Supporting Information For

Evolution of catalysts directed by genetic algorithms in a plug-based microfluidic device tested with oxidation of methane by oxygen

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Experimental Section

Chemicals and Materials

All compounds were used as received unless specified otherwise. Europium (III) chloride, iron (II) and iron (III) sulfate, iron (III) nitrate, nickel (II) sulfate, potassium tetrachloroplatinate, sodium molybdate, sodium tungstate, pyridine, deuterated water and deuterated sulfuric acid were obtained from Acros. Hydroge tetrachloroaurate was obtained from Alfa Aesar. 2,2,3,3-D4-sodium-3-trimethylsilylpropionate (TMSP), and methanol-D4 were obtained from Cambridge isotopes. Bipyridine was obtained from EMD biosciences. Iodine, sodium iodide, potassium thiocyanate, silver nitrate, sodium chromate, sodium sulfate, zinc (II) sulfate, methanol, acetic acid, trifluoroacetic acid, hydrochloric acid and sulfuric acid were obtained from Fisher Scientific. Cobalt (II) sulfate, copper (II) sulfate, iron (III) chloride, manganese (II) sulfate, vanadyl sulfate, iridium (III) chloride, palladium (II) chloride, rhodium (III) chloride, ruthenium (III) chloride, sodium acetate, bipyrimidine, picolinic acid and 2,6-pyridinedicarboxylic acid (PDCA) were obtained from Sigma-Aldrich. Formic acid was obtained from Spectrum Chemicals. 1H, 1H- tridecafluoro-1-heptanol was obtained from TCI chemicals. FC-3283 was obtained from 3M. N1,N3-dimethylisophthalamide (L1), N-propylpicolinamide (L2), N-pentylpyridine-2-sulfonamide (L3), N-(pyridin-2-ylmethyl)pivalamide (L4), N-(quinolin-8-yl)acetamide (L5), and N-

propylquinoline-8-sulfonamide (L6) were from a preexisting library. The polyoxometalate $H_5PMo_{10}V_2O_{40}$ (POM-V2) was synthesized according to published protocols.[1] Ultra high purity oxygen and methane, and nitrogen were obtained from Airgas. Teflon PTFE tubing TT-30 (360 μm ID) was obtained from Weico wire. The smaller Teflon PFA tubing (0.03" ID, 1/16" OD, referred to as 750 μm ID tubing) was obtained from Upchurch, and the larger Teflon PFA tubing (0.04" ID, 0.08" OD referred to as 1000 μm ID tubing) was obtained from Stranco. All stainless steel tubing, valves, and gauges were obtained from Swagelok.

Methods

Genetic Algorithm (GA) Protocol: Each individual consisted of three genes: Gene A was composed of catalyst species, Gene B was composed of cocatalyst species, and Gene C was composed of ligand species. The 48 individuals for Generation 1 (G1) were produced by randomly selecting catalysts, cocatalysts, and ligands from the compounds listed in Table S1. Three catalysts species were selected for Gene A, five cocatalysts species were selected for Gene B, and two ligand species were selected for Gene C. The catalysts, cocatalyst, and ligand species selected for a particular gene in a particular individual could be duplicate compounds.

A Microsoft Excel Macro was used to generate the 48 individuals in all further generations. In generations 2- 4 the top 10 individuals (or 11 in the case of a tie for tenth place) in terms of fitness (see Analysis of Plugs to Assign Fitness, below) were paired with 10 (or 11) randomly chosen individuals from the entire population. Each pair then underwent mating, which consisted of crossover at up to three randomly chosen positions, to generate new individuals for the next generation. Additional individuals (16) were randomly chosen from the population and were mutated at up to four positions to generate new individuals for the next generation. The remaining 22 (or 21) individuals for the next generation were generated by randomly selecting catalysts, cocatalyst, and ligand species for Genes A, B, and C, to represent migration. This selection process resulted in relatively low selection pressure, which facilitated continued exploration of the solution space.

