Chemists frequently encounter problems associated with trace palladium in synthetic samples because palladium is presumably the most frequently used transition metal in synthetic organic chemistry. We previously reported a colorimetric method for trace palladium quantification, the only high throughput method implemented in the pharmaceutical industry. However, slight changes from the published reaction conditions have caused reproducibility problems, with little understanding of underlying molecular mechanisms. In the current study, we took a combinatorial approach to investigate the method and found that strong basicity was a culprit for the lack of reproducibility. We changed the reaction conditions and procedure accordingly, which substantially improved reproducibility. We discovered that the reaction followed Michaelis-Menten kinetics, allowing for predicting reaction rates on the basis of the substrate concentrations. The current method showed good correlation with inductively coupled plasma mass spectroscopy when 14 synthetic samples with unknown amounts of trace palladium were quantified.
Third-generation method for high throughput quantification of trace palladium by color or fluorescence

Lydia Lukomski, Ivanna Pohorilets, and Kazunori Koide*
Department of Chemistry, University of Pittsburgh
219 Parkman Avenue, Pittsburgh, Pennsylvania 15260, United States
koide@pitt.edu

Abstract
Chemists frequently encounter problems associated with trace palladium in synthetic samples because palladium is presumably the most frequently used transition metal in synthetic organic chemistry. We previously reported a colorimetric method for trace palladium quantification, the only high throughput method implemented in the pharmaceutical industry. However, slight changes from the published reaction conditions have caused reproducibility problems, with little understanding of underlying molecular mechanisms. In the current study, we took a combinatorial approach to investigate the method and found that strong basicity was a culprit for the lack of reproducibility. We changed the reaction conditions and procedure accordingly, which substantially improved reproducibility. We discovered that the reaction followed Michaelis-Menten kinetics, allowing for predicting reaction rates on the basis of the substrate concentrations. The current method showed good correlation with inductively coupled plasma mass spectroscopy when 14 synthetic samples with unknown amounts of trace palladium were quantified.

Introduction
To comply with government’s regulations (e.g., USP <232> in the United States),¹ the pharmaceutical industry must ensure that heavy metals are below their toxic levels in active pharmaceutical ingredients (APIs). Among heavy metals, palladium is the most frequently used transition metal in chemical synthesis² and must be below 10 ppm in APIs, according to USP <232>. Therefore, process chemists must develop a specific and highly efficient palladium-scavenging protocol for each API to meet the safety regulation. This is accomplished through screenings of dozens of potential workup protocols and scavengers, among other purification techniques.³⁻¹⁰ This process generates several dozens of samples in which to quantify palladium. Meanwhile, new scavengers continue to emerge,⁷⁻¹² adding options to explore.
Trace metal analysis has been performed using inductively-coupled plasma mass spectrometry (ICP-MS).\textsuperscript{13-17} Unfortunately, ICP-MS is a highly specialized, costly method for analyzing palladium. The instrument is large and expensive, requires highly skilled experimentalists, a long calibration time upon starting, and expensive materials to maintain operation. Additionally, ICP-MS often becomes the bottle neck of the entire API purification process because scavenger screenings are high throughput while ICP-MS is low throughput, thus slowing the process.

As such, high throughput platforms are needed to streamline the API production process. A grand challenge is to shift from the established, but time-consuming, paradigm to high throughput experimentation, even at the expense of accuracy to some extent.\textsuperscript{18} Fluorometric and colorimetric methods are high throughput and easy to master. Moreover, fluorescence and/or UV-vis plate readers are inexpensive, robust, and do not require calibration. Due to the ease of use and portability of fluorometers, a colorimetric or fluorometric method can be employed on site (i.e., in the laboratory where APIs are purified), eliminating the need to transport synthetic samples.

![Scheme 1. Pd-catalyzed deallylation of resorufin allyl ether.](image)

We developed a high throughput method for quantifying trace palladium in APIs by fluorescence or color (Scheme 1).\textsuperscript{19, 20} In this method, resorufin allyl ether (RAE) undergoes palladium-catalyzed allylic C-O bond cleavage (Tsuji-Trost type reaction) to produce fluorescent, magenta resorufin in its anion form. The correlation between the resorufin concentration and fluorescence signals with our instrument (see the experimental procedure section for detail) is shown in Figure 1. The reaction is accelerated by tri(2-furyl)phosphine (TFP) and sodium borohydride (NaBH\textsubscript{4}). The roles of TFP are two-fold: first, it binds to palladium to form catalytically active
species\textsuperscript{21-23}; second, it acts as a nucleophile (manuscript submitted for publication; please find the reviewers-only material). The reaction autonomously stalls due to the air-oxidation of palladium(0) when NaBH\textsubscript{4} is used up and can be re-started upon the addition of more NaBH\textsubscript{4}, broadening the dynamic range with respect to palladium concentration. This is convenient when the signal intensity continuously increases over time, because it is difficult to start and analyze dozens of reactions in wells. In other words, autonomous stalling provides unorthodox, time-independent data after a certain period for the catalysis-based method. This method has been implemented in the multiwell-format\textsuperscript{19} and in flow chemistry in the pharmaceutical industry.\textsuperscript{20} Others have used similar O-deallylation reactions with different fluorophores or prodrugs as analytical\textsuperscript{24} or biological tools.\textsuperscript{25-33}

