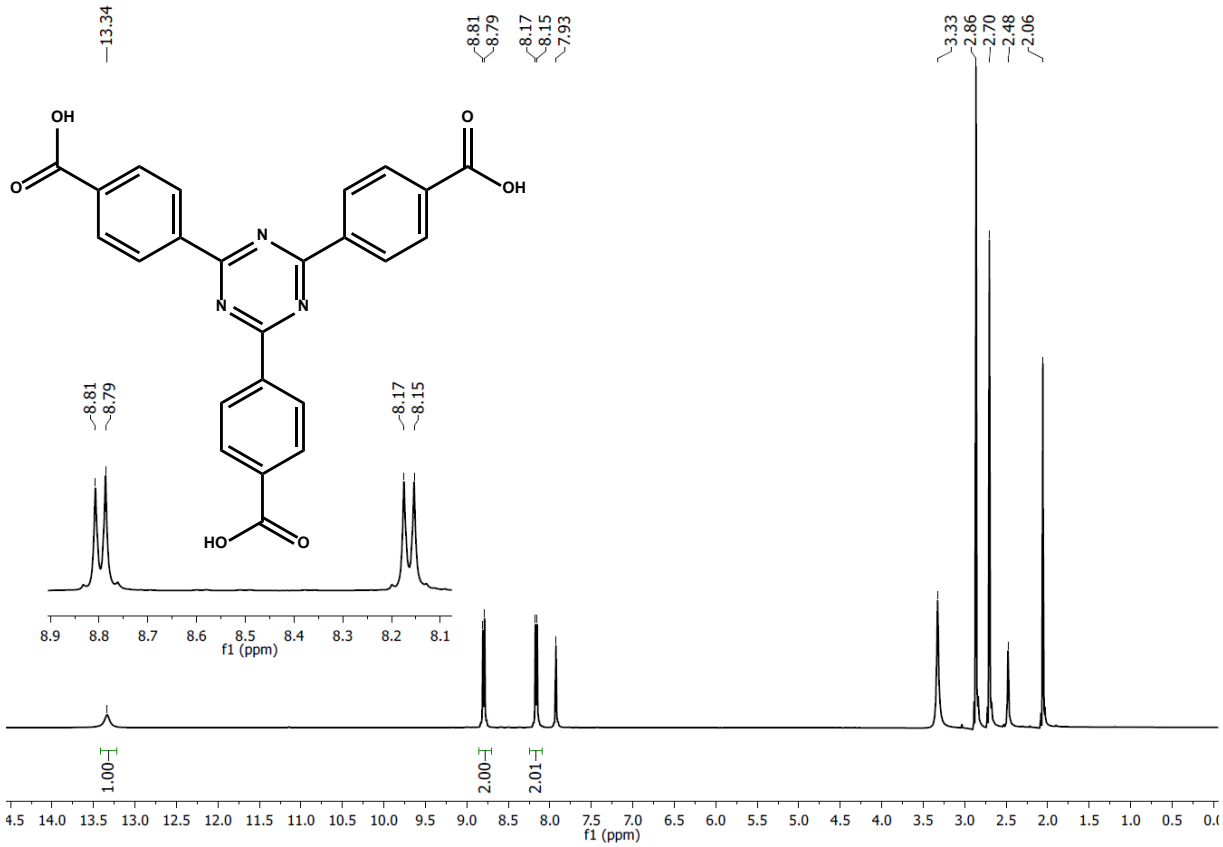

Figure S4: ^{1}H NMR spectra of 2,4,6-Tris(4-carboxyphenyl)-1,3,5-triazine product (DMSO-d_6). δ = 13.34 (s, 3H). 8.79 (d, 6H), 8.15 (d, 6H).[1]

5. References

1. Park, J.; Feng, D.; Zhou, H.-C. Dual Exchange in PCN-333: A Facile Strategy to Chemically Robust Mesoporous Chromium Metal–Organic Framework with Functional Groups *J. Am. Chem. Soc.* **2015,** *137,* 11801-11809.