For generations 5-8, the top 10 (or 11) individuals in terms of fitness were paired with 10 (or 11) individuals selected from the population using a weighted random selection. In this weighted random selection, the activity of each individual correlated with the probability of being selected; that is, if individual A had a higher fitness score than individual B it was more likely to be selected for the mating pair. During mating, up to four crossover events occurred between pairs. The remaining 38 (or 37) individuals for the next generation were generated by first using the same weighted random selection method to select individuals, and then mutating these individuals at up to five spots to generate individuals for the next generation. During mutation, the chance of a mutated slot being filled with a blank solution was significantly increased as compared to generations 1-4. This selection process increased the selection pressure, and helped to eliminate unnecessary components of the catalyst-cocatalyst-ligand system, thus leading to increased optimization of conditions.

Preparation of Well Plates: Row A of the well plate contained catalyst conditions 1-12, B: 13-24, D: 25-36 and E: 37-48. Rows C and F contained the carrier fluid and indicator solutions. The carrier fluid was composed of 2 μL/mL 1H,1H-perfluoroheptanol (PFH) in purified FC-3283. The indicator solution was 30 mM Na_2CrO_4, 30 mM 2,6-pyridinedicarboxylic acid (PDCA), and 0.50 M H_2SO_4. Each catalyst solution was composed of three potential catalyst species (30 μL of each giving a final concentration of 15 mM), five potential cocatalyst species (6.67 μL, final concentration of 10 mM), and two potential ligand species (38.3 μL, final concentration of 5.75 mM). All stock solutions, and thus the final solutions, were in 50 mM H_2SO_4. 12-Channel pipettes were used to dispense the desired solutions into the wells.

Formation of Reactors: To generate plugs in 1000 μm ID Teflon PFA tubing while minimizing cross contamination, a tip made of 360 μm ID Teflon PTFE tubing was attached to the piece of 1000 μm ID Teflon tubing. This was done by passing the 360 μm ID tubing completely through a small segment of 750 μm ID Teflon PFA tubing. The 360 μm ID tubing fit tightly within the 750 μm ID tubing. This tube-within-a-tube was inserted into one end of the 42” long piece of 1000 μm ID tubing, whose tip had been

stretched to accommodate the smaller tubing. Besides minimizing cross contamination, using the 360 μm ID tubing also allowed for control over solution volumes. The 750 μm ID tubing was used to avoid problems with sealing and allowed for a seamless transition of the contents of the 360 μm ID tubing into the 1000 μm ID tubing. Once this whole configuration was assembled, the opposite end of the 1000 μm ID tubing was attached to a 1 mL Hamilton gas tight syringe. The syringe was filled with degassed FC-3283, which was then used to fill the tubing. Next, the syringe was connected to the syringe pump, and the tip of the tubing was inserted into the starting well position. A labview program was written to couple aspiration by the syringe with the stage that moves the well plate in three dimensions[2] (Movie S1 and Figure S1). The computer controlled syringe pump was used to aspirate approximately 10 "dummy" plugs in order to stabilize plug formation, and then the aspiration of the desired sequence of plugs was initiated. Individuals 1-24 (in rows A and B on the plate) and 25-48 (in rows D and E on the plate) were simultaneously aspirated into two sets of the tubing in quadruplicate. After all plugs were formed, the ends of the tubing were clamped using hemostats. The ends of the tubing were then sealed by heating with a butane torch and clamping shut using tweezers. Next, images of the plugs were obtained on a Leica stereomicroscope, and then the tubing was inserted into the stainless steal reactors. Each reactor was sealed and pressurized in 5 bar increments up to 50 bar by using a 4:46 mixture of O_2:CH_4. The reactors were then submerged in a silicon oil bath and heated to 120 °C. After 3-5 hours, the reactors were removed from the bath and allowed to cool for 15 minutes. Earlier generations required longer reaction times to observe sufficient reactivity, but later generations required shorter reaction times to prevent signal saturation of the indicator plugs. The reactors were then slowly depressurized over approximately 60 minutes. Images of the plugs were acquired again.