DMSO was used in our previous and current methods because it dissolves most APIs and thus is frequently used to prepare stock solutions for trace metal analysis. However, excess DMSO was avoided because it decelerated the deallylation reaction in the previous system, although the upper limit of DMSO amount was not specified.\textsuperscript{19} We chose EtOH as the majority of the assay mixture to accommodate both aqueous and organic layers during the extraction process after a palladium-catalyzed synthetic reaction is employed. A small fraction of water is necessary to dissolve NaBH\textsubscript{4}. Resorufin is strongly fluorescent only above pH 4.8,\textsuperscript{34} necessitating buffering or basifying the assay solutions. Although phosphate ions accelerate the palladium-catalyzed allylic C-O bond cleavage (submitted for publication), phosphate reagents are not sufficiently soluble when the assay solution contained only a small fraction of water. Instead, NH\textsubscript{4}OAc was chosen to neutralize acids that may be part of palladium-contaminated APIs after sample digestion with acid.

The concentrations for most components were optimized by evaluating one component at a time.\textsuperscript{19} Recently, our group began applying the technology to real-world samples. These studies required perturbations of components’ concentrations and order of addition of chemicals, which afforded nonreproducible results. These problems made us realize that the reaction conditions might not be optimal because some reagents react with each other, necessitating the simultaneous perturbation of multiple parameters. Therefore, we decided to develop a more robust method while investigating how the relative concentrations of reagents impact reproducibility and sensitivity. This work has resulted in a more sensitive method for quantifying palladium. It has also provided a blueprint for reproducibility, and no experiments failed during this study after the blueprint emerged.

Result

Problems with the previous method. Our original aims were to improve the sensitivity of the previous colorimetric method and to improve the reproducibility. Our previous conditions are summarized in entry 1 (Table 1): At the outset of this study, we performed experiments with lower concentrations of NH\textsubscript{4}OAc because this salt is difficult to dissolve in EtOH. Specifically, the reactions were performed under the reaction conditions shown in entry 2.1 (Figure 2a, circles); although the signals were weak, a linear correlation between the palladium concentration and fluorescence signal (excitation 525 nm, emission 570–640 nm) was observed. After 16 min, the reactions autonomously stalled. Because the color change was not visibly obvious, as shown in the photograph, we restarted the catalysis with additional NaBH\textsubscript{4} (stop-and-go). As expected from our previous work,\textsuperscript{19} both the color and fluorescence signals intensified (entry 2.2). The second addition of NaBH\textsubscript{4} further increased both the color and
fluorescence intensities (entry 2.3). However, in the 0–0.8 nM palladium range (the first three columns), the solutions changed from yellow to purple, making it difficult to visibly distinguish the magenta color from the purple color. We were also aware that too many parameters, such as time and the concentrations of NaBH₄, NaOH, and H₂O, varied in entries 2.1, 2.2, and 2.3. When the NaBH₄ concentration was 50 mM from the beginning (entry 3), all the solutions turned purple (Figure 2b).

Table 1. All the reactions were performed at 24 °C with 0–250 nM PdCl₂.

| Entry | RAE (μM) | TFP (μM) | NaBH₄ (mM) | NaOH (mM) | NH₄OAc (mM) | DMSO (% v/v) | H₂O (% v/v) | EtOH (% v/v) | Time (min) |
|-------|----------|----------|------------|-----------|-------------|---------------|-------------|--------------|------------|
| 1     | 29       | 200      | 2–75       | 235       | 626         | 7             | 12          | 81           | 60         |
| 2.1   | 30       | 200      | 10         | 50        | 300         | 6             | 12          | 82           | 16         |
| 2.2   | 27       | 181      | 45         | 405       | 272         | 5.5           | 20          | 74.5         | 16         |
| 2.3   | 23       | 167      | 75         | 700       | 250         | 5             | 26          | 69           | 16         |
| 3     | 30       | 200      | 50         | 500       | 300         | 6.5           | 12          | 81.5         | 16         |
| 4     | 30       | 180      | 50         | 20–420    | 250         | 6             | 20          | 74           | 16         |
| 5     | 30       | 180      | 50         | 70        | 0–375       | 6             | 9           | 85           | 16         |
| 6     | 30       | 180      | 50         | 120       | 0–375       | 6             | 10          | 84           | 16         |

Figure 2. (a) Sequential additions of NaBH₄. (b) Starting with high concentration of NaBH₄.

Because the observations above were not easily interpreted, we redesigned an experiment so that only the NaOH concentration would vary (entry 4 and Figure 3a). Both the lower and higher NaOH concentrations decelerated the deallylation reaction. We initially thought that at lower NaOH concentrations, NaBH₄ degraded rapidly and thus was unable to reduce air-oxidized palladium species. At higher NaOH concentrations, once again the reactions
turned purple. If the method can tolerate only a narrow range of NaOH concentrations, that would make the method difficult to employ. As we discuss below, this was not the case.

