

Supplementary Information for

Patterning Precipitates of Reactions In Paper

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Experimental

Reagents. We obtained all chemical reagents from Sigma-Aldrich (Milwaukee, WI) or VWR International (West Chester, PA) and used them without further purification. Stock solutions of the reagents were prepared in deionized water. The solutions of $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ (99.9%, Aldrich) were filtered prior to use to remove an insoluble white solid.

Preparation of the Patterned Paper. We designed 2-D patterns in Microsoft PowerPoint and printed them onto 20 cm \times 20 cm sheets of Whatman No. 1 chromatography paper (Sigma-Aldrich) in either color laser toner or solid wax. For the laser toner, we used a Xerox Phaser Model 6250 color laser printer, selected the print quality setting of “photo” to deposit the most toner possible per print, and printed each pattern three times on the same sheet of paper to ensure that the hydrophobic barrier was thick enough to be impervious to water. Heating the sheet with a heat gun (set at ~ 200 °C) for 1 minute sealed any holes in the layer of toner. For producing the templates with wax, we used a Xerox Phaser Model 8560 solid-ink printer and printed the design on the chromatography paper in duplicate. We then heated the wax with a heat gun (~ 150 °C) until it melted and flowed into the paper such that it permeated the entire 180- μm sheet (~ 10 – 20 s). More information on a variation of this procedure can be found in a previous study.¹ By the end of our investigation, we determined that the templates produced with reflowed wax gave superior results to the templates produced with color toner, although both types of templates were functional (see Results and Discussion section).

Assembly of the Stamps. We used scissors to trim the desired template (from the printed sheet) and mounted it on a silicone rubber stopper (typically, size 8, VWR International) with adhesive tape (Scotch-brand transparent tape, 3M Company). To reduce how often the stamp required re-inking, several “reservoir” layers of chromatography paper were inserted between the

patterned (stamp) layer and the stopper to hold an increased quantity of the ink. After one side of the paper stack was fixed with tape, we wet the sheets using a 100 μL micropipettor. Any excess solution—ink that did not seep into the stamp—was blotted with a lintless wipe, and the stack was sealed to the stopper on all four sides with waterproof tape to ensure that the stack was secure and that ink could not leak out of the sides. We performed the first several stamping repetitions on scrap paper in case the stamp was over-inked.

General Procedure for Patterning Precipitates in Paper by Stamping. The substrate sheet was wet with the second solution of reagent using a surface coverage of roughly 10–15 $\mu\text{L}\cdot\text{cm}^{-2}$. A heat gun set to warm ($\sim 40\text{ }^\circ\text{C}$) was used to dry the substrate layer (this step typically required 2–3 min.). To make each replicate of the pattern, we pressed the inked stamp against the dry substrate sheet for ~ 1 sec. The stamping was repeated as desired or until the stamp ran dry. Once stamping was complete, the substrate sheet was rinsed with water and allowed to air dry.

Precipitation of Paramagnetic Salts in Paper. We precipitated $\text{Gd}(\text{OH})_3$ in both Chromatography Paper No. 1 and No. 3 (which is thicker and more absorbent) from Whatman, Inc. (Sanford, ME). The paper was cut into square pieces measuring 1 cm \times 1 cm that were placed on a plastic Petri dish. A saturated solution of GdCl_3 was dispensed in 5 μL aliquots onto the pieces until they became saturated and would hold no more solution. A lintless tissue blotted away any excess liquid on the surface of the pieces, and the Petri dish was placed in an oven at $70\text{ }^\circ\text{C}$ for 30 minutes. At this stage, the dried pieces adhered to a standard NdFeB bar magnet, but if they were exposed to water, the GdCl_3 redissolved and the paper lost its magnetic response. A solution of 3 M NaOH was dispensed in 5 μL aliquots onto the dried paper with GdCl_3 until the paper again became saturated with liquid. The wet pieces were allowed to stand

for 10 minutes and were then washed under a stream of tap water for two minutes. After they had dried in an oven at 70 °C for 30 minutes, the pieces adhered to a bar magnet and retained this property underwater or after being washed with water.