Because this system is diffusion-based, the gas must enter the catalyst plugs through the Teflon tubing from the external chamber faster than the rate of reaction (as described by the Damköhler number). It also requires that the rate of product (e.g. methanol) transport between plugs through the carrier fluid is faster than the rate of loss of product through the Teflon tubing into the external chamber. The diffusion coefficient of oxygen through PFA (2.2×10^{-7} cm^2/sec at 25 °C)[3] results in a timescale for diffusion on

the order of 10 minutes, while diffusion through a fluorocarbon with viscosity comparable to FC-3283 (5.7×10^{-5} cm^2/sec at 37 ℃)[4] results in a timescale of diffusion between plugs on the order of 1 minute. The high initial pressure differential between outside and inside and the higher temperature during the reaction should lead to even faster diffusion times into the catalyst plugs suggesting that over the hours-long reaction time the reaction should not be significantly limited by the presence of reagent gas. The time scale difference for transport between plugs and transport into and out of the tubing also suggests that product transport to indicator plugs should dominate loss to the external chamber. This effect should be further enhanced by the fact that the methanol also has to compete with the fluorocarbon for transport through the PFA (it would also compete against the water vapor and gasses, but this is also the case for transport through the fluorocarbon).

Preparation of the reagents in the plate from scratch took ~2 hours, and would be dependent of the chemistry being investigated and availability of pre-made solutions. Automated transfer of solutions into plugs took ~ 1 hour. Reaction and cool-down time was ~ 6 hours. Visual inspection of results could be done in minutes, but we opted to document all of the results by acquiring images, organizing and archiving them and scoring. This process took about 1.5 hours. Analysis of numerical data by GA to design the next generation of catalysts was done automatically in minutes. While the reaction itself is the longest step, automated preparation of plates and automated analysis could speed up this process further to some extent. Increasing the number of plugs per experiment, setting up parallel reactors and staggering the experiments in time (to perform multiple GA-driven evolution experiments) would maximize the output further.

Analysis of Plugs to Assign Fitness: The results of each generation of the GA were analyzed by using the standardized plugs in Figure S3 as a guide. A score of zero to four based on the color of the indicators plugs was assigned to quantify the activity of each catalyst plug. The average score for each individual determined the fitness for that individual in the population. This fitness score was then used in the GA protocol to determine selection for the next generation. The color change of the plugs was observed by eye. For one generation, the images of the plugs were also analyzed using the b channel of

the Lab color setting in Photoshop to determine the color transition independently from visual observation. This method was used to obtain color information in the yellow to blue range, and gave comparable results to measurements made by eye. In this study, semi-quantitative visual observation (scoring) was sufficient to monitor catalyst activity and was faster and easier to perform than automated image analysis. If more quantitative data are required, UV-Vis systems that are compatible with microfluidic systems do exist,[5] but implementing such an integrated system was beyond the scope of this project.

If plug merging had occurred such that no clear assignment could be made, no score was given. Plug merging could occur at any stage of the experiment if the tubing was overly agitated. In particular, the temperature and pressure changes during the reaction could cause nonuniform shifting of the plugs, and the most plug merging occurred during this time. Over the eight generations, 10% of the catalyst plugs had experienced merging that prevented analysis of the catalyst activity. If the catalysts plugs showed merging before the reaction they were not analyzed, and if indicator plugs showed discoloration or abnormal size change after the reaction they were not analyzed. It was not possible to completely eliminate merging in this system, but running the reactions in quadruplicate alleviated this issue. Over 94% of reactions had at least 3 useful plugs per quadruplicate, and only 1.3% of conditions had only one useful plug to analyze.

Purification of Carrier Fluid: FC-3283 was filtered through basic alumina and silica and then distilled using a standard distillation setup. Fractions were collected, and ^{1}H NMR was used to determine the most pure fractions, which were then used as the carrier fluid in all experiments.