With the tentatively optimal NaOH concentration (70 mM), we investigated the effect of NH$_4$OAc concentration (entry 5 and Figure 3b). When the NH$_4$OAc concentration was above 100 mM, there was no difference between palladium-free solutions and 30 nM palladium solutions. When the NH$_4$OAc concentration was below 100 mM, 30 nM palladium solutions showed increased signals. Importantly, the lower NH$_4$OAc concentrations increased the background signals with no palladium and turned the solutions purple. When the NaOH concentration was increased from 70 mM to 120 mM (entry 6 and Figure 3c), we observed the same trend. Figure 3b and Figure 3c show that the reaction was faster when the NH$_4$OAc concentration was lower. However, for visual estimation of relative palladium concentration, the palladium-independent purple color with less NH$_4$OAc was unfavorable because the readout of this system with higher palladium concentrations is magenta color. Therefore, NH$_4$OAc must be at a certain concentration to prevent background signal increase.

![Figure 3](image)

**Figure 3.** (a) Effect of NaOH concentration. (b) Effect of NH$_4$OAc concentration with 70 mM NaOH. (c) Effect of NH$_4$OAc concentration with 120 mM NaOH.

Three components are at play: (1) NH$_4$OAc reacts with NaOH to form NH$_3$, NaOAc, and H$_2$O, (2) NaOH is necessary to stabilize NaBH$_4$ in water,$^{35}$ and (3) NaBH$_4$ reacts with NH$_4$OAc to form NaBH$_3$OAc, NH$_3$, and H$_2$. At this point in this study, we realized that the traditional approach through optimizing one parameter at a time might not be the best. As such, we simultaneously titrated NH$_4$OAc, NaOH, and NaBH$_4$ using ten 96-well plates (Figure 4a). It is important to note that we started reading the 96-well plate with 1.6 M NaOH and ended with the 0.16 M NaOH plate, for which ~4-min gap between plates was inevitable. Palladium-free reaction mixtures showed stronger signals over time. Apparently, NaOH converts RAE to a purple compound in the absence of palladium.
Also, within a 96-well plate with the same NaOH concentration, higher NH₄OAc concentrations slowed the NaOH-mediated formation of a purple compound more effectively, presumably because NH₄OAc neutralizes NaOH. Because the reaction times of the plates on the right side of the figure were longer, the signals were stronger despite the lower NaOH concentrations. With 200 nM palladium, fluorescence signals were higher with more NaBH₄. Particularly with 80 mM NaBH₄ and 1.6 M NaOH, the signals were the highest in the entire panel. However, the same panel showed the greatest sensitivity to the NaBH₄ concentration. This is undesirable for the development of robust and reproducible technology for palladium quantification because NaBH₄ in aqueous solution degrades even when stabilized by NaOH,³⁵ and the actual NaBH₄ concentration in reaction solutions may vary in day-to-day operations.

It is crucial to neutralize acids stemming from palladium-containing solutions for reproducibility. Therefore, although lower concentrations of NH₄OAc show stronger signals, we chose 200 mM NH₄OAc. With this concentration, we performed the next round of a combinatorial assay for NaOH and NaBH₄ (Figure 4b); lower signals were observed with the combination of low NaBH₄ concentration and high NaOH concentration. We also found that when the NaOH concentration was above the NH₄OAc concentration, the result depended too highly on the NaBH₄ concentration, which is undesirable for the reason we stated above. Therefore, we conclude that the NaOH concentration must be below the NH₄OAc concentration, which is also corroborated with the photograph in Figure 4c; palladium-free solutions showed a purple color when the NaOH concentration was higher than NH₄OAc concentration.

One of the advantageous features of the stop-and-go method is that the users can analyze the data many hours later or even the next day because once the assay stalls, the signals remain the same afterward. This is not the case when the NaOH concentration is above the NH₄OAc concentration, because in the presence of palladium, the solutions turned blue (Figure 4d). The UV-vis spectrum of the solution in well E5 (200 nM PdCl₂, 1.6 M NaOH, 360 mM NH₄OAc) suggested that the blue color may be due to the formation of resazurin by the palladium-O₂ catalysis. Therefore, although these reaction conditions showed expected signal immediately after the assay was complete, we recommend that users do not add more NaOH than NH₄OAc.
**Figure 4.** (a) Combinatorial screening for NaOH, NH$_4$OAc, and NaBH$_4$. (b) and (c) Combinatorial screening for NaOH and NaBH$_4$ with 200 mM NH$_4$OAc. (c) Photograph of (b). (d) 24 h after the screening in (b).

**The order of addition.** For convenience, the deallylation should start as soon as the last solution is added to wells. Because NaBH$_4$ gradually reacts with water or NH$_4$OAc to become inactive, NaBH$_4$ must be added last. Although the palladium-catalyzed deallylation of RAE is accelerated by TFP and NaBH$_4$, the reaction slowly proceeds in the absence of NaBH$_4$ because kinetically inferior TFP can still reduce oxidized palladium species to palladium(0). Therefore, we decided that the last solution to add (starter solution) would be a mixture of TFP, NaBH$_4$, and NaOH. In the new order of addition, each well would be treated with (1) a mixture of RAE and NH$_4$OAc, (2) palladium, and (3) a mixture of TFP, NaBH$_4$, and NaOH in this order. This order of addition has substantially improved reproducibility throughout the remainder of this study.