Precipitation of Horseradish Peroxidase (HRP) in Paper. We established that our commercial sample of HRP (Type II, Sigma-Aldrich) would form precipitates with ammonium sulfate by dispensing 10 μL of a concentrated solution (10 mg/mL) into 200 μL of a saturated aqueous solution of $(\text{NH}_4)_2\text{SO}_4$. An auburn-colored precipitate formed that we subsequently isolated by centrifuging the sample ($16,000 \times g$ for 90 s) and decanting the supernatant. The precipitate dissolved in 100 μL of deionized water, and centrifugation of this sample did not yield a solid pellet. Aliquots (20 μL) of a saturated solution of ammonium sulfate dispensed onto square pieces (2×2 cm) of chromatography paper evaporated at room temperature over the course of 30 minutes. We dispensed 1 μL aliquots of the HRP stock (5 mg/mL) onto the salted pieces of paper, and the same auburn precipitate described above formed in the paper. The pieces of paper were flash frozen by placing them in a plastic boat floating in a bath of liquid nitrogen (the nitrogen never directly came into contact with the paper), and the frozen samples were dried under vacuum. The freeze-drying step was critical to the method; we sometimes noticed complete loss of activity if the samples were simply allowed to air-dry (on standing at room temperature).

Activity of Horseradish Peroxidase. We monitored the activity of HRP based on the method of Banerjee, et al.² In a typical experiment, a piece of paper containing the precipitated HRP was immersed in 10 mL of water and agitated on a rocker for 5 minutes to ensure complete dissolution of the enzyme. A 200 μL aliquot of this solution was added to a mixture of 100 μL of 500 mM sodium acetate (pH 5.5), 100 μL of 17 mM potassium iodide, and 500 μL of

deionized water. After the addition of 100 μL of 10 mM H_2O_2 as a stoichiometric oxidant, the activity of the enzyme was monitored by following the growing absorption of I_3^- at 353 nm. The UV-vis spectrum was recorded every 30 seconds for 5 minutes by an automated program used to control a Hewlett-Packard HP8453 UV-visible spectrophotometer. This software then calculated a linear fit for the data based on an assumption of zero-order (saturation) kinetics and reported a value for the activity of the sample in absorption units per second (AU/sec).

To compare the precipitation method to alternative methods for storing enzymes on paper, we also dispensed 1 μL aliquots of the HRP stock solution onto paper wet with 5 μL of a 10% aqueous solution of trehalose and freeze-dried and assayed these samples as described above. The activity of the HRP in the stock solution was measured by dispensing 1 μL of the stock directly into 10 mL of deionized water and performing the same assay as described above.

Precipitation of a Catalyst on a Simulated Banknote. A scanned image of a one-dollar U.S. Federal Reserve note was altered in Adobe Photoshop by replacing the likeness of George Washington on the observe side of the note with a likeness of Alphonse Capone smoking a cigar. The image was printed in color laser toner onto a sheet of chromatography paper and the excess paper was trimmed away with a paper cutter. A 10 μL aliquot of 3 M NaBH_4 dispensed onto the tip of the cigar dried within minutes. A template to stamp the shape of a circle (diameter = 1 mm) was prepared in laser toner in the manner described above. Once loaded with a 0.25 M solution of PdCl_2 in 3 M HCl (to improve solubility of the PdCl_2), the stamp was pressed against the tip of the cigar to pattern a small circle of Pd^0 into the paper. The sheet was rinsed with water and immersed in a tank of 1 % aqueous H_2O_2 . Bubbles of oxygen (from the disproportionation of H_2O_2) formed at the tip of the cigar and adhered to the paper (Figure S2b).