NMR Experiments: All 1000 μm ID tubing used for the NMR experiments was pretreated to eliminate leaching of acid. To pretreat, the tubing was filled with 0.5 M NaOH, sealed, enclosed in stainless steel tubing, and heated at 180 °C for five hours. After cooling, the 1000 μm ID tubing was emptied and rinsed with Millipore H_2O until the rinse water was at a neutral pH. Unless otherwise specified, for each reaction, 10 cm of tubing was filled with approximately 6 cm of catalyst solution and then sealed. 50 mM D_2SO_4 in D_2O was used as the solvent. After filling, the tubing was connected on

either end to another piece of tubing containing approximately 5 cm of the solvent to minimize evaporation from the catalyst solution. This configuration was then inserted into the reactor, pressurized in five bar increments with the desired gas ratio, and heated to the desired temperature. After the reaction, the catalyst solution was kept in the tubing, and the entire tube was inserted into an NMR tube containing a 0.5 mg/mL solution of TMSP in D_2O. This setup was allowed to sit for at least 12 hours so that the catalyst mixture could depressurize. The outer solution served as a standard solution for quantitative measurements of the product. The diameters of the NMR tube and Teflon tubing were used to calculate the volume ratio so that the concentrations could be accurately determined. Control experiments showed that this approach gave results that were in good agreement with expected values. The results were also in agreement with measurements taken when the solutions were premixed and no insert was used (Figure S5). An acquisition time of 5 seconds and delay time of 10 seconds was used; further extending the delay time had little effect on the integration. Sodium acetate was used in these calibration experiments to avoid the use of volatile liquids such as methanol. Keeping the catalyst mixture sealed prevented loss of methanol and formic acid due to evaporation and prevented contamination of NMR tubes.

References

(1) Tsigdinos, G. A.; Hallada, C. J. *Inorg. Chem.* **1968**, *7*, 437-441.
(2) Chen, D.; Du, W. B.; Liu, Y.; Liu, W. S.; Kuznetsov, A.; Mendez, F. E.; Philipson, L. H.; Ismagilov, R. F. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 16843-16848.
(3) Extrand, C. W.; Monson, L. *J. Appl. Polym. Sci.* **2006**, *100*, 2122-2125.
(4) Navari, R. M.; Rosenblum, W. I.; Kontos, H. A.; Patterson, J. L. *Res. Exp. Med.* **1977**, *170*, 169-180.
(5) Gotz, S.; Karst, U. *Anal. Bioanal. Chem.* **2007**, *387*, 183-192.