**Autonomous stalling.** Next, we aimed to determine the NaBH$_4$ concentration range in which the deallylation autonomously stalls (Figure 5a). When the NaBH$_4$ concentration was ≤2.5 mM, the reaction stalled in 45–78 min. When higher sensitivity is desired, the NaBH$_4$ concentration should be ≥5 mM. Figure 5b shows that when the NaBH$_4$ concentration is above 10 mM, the reaction rates remain the same.

**Figure 5.** [NaBH$_4$]-dependent autonomous stalling. Conditions: 180 μM TFP, 30 μM RAE, 0, 0.625, 1.25, 2.5, 5, 10, 20, or 40 mM NaBH$_4$, 100 mM NaOH, 200 mM NH$_4$OAc, 6% DMSO and 15% water in EtOH, 31 mM PdCl$_2$, 24 °C, 87 min (read...
fluorescence every 3 min), \( n = 2 \). (a) Time-dependence is shown. (b) \([\text{NaBH}_4]\)-dependence is shown at 40 min point. \( F_{40} - F_0 \) values are shown as the \( y \)-axis (\( F_{40} \) or \( F_0 \) = fluorescence signal at \( t = 40 \) or 0 min). (c) Reaction conditions: 0–300 \( \mu \)M TFP, 30 \( \mu \)M RAE, 1.0 mM \( \text{NaBH}_4 \), 100 mM \( \text{NaOH} \), 200 mM \( \text{NH}_4\text{OAc} \), 5–17.5\% DMSO and 20\% water in EtOH, 30 nM Pd, 24 \( ^\circ \)C, 60 min, \( n = 1 \).

**DMSO and TFP concentrations.** DMSO is an excellent solvent to dissolve APIs with a wide range of polarity and is convenient for the storage due to its low volatility and high stability. However, excess DMSO may be detrimental to the current method because DMSO can bind to palladium to form resting-state palladium species. Therefore, we hypothesized that tolerable DMSO concentrations might depend on TFP concentration. To determine the optimum concentration range for these two components, we titrated them in a combinatorial manner (Figure 5c) to find that 5–10\% DMSO and 100–220 \( \mu \)M TFP were acceptable.

**Metal selectivity and interference.** When a palladium-catalyzed reaction is employed, palladium is obviously the major concern with respect to metal contamination. However, other metal contaminations may be present because other reagents, glassware, or water used during purification may contain copper and iron, among other heavy metals. The previous method was fairly selective for palladium, but platinum produced approximately one-fifth of the signal compared to palladium.\(^{19}\) Figure 6a shows that the current method is more selective for palladium (41810 A.U.), with platinum (9242 A.U.) being second most reactive. More specifically, after the background (no metal; 5677 A.U.) subtraction, we determined that the selectivity between palladium and platinum was 10:1.

We then tested whether other metals would interfere with the quantification of palladium. In the presence of other metals at 345 nM, 30 nM palladium was detected fairly accurately, with the exception of silver ions (Figure 6b and Figure 6c). The reason for the interference is currently unknown. Given the aforementioned reactivity of platinum, it was not surprising that the mixture of 30 nM palladium and 345 nM platinum generated stronger signal than 30 nM palladium alone. Therefore, in instances where both palladium- and platinum-catalyzed reactions are performed in the same synthetic sequence, it would be prudent that a more palladium-selective fluorometric method\(^{18}\) or a platinum-selective fluorometric method (manuscript in preparation) be employed. Also, if silver is suspected as a contaminant in large excess relative to palladium, ICP-MS analysis for silver ions may be necessary.
Figure 6. (a) Metal selectivity. Conditions: 180 μM TFP, 30 μM RAE, 1.0 mM NaBH₄, 100 mM NaOH, 200 mM NH₄OAc, 5% DMSO and 17% water in EtOH, Metal: no metal or 30 nM Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Re, Os, Ir, Pt, Au, Hg, Pb, 24 °C, 60 min, n = 3. (b) Metal interference. Conditions: 180 μM TFP, 30 μM RAE, 1.0 mM NaBH₄, 100 mM NaOH, 200 mM NH₄OAc, 5% DMSO and 17% water in EtOH, Metal: 30 nM Pd and no metal or 345 nM Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Re, Os, Ir, Pt, Au, Hg, or Pb, 24 °C, 60 min, n = 3. (c) UV-vis data of Figure 6b.

Quantification. Synthetic samples prepared by palladium-catalyzed reactions may contain 10–2000 ppm palladium in the solid state. If 1 mg of a synthetic material as the solid is dissolved in 1 mL liquid, the resulting palladium concentrations will be 10–2000 ppb. In the metal analysis, if an aliquot (20 μL) is added to an assay solution (180 μL), the resulting mixture will contain 1–200 ppb (9.4–1880 nM) palladium.