Generation of a 3-D Cage of Oxygen Bubbles. A template designed in Microsoft PowerPoint and printed onto a sheet of Whatman No. 1 chromatography paper consisted of a ring of exposed paper (on a field of blue toner) with an outer diameter of 10 cm and a thickness of 1 cm. The template was heated at ~ 200 °C for ~ 1 min. to seal any holes in the hydrophobic barrier. The paper was backed with waterproof tape (Scotch-brand transparent duct tape, 3M Company) and affixed to a pliable, flat surface (a thin plastic tray). A second, unpatterned sheet of Whatman No. 1 chromatography paper was wet completely with aqueous 3 M NaBH₄. The sheet was dried with a heat gun set to ~ 40 °C and set flat against a hard, flat plastic surface. The template was inked with 0.25 M PdCl₂ in 3 M HCl, inverted, and pressed against the dry sheet that contained NaBH₄. After pressing down the template to ensure conformal contact at all points of the template, the template was lifted and discarded. A black circle (of Pd⁰) remained on the substrate layer, which was then washed with tap water and dried with the heat gun.

A layer of waterproof tape (Scotch-brand transparent duct tape, 3M Company) applied to the reverse side of the paper limited the formation of oxygen bubbles on the underside of the substrate. A liberal portion of paste (UHU-brand glue stick) was applied to the taped side of the paper, and the entire sheet was glued to the bottom of a plastic tank. The sides of the paper were then taped down (Scotch-brand transparent tape, 3M Company) to limit penetration of the solution of hydrogen peroxide to the underside of the template and to prevent the catalyst-bearing paper from floating. (Once the bubbles of oxygen form, they can adhere to the paper and cause it to float). A 1% solution of hydrogen peroxide was then poured into the tank. Bubbles of oxygen formed immediately, but a stable cage did not form until the waves in the liquid had calmed after its addition (roughly, 30 s to 1 min.). The floating objects sequestered in the cage were constructed by adding a single steel ball bearing (diameter = 2 mm) as ballast to a 600 μ L

plastic microcentrifugation tube with ~400 μL of water dyed with Allura Red AC. Under these conditions, the tubes floated in an upright position (see Figure 3b). When one of the floating tubes impacted the rising “wall” of bubbles, the force of the bubbles pushed the tube back towards the center of the cage.

Table S1. Solubility data for the ionic compounds used in this study. Values correspond to solubility in water at 20–25 °C. The solubility values are reported as they appear in the reference.

Substance	CAS Number	K_{sp}	Solubility (in H ₂ O)	Ref.
Cu(NO ₃) ₂	[3251-23-8]		1.45 g/g	3
Cu(OH) ₂	[20427-59-2]	2.20×10^{-20} M ³		4
Fluorescein	[2321-07-5]		0.3 mg/mL	5
Fluorescein, disodium salt	[518-47-8]		40 mg/mL	5
Gd(OH) ₃	[16469-18-4]	$\sim 10^{-26}$ M ⁴		6
Pb(OAc) ₂	[301-04-2]		443 mg/g	3
PbI ₂	[10101-63-1]	9.8×10^{-9} M ³		3
PdCl ₂	[7647-10-1]		1.9 M [†]	7
KI	[7681-11-0]		1.48 g/g	3
Rhodamine 6G chloride	[989-38-8]		20 mg/mL	5
Rhodamine 6G perchlorate	[13161-28-9]		<0.1 mg/mL	5
NaBH ₄	[16940-66-2]		0.55 g/g	3
NaOH	[1310-73-2]		1.00 g/g	3

[†] This value corresponds to the concentration of PdCl₂ when saturated in 3 M HCl.

Figure S1. Pieces of paper containing precipitated $\text{Gd}(\text{OH})_3$ adhere to a NdFeB bar magnet. The pieces retain their paramagnetism in water because of the poor solubility of gadolinium hydroxide.