Supporting Figures and Tables

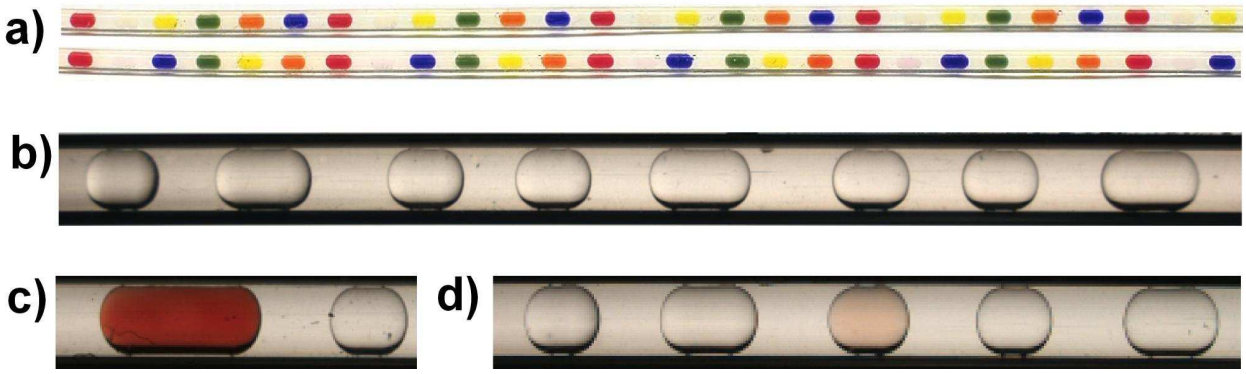


Figure S1: A computer controlled syringe pump coupled with a stage that moves the well plate in three dimensions reliably and reproducibly generated plugs of different compositions (a) and sizes (b). a) Plugs containing six different dye solutions were generated in two different sequences (top and bottom). The standard deviation of plug volume was less than 3% (measured over 160 plugs, data not shown). b) A representative section of tubing from the formation of 200 plugs of different sizes using the automated setup. In this experiment solutions were used that detect cross contamination events. The pattern demonstrated is the one that was used for the GA screening. The larger plugs (1.5 μL) represent catalyst plugs and are composed of 10 mM $Fe(NO_3)_3$; the smaller plugs (1.1 μL) represent indicator plugs and are composed of 60 mM KSCN. Cross contamination between plugs is indicated by the formation of $K_3Fe(SCN)_6$, which is red. c) Plug merging results in an obvious color change. d) Breakoff and transfer of small droplets formed during the transition from the 360 μm ID tubing into the larger 1000 μm ID tubing also results in a visible color change. Throughout the course of plug formation, tube sealing, and image capture, there were only two cross contamination events in 200 plugs. The tubing used in a)-d) had an inner diameter of 1000 μm.

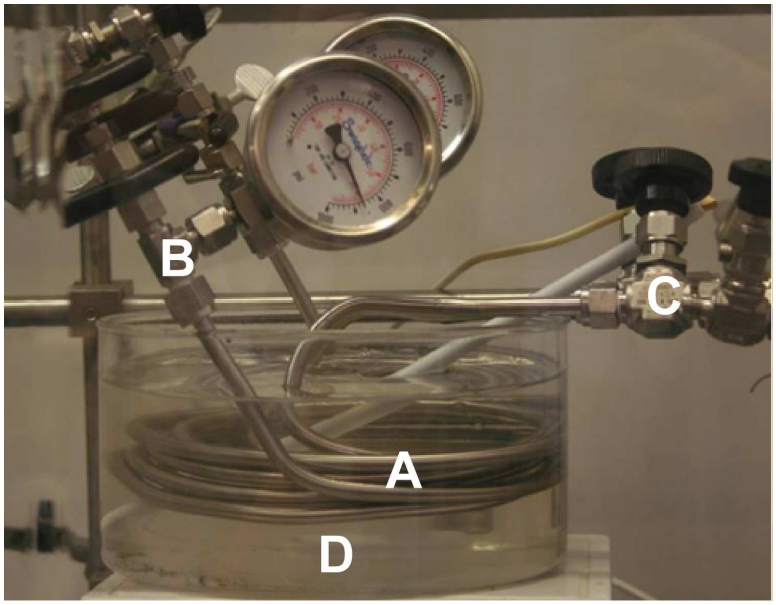


Figure S2: The reaction setup used for all experiments. A blast shield was used but was not shown for clarity. The plug containing Teflon tubing was inserted through the left end of the coiled tubing (A), and the piece (B) containing the pressure gauge and pressure release valve was attached. Gasses were introduced through valves (C) connecting a network of tubes on the right. After pressurization the coils were lowered into the preheated oil bath (D) to initiate the reaction.