With this in mind, we examined the correlation between palladium concentrations and fluorescence intensities. First, with 180 μM TFP, 30 μM RAE, 1.0 mM NaBH₄, 200 mM NH₄OAc in 5:17:78 DMSO/H₂O/EtOH, the reactions were carried out for 1 h in the 0–2000 nM palladium range (Figure 7a), revealing
that the method was quantitative up to 250 nM. By lowering the NaBH₄ concentration from 1 mM to 0.5 mM, the dynamic range broadened, at the expense of sensitivity (Figure 7b). The sensitivity could be improved by the second addition of NaBH₄ (stop-and-go) to increase the NaBH₄ concentration to 19 mM. To omit the second addition of NaBH₄ for procedural simplicity, we started the reactions with 20 mM NaBH₄, with the expectation that the data at earlier points might be more linear because less substrate (RAE) would be consumed. However, even the 5-min data point showed a non-linear trend (Figure 7c). These data were reproducible; Figure 7d shows the result under the same conditions on a different day. This figure also compares the current method with our previous method, indicating that the current method is 54 times more sensitive in the 0–32 nM palladium concentration range.

(a) 30 μM RAE, 1 mM NaBH₄, 60 min
(b) 30 μM RAE, 0.5 then 19 mM NaBH₄
(c) 60 μM RAE, 20 mM NaBH₄
(d) 60 μM RAE, 20 mM NaBH₄, 30 min

Figure 7. Correlation between palladium concentration (0–2000 nM) and fluorescence intensity. Common conditions for the four graphs: 180 μM TFP, 200 mM NH₄OAc, 5–9% DMSO and 17% water in EtOH, 24 °C. Variables: (a) 30 μM RAE, 1.0 mM NaBH₄, 100 mM NaOH, 24 °C, 1 h, n = 3. (b) 30 μM RAE, 0.5 mM NaBH₄, 25 mM NaOH, 30 min, n = 4. Then add more NaBH₄. (c) 60 μM RAE, 20 mM NaBH₄, 75 mM NaOH, 60 min, n = 3. (d) 60 μM RAE, 20 mM NaBH₄, 75 mM NaOH, 30 min, n = 3.

We hypothesized that this reaction might follow Michaelis-Menten kinetics because there may be pre-equilibrium among palladium, TFP, and RAE to form the catalyst-substrate complex. To test this hypothesis, we measured the velocity as a function of substrate concentration (Figure 8). To slow the reaction to accurately measure fluorescence intensities for the first several minutes, we lowered the NaBH₄ concentration to 0.5 mM.
Indeed, we observed the saturation kinetics, indicating that the palladium-catalyzed deallylation fits Michaelis-Menten kinetics.

![Fluorescence Intensity vs RAE](image)

**Figure 8.** Saturation kinetics. Conditions: 7.5–960 µM RAE, 180 µM TFP, 0.5 mM NaBH₄, 75 mM NaOH, 200 mM NH₄OAc, 8.7% DMSO and 17% H₂O in EtOH, 31 nM PdCl₂, 24 °C.

**Validation of the method.** The purpose of implementing a high throughput screening method for palladium is not to accurately measure the absolute concentrations of palladium in APIs but to estimate the relative concentrations of palladium when process chemists screen for dozens of scavenging methods. One of major challenges is to semi-quantify palladium at as low as 10 ppm and as high as 2000 ppm. We must consider the solubility of APIs and stability of palladium when solutions of APIs are prepared for analysis. If an API substance (1 mg) with X ppm palladium in the solid state is dissolved in 500 mM HCl in 1:4 DMSO/H₂O (1.0 mL), the resultant concentrations are 1 mg API/mL and X ppb (9.4X nM) palladium. When this solution (20 µL) is added to a reaction mixture (180 µL) in a well, the palladium concentration is 0.1X ppb (0.94X nM). Therefore, when the palladium concentration in the well is determined to be Y nM, the palladium content in the original solid API is Y/0.94 ppm.

To target the 0–250 nM palladium range in the assay solutions with various amounts of palladium in mock APIs, we chose to subject 1.0 and 0.1 mg/mL mock API solutions to the reaction conditions. In a double-blinded manner, a member in our research group, who was not part of this study, prepared solutions of drugs spiked with known amounts of palladium. Four or five days after the samples were prepared, we analyzed these samples in three separate experiments. We used freshly diluted palladium solutions with known concentrations to create a calibration curve. The results are summarized in Table 2. In general, the method underestimated palladium concentrations, presumably because we compared four- to five-day old palladium solutions with freshly prepared palladium standard solutions. Nonetheless, the graph shown in Figure 9 indicates that our method can determine the relative concentrations of palladium.
Table 2. Evaluation of the current method with mock APIs.

| Material ID | Pd (ppm) in solid determined by the current method | Actual Pd (ppm) in solid |
|-------------|--------------------------------------------------|--------------------------|
| 6-bromoindole A | 95 | 45 |
| 6-bromoindole B | 85 | 405 |
| 6-bromoindole C | <1 | 0 |
| tyrosine A | 19 | 15 |
| tyrosine B | 4 | 5 |
| tyrosine C | 73 | 45 |
| Shikimic acid A | 10 | 15 |
| Shikimic acid B | 209 | 405 |
| Shikimic acid C | 871 | 1215 |
| uridine A | <1 | 0 |
| uridine B | >2000 | 1215 |
| uridine C | 96 | 45 |
| biotin A | 26 | 15 |
| biotin B | 941 | 405 |
| biotin C | <1 | 5 |
| brucine A | <1 | 0 |
| brucine B | 219 | 135 |
| brucine C | 53 | 45 |
| yohimbine A | >2000 | 1215 |
| yohimbine B | 116 | 135 |
| yohimbine C | 3 | 5 |
| progesterone A | 33 | 45 |
| progesterone B | 44 | 135 |
| progesterone C | 12 | 5 |
| Imatinib A | <1 | 5 |
| Imatinib B | 140 | 135 |
| Imatinib C | 1210 | 405 |