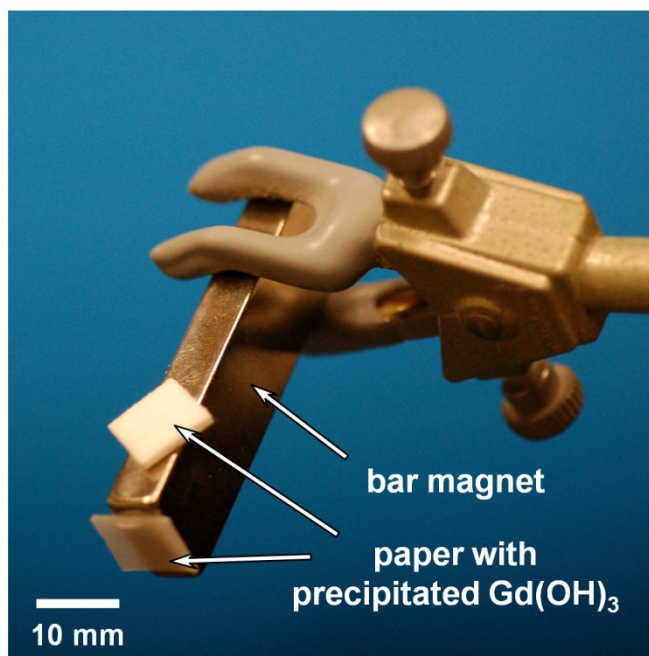
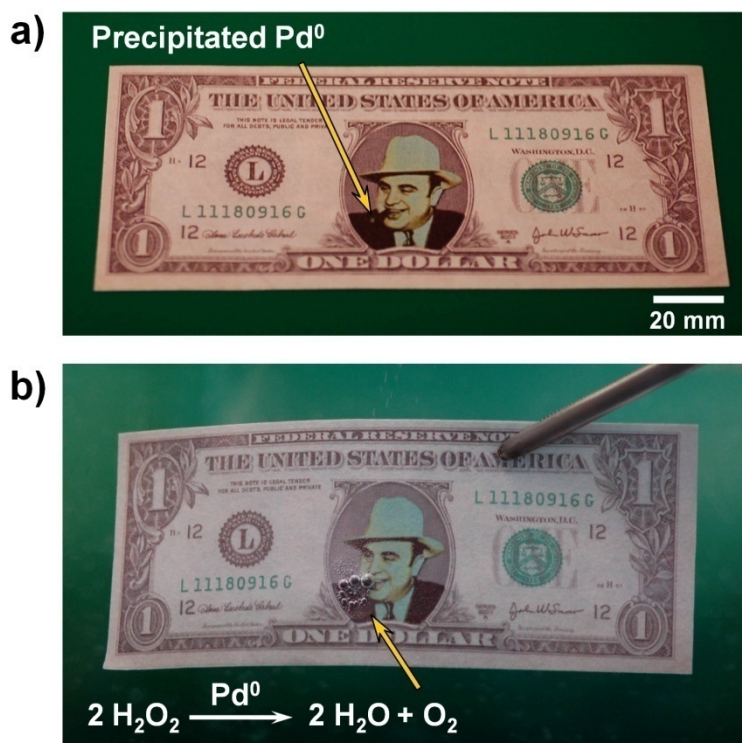


Figure S2. a) A simulated banknote patterned with a catalytic region for verification of authenticity by a chemical reaction. The tip of the cigar in the likeness of Alphonse Capone has been stamped with a circle of Pd⁰. b) The simulated banknote immersed in a 1% solution of aqueous hydrogen peroxide. The Pd⁰ catalyzes the disproportionation of hydrogen peroxide to generate bubbles of oxygen that adhere to the paper.



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- (7) We measured the solubility of PdCl₂ in 3 M HCl using UV-vis spectroscopy and the Beer-Lambert relation. The molar extinction coefficient of the complex of palladium at 472 nm was $162 \pm 2 \text{ M}^{-1} \text{ cm}^{-1}$ (95% confidence interval).