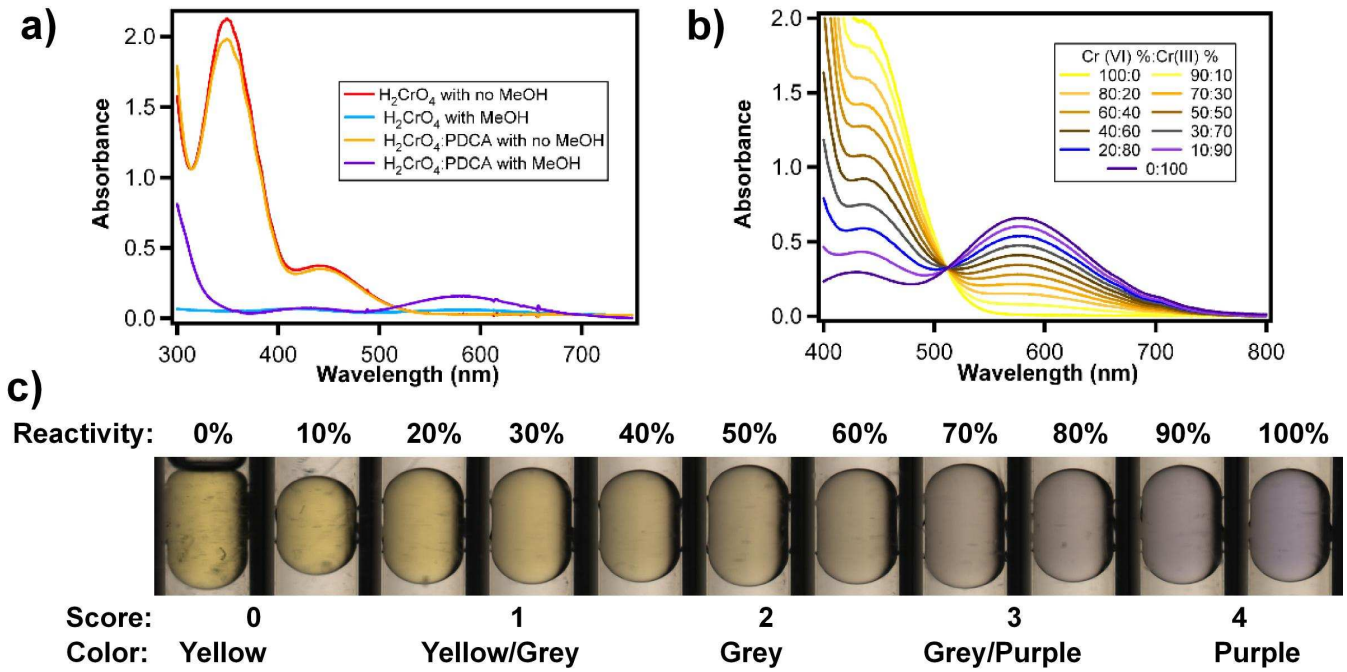
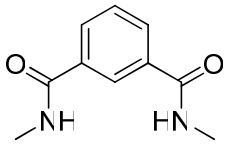
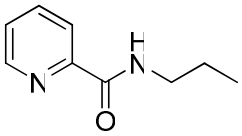
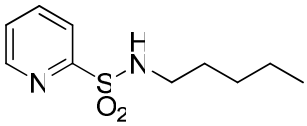
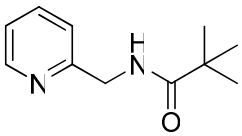
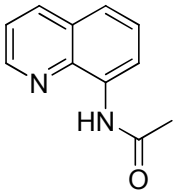
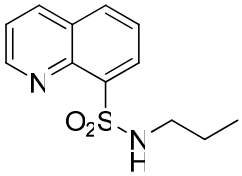
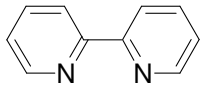
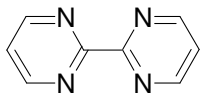
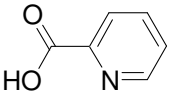
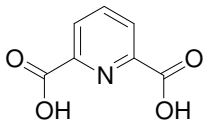


Figure S3: An *in situ* colorimetric indicator system was developed to detect production of methanol from catalyst plugs. a) The difference in UV-Vis absorption between reacted ("with MeOH") and unreacted ("with no MeOH") indicator solutions, in the presence or abscence of 2,6-pyridine dicarboxylic acid (PDCA). The PDCA gives approximately a five fold enhancement at 580 nm. b) Transition in UV-Vis absorption over the course of the reaction. As the reaction proceeds, the absorbance at 580 nm increases and gives a quantitative measure of reactivity. c) Microphotographs showing the color transition in plugs and the corresponding scoring system used to measure extent of reactivity. The solutions used in b) and c) were generated by mixing different ratios of Cr(VI) and Cr(III) solutions containing PDCA.