Figure 9. Correlation between ICP-MS and the current method with mock API samples.
Finally, we analyzed synthetic samples that were produced by palladium-catalyzed reactions in our laboratory for unrelated projects. The same stock solutions of these samples were also analyzed by ICP-MS as reference. Table 3 and Figure 10 summarize the results. In general, the current method is not yet optimal for samples containing more than 1000 ppm palladium. The plateaus we observed in Figure 7 are due to the full consumption of RAE (cf. Figure 1). Further dilutions or other strategies may be required in the future. Otherwise, the current method is adequately comparable to ICP-MS. Interestingly, unlike the mock samples above, these real-world samples afforded closer data to those produced by ICP-MS analysis. Moreover, there were three samples that must be contaminated with trace palladium because they were synthesized by Pd/C-catalyzed hydrogenation reactions. ICP-MS could not detect the metal, but the current method could. This may be because unlike spiked mock samples, the synthetic compounds, during weeks of storage period, might bind to palladium ions stronger to stabilize and/or solubilize palladium in catalytically active forms. This hypothesis warrants further extensive studies in the future.

Table 3. Comparison between the current method and ICP-MS using actual synthetic samples with unknown amounts of palladium. The values were calculated for palladium concentrations in synthetic compounds in the solid state.

| Sample ID          | Pd (ppm) measured by the current method | Pd (ppm) measured by ICP-MS |
|--------------------|-----------------------------------------|-----------------------------|
| BMK1030            | 1149                                    | 600                         |
| RKB9101 crude      | 1306                                    | 648                         |
| RKB9101 after CC   | 438                                     | 365                         |
| RKB9102 impure     | 3293                                    | 3321                        |
| RKB9102 after resin| 594                                     | 146                         |
| PLM 3108A          | 3                                       | <1                          |
| PLM 3108B          | 2                                       | <1                          |
| PLM 3109A          | 1                                       | 1                           |
| PLM 3109B          | 2                                       | <1                          |
| RKB9019            | 135                                     | 63                          |
| JAB7140            | 5808                                    | 9776                        |
| JAB7140 purified   | 175                                     | 237                         |
| JAB7141            | 9480                                    | 5979                        |
| JAB7141 purified   | 113                                     | 159                         |
Figure 10. Correlation between ICP-MS and the current method with synthetic samples.

Discussion

We set a goal of understanding how the multiple components in the fluorometric/colorimetric method may influence the outcome. Addition of NaOH was inevitable because NaBH₄ was essential for the palladium-catalyzed allylic C-O bond cleavage under air atmosphere¹⁹, and NaBH₄ must be stabilized by NaOH in water. NaBH₄ and NaOH react with NH₄OAc, and the resulting basicity influences the stability of NaBH₄. NH₄OAc was found to retard the reaction because it quenches NaBH₄, but it was necessary to avoid acidic conditions in the assay. We titrated multiple components in a combinatorial manner for the first time since we began developing and applying fluorometric or colorimetric methods for palladium. When there was more NaOH than NH₄OAc, the excess NaOH turned the solution purple in the absence of palladium. Although the UV-vis spectrum of the purple solution resembled that of resorufin, RAE was not converted to resorufin because neutralization with additional NH₄OAc or acid reversed the UV-vis spectrum back to that of RAE. The reversible reaction between RAE and NaOH was further supported by ¹H NMR analysis. Unfortunately, we were unable to determine the structure of the transient product under basic conditions due to its poor solubility. Based on the previously observed mode of reactivity of related resorufin derivatives,⁴⁶ we speculate that NaOH undergoes reversible conjugate addition to form keto-1, which may be in equilibrium with its tautomer, enol-1 (Scheme 2).

![Scheme 2. Proposed mechanism for the reaction of RAE with NaOH.](image-url)

Although the hypothesized mechanism may be intriguing, this is detrimental to achieving the goal of developing a palladium-specific quantification method. Thus, it is important to ensure that the NH₄OAc concentration exceeds the NaOH concentration. Our palladium stock solutions were stored in 500 mM HCl, which partially eliminated NaOH in assay solutions. Because of this background reaction identified in the current study, we changed the order of addition of reagents to avoid such an undesired reaction.
The stop-and-go strategy is convenient for those who have no access to continuous monitoring of the assay. For the current study, because we had access to an instrument that could read a 96-well plate every few minutes, we chose to opt for procedural simplicity. Figure 5b shows that the reaction rate (i.e., sensitivity of the assay) can be fine-tuned by changing the NaBH₄ concentration. The optimum ranges of TFP and DMSO concentrations were 100–220 μM and 5–10% v/v, respectively. Too much TFP slowed the reaction, presumably because TFP began to form coordinatively saturated (i.e., unreactive) palladium species in situ (manuscript under review). DMSO can bind and form catalytically inactive palladium species, which is likely why more than 10% DMSO substantially retarded the reaction.

Although platinum showed reactivity under the reaction conditions, palladium was about 10 times more reactive. Only silver (12 equivalents relative to palladium) showed significant interference, the molecular mechanism of which is currently unknown.

Achieving a broad dynamic range for any fluorometric or colorimetric method is a formidable challenge in chemistry and biology. For example, a commercial kit for hydrogen peroxide by fluorescence can be effective between 0.1 mM and 1 mM concentrations. Our method was quantitative in the 2–250 nM palladium concentration range. Therefore, if the palladium concentration in an API is expected to be above 250 ppm in the solid state, we recommend that the stock solutions of the sample be diluted to 0.1 mg/mL or lower. Comparison of the current method and our previous method

revealed that the initial reaction rate is 54 times faster (i.e., the current method is 54 times more sensitive; Figure 7d).

We evaluated the current method with drugs or drug-like compounds spiked with known amounts of palladium. Presumably because the spiking was performed 4–5 days prior to the assays, the current method underestimated palladium concentrations on average. It is important to note that the method showed the expected relative concentrations of palladium, which may fulfill users’ needs in high throughput screenings of palladium scavenging to determine the relative efficiency of the scavengers. With 14 synthetic samples prepared by palladium-catalyzed reactions, we compared the current method with ICP-MS. In this case study, unlike the mock samples spiked with known amounts of palladium, the current method slightly (but not significantly) overestimated palladium concentrations on average. Nonetheless, the relative concentrations of palladium boded well with ICP-MS. We believe these results are remarkable, considering that the samples were not digested. If we develop a protocol to fully restore the palladium reactivity under the reaction conditions through digestion (e.g., microwave digestion

), the accuracy may be further improved.

Finally, we discovered that the palladium-catalyzed Tsuji-Trost reaction under the current reaction conditions fit Michaelis-Menten kinetics. Michaelis-Menten kinetics in transition-metal-catalyzed reactions were discussed in literature, although scarcely. This is surprising, since Pirrung and co-workers stated that there might be many such reactions. The saturation kinetics experiment depicted in Figure 8 shows that increasing the RAE concentration up to ~120 μM will linearly increase the reaction rate. Scheme 3a (general equation for Michaelis-Menten kinetics) can be applied to Scheme 3b, in which the π-allylpalladium complex is equivalent to an enzyme-substrate complex depicted in the original paper by Michaelis and Menten.
(a) Typical enzyme-catalyzed reactions with Michaelis-Menten kinetics.

(b) Analogy of the current method to Scheme 3a.

Conclusion

We developed a more robust method with lower background signals to semi-quantify palladium at nanomolar concentrations. The method proved to be useful for real-world samples. We also discovered that the reaction follows Michaelis-Menten kinetics.

Acknowledgement

L.L. is a recipient of the Summer Undergraduate Research Fellowship from the Chemistry Department of the University of Pittsburgh. This work was supported by the US National Science Foundation (CHE-1506942).

References

1. Pohl, P.; Bielawska-Pohl, A.; Dzimitrowicz, A.; Jamroz, P.; Welna, M., Impact and practicability of recently introduced requirements on elemental impurities. *Trac-Trends Anal. Chem.* 2018, 101, 43–55.

2. Bostrom, J.; Brown, D. G.; Young, R. J.; Keseru, G. M., Expanding the medicinal chemistry synthetic toolbox. *Nature Reviews Drug Discovery* 2018, 17, 709–727.

3. Reginato, G.; Sadler, P.; Wilkes, R. D., Scaling up metal scavenging operations for pharmaceutical pilot plant manufactures. *Org. Process Res. Dev.* 2011, 15, 1396–1405.

4. Wang, L.; Green, L.; Li, Z.; McCabe Dunn, J.; Bu, X.; Welch, C. J.; Li, C.; Wang, T.; Tu, Q.; Bekos, E.; Richardson, D.; Eckert, J.; Cui, J., Screening binary systems of chelating agents combined with carbon or silica gel adsorbents: The development of a cost-effective method to remove palladium from pharmaceutical intermediates and APIs. *Org. Process Res. Dev.* 2011, 15, 1371–1376.

5. Magano, J., Large-scale process transitions of transition metal removal techniques in the manufacture of pharmaceuticals. In *Transition metal-catalyzed couplings in process chemistry: Case studies from the pharmaceutical industry*, Magano, J.; Dunetz, J. R., Eds. Wiley-VCH Verlag GmbH & Co. KGaA 2013; pp 313–355.

6. Mondal, B.; Wilkes, R. D.; Percy, J. M.; Tuttle, T.; Black, R. J. G.; North, C., Towards a quantitative understanding of palladium metal scavenger performance: an electronic structure calculation approach. *Dalton Trans.* 2014, 43, 469–478.

7. Miyamoto, H.; Sakamoto, C.; Takekoshi, E.; Maeda, Y.; Hiramoto, N.; Itoh, T.; Kato, Y., Effective method to remove metal elements from pharmaceutical intermediates with polychelated resin scavenger. *Org. Process Res. Dev.* 2015, 19, 1054–1061.