Table S1. Compounds used to compose the genes

Numerical Value[a]	Gene A, catalyst	Gene B, cocatalyst	Gene C, ligand Structure	Name	Abbreviation
1	K_2PtCl_4	$CoSO_4$		N1,N3-dimethylisophthalamide	L1
2	$PdCl_2$	$CuSO_4$		N-propylpicolinamide	L2
3	$HAuCl_4$	$FeSO_4$		N-pentylpyridine-2-sulfonamide	L3
4	$AgNO_3$	I_2 + NaI		N-(pyridin-2-ylmethyl)-pivalamide	L4
5	$RuCl_3$	$MnSO_4$		N-(quinolin-8-yl)-acetamide	L5
6	$RhCl_3$	Na_2MoO_4		N-propylquinoline-8-sulfonamide	L6
7	$IrCl_3$	$NiSO_4$		Pyridine	Pyr
8	$EuCl_3$	$H_5PMo_{10}V_2O_{40}$		Bipyridine	Bipy
9	Blank	$OVSO_4$		Bipyrimidine	Bipym
10	Blank[b]	Na_2WO_4		Picolinic acid	Pic

11	Blank[b]	$ZnCl_2$		2,6-pyridinedicarboxylic acid	PDCA
12	Blank[b]	Blank		Acetic acid	AcOH
13	Blank[b]	Blank		Trifluoroacetic acid	TFA
14	Blank[b]	Blank			Blank
15	Blank[b]	Blank[b]			Blank
16	Blank[b]	Blank[b]			Blank
17	-	Blank[b]			Blank
18	-	Blank[b]			Blank
19	-	Blank[b]			Blank[b]
20	-	Blank[b]			Blank[b]
21	-	Blank[b]			Blank[b]
22	-	Blank[b]			Blank[b]
23	-	-			Blank[b]
24	-	-			Blank[b]
25	-	-			Blank[b]
26	-	-			Blank[b]

[a] The number used to represent the corresponding component for each gene. [b] These slots were used only for G5-G8.

Movie S1: A demonstration of the formation of the catalyst and indicator plugs in the Teflon tubing using the computer controlled syringe pump and moveable stage.

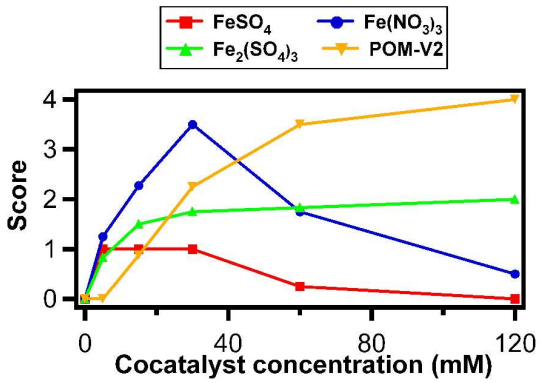


Figure S4: Indicator reactions with 30 mM K_2PtCl_4 and varying concentrations of different cocatalyst species were carried out in plugs for 3 hours at 120 °C and with an O_2:CH_4 ratio of 4:56. Initial pressure before heating was 50 bar. Fe(III) (blue circles and green triangles) shows greater activity than Fe(II) (red squares). POM-V2 (yellow inverted triangles) shows greater activity than both Fe species at higher concentration. Use of nitrate can lead to overoxidation by Fe(III) (blue circles). The activities, as measured by the score from the indicator plugs, of Fe (III) and POM were comparable particularly at lower concentrations.