8. Phillips, S.; Holdsworth, D.; Kauppinen, P.; Namara, C. M., Palladium impurity removal from active pharmaceutical ingredient process streams. *Johnson Matthey Technol. Rev.* 2016, 60, 277–286.

9. Yamada, T.; Matsuo, T.; Ogawa, A.; Ichikawa, T.; Kobayashi, Y.; Masuda, H.; Miyamoto, R.; Bai, H. Z.; Meguro, K.; Sawama, Y.; Monguchi, Y.; Saijiki, H., Application of thiol-modified dual-pore silica beads as a practical scavenger of leached palladium catalyst in C-C coupling reactions. *Org. Process Res. Dev.* 2019, 23, 462–469.

10. Phillips, S.; Holdsworth, D.; Kauppinen, P.; Mac Namara, C., Palladium impurity removal from active pharmaceutical ingredient process streams. *Johnson Matthey Technol. Rev.* 2016, 60, 277–286.

11. Yamada, T.; Kobayashi, Y.; Ito, N.; Ichikawa, T.; Park, K.; Kunishima, K.; Ueda, S.; Mizuno, M.; Adachi, T.; Sawama, Y.; Monguchi, Y.; Saijiki, H., Polyethyleneimine-modified polymer as an efficient palladium scavenger and effective catalyst support for a functional heterogeneous palladium catalyst. *ACS Omega* 2019, 4, 10243–10251.

12. Cao, J.; Xu, G.; Xie, Y.; Tao, M.; Zhang, W., Thiourea modified polyacrylnitrile fibers as efficient Pd(II) scavengers. *RSC Adv.* 2016, 6, 58088–58098.

13. Olesik, J. W., Elemental analysis using ICP-OES and ICP/MS - an evaluation and assessment of remaining problems. *Anal. Chem.* 1991, 63, 12A–21A.

14. Lewen, N.; Mathew, S.; Schenkenberger, M.; Raglione, T., A rapid ICP-MS screen for heavy metals in pharmaceutical compounds. *J. Pharm. Biomed. Anal.* 2004, 35, 739–752.
46. Kitson, T. M., The oxidative addition reaction between compounds of resorufin (7-hydroxy-3H-phenoxazin-3-one) and 2-mercaptoethanol. *Bioorg. Chem.* 1998, 26, 63–73.
47. Pinheiro, F. C.; Barros, A. I.; Nobrega, J. A., Microwave-assisted sample preparation of medicines for determination of elemental impurities in compliance with United States Pharmacopeia: How simple can it be? *Anal. Chim. Acta* 2019, 1065, 1–11.
48. Corey, E. J.; Noe, M. C., Kinetic investigations provide additional evidence that an enzyme-like binding pocket is crucial for high enantioselectivity in the bis-cinchona alkaloid catalyzed asymmetric dihydroxylation of olefins. *J. Am. Chem. Soc.* 1996, 118, 319–329.
49. Pirrung, M. C.; Liu, H.; Morehead, J. A. T., Rhodium chenzymes: Michaelis-Menten kinetics in dirhodium(II) carboxylate-catalyzed carbonoid reactions. *J. Am. Chem. Soc.* 2002, 124, 1014–1023.
50. Joshi, A. M.; MacFarlane, K. S.; James, B. R., Kinetics and mechanism of H₂ hydrogenation of styrene catalyzed by [RuCl(dppb)(μ-Cl)]; (dppb = 1,4-bis(diphenylphosphino)butane). Evidence for hydrogen transfer from a dinuclear molecular hydrogen species. *J. Organomet. Chem.* 1995, 488, 161–167.
51. Michaelis, L.; Menten, M. L., Die Kinetik der Invertinwirkung. *Biochem. Z.* 1913, 49, 333–369.
52. Johnson, K. A.; Goody, R. S., The original Michaelis constant: Translation of the 1913 Michaelis–Menten paper. *Biochemistry* 2011, 50, 8264–8269.
Supporting Information
for
Third-generation method for high throughput quantification of trace palladium by color or fluorescence
Lydia Lukomski, Ivanna Pohorilets, and Kazunori Koide*
Department of Chemistry, University of Pittsburgh
219 Parkman Avenue, Pittsburgh, Pennsylvania 15260, United States
koide@pitt.edu

Instrumentation
All fluorescence measurements (excitation 525 nm, emission 580–640 nm) were carried out using a Promega Biosystems Modulus II Microplate Reader.

Reagents
Water in this study was purified by a Barnstead Nanopure Diamond Lab Water System and distilled. DMSO was used without purification. EtOH was USP-grade 200 proof. A 1 mg/mL (1000 ppm = 9.4 mM) palladium standard solution in 10% HCl (2.7 M HCl) for atomic absorption spectroscopy (CAS#7440-30-5, catalog #196171000) was purchased from Fisher and stored at 24 °C. NaBH₄ pellets (1.00 g per pellet) were used for this study. 10 M NaOH in water was purchased from Fisher.

General procedures
All the experiments were performed at 24 °C unless stated otherwise.

Preparation of stock solutions
Preparation of 0.5 M HCl in 1:9 v/v DMSO/water (solution A): Mix 556 mM HCl in water (90.0 mL) and DMSO (10.0 mL) in an amber glass bottle.
Preparation of 0.5 M HCl in 1:4 v/v DMSO/water