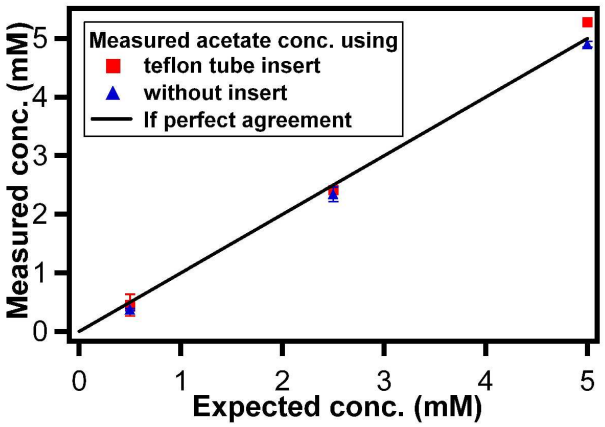


Figure S5: Keeping the catalyst solution in the Teflon tubing during NMR measurements eliminated potential sample loss due to evaporation during transfer from the reaction vessel to the NMR tube. It did not affect the accuracy of the NMR measurements. Calibration experiments with sodium acetate as the standard showed near perfect agreement between the measured and the expected concentrations.

Table S2. Effect of counterions on catalyst activity

Entry[a]	Iron source	Acid (conc. (mM))	Salt (conc. (mM))	Average Score
1	$Fe_2(SO_4)_3$	H_2SO_4 (50)	-	3.1
2	$Fe_2(SO_4)_3$	H_2SO_4 (50)	Na_2SO_4 (50)	2.1
3	$Fe_2(SO_4)_3$	H_2SO_4 (50)	Na_2SO_4 (100)	1.4
4	$Fe_2(SO_4)_3$	H_2SO_4 (50)	Na_2SO_4 (250)	0
5	$Fe_2(SO_4)_3$	H_2SO_4 (100)	-	3.3
6[b]	$Fe_2(SO_4)_3$	H_2SO_4 (50)	-	2
7[b]	$FeCl_3$	HCl (100)	-	0.5

[a] Unless otherwise stated all reactions were performed using 30 mM K_2PtCl_4 and 30 mM concentration of Fe(III) at 120 °C for 4 hours and a 4:46 ratio of O_2:CH_4 at 50 bar of initial pressure before heating. [b] Here, the reaction time was 3.5 hours.

Table S3. Extent of overoxidation of methanol and testing product inhibition.

Experiment[a]	[Pt(II)]:[Fe(III)] (mM)[b]	Additive and (amount (mM))	CH_3OH recovery / TON[c]	HCOOH recovery/ TON[c]	Total recovery/ TON[c]
Overoxidation of methanol	0:0	CH_3OH (5)	59.5%	10.5%	70%
	0.05:0.00[d]	CH_3OH (5)	28%	2%	30%
	0.00:0.75[d]	CH_3OH (5)	44%	16%	60%
	0.05:0.75[d]	CH_3OH (5)	36.5%	12%	48.5%
Formic acid inhibition	0.05:0.75	HCOOH (5)	21.63	92.71%	-
	0.05:0.75	HCOOH (50)	1.64	2.28%	-
Methanol inhibition	0.05:0.75	CD_3OD (5)	22	25	47
	0.05:0.75	CD_3OD (50)	33	23	56
Standard	0.05:0.75	none	23	26	49

[a] Unless otherwise stated all reactions were performed at 180 °C for 6 hours and a O_2:CH_4 ratio of 4:46 at a pressure before heating of 50 bar. [b] K_2PtCl_4 and $Fe_2(SO_4)_3$ were the metal sources and were dissolved in 50 mM D_2SO_4. [c] Values given as % are recovery values and other numbers are TONs. [d] N_2 was used instead of CH_4 so no background production could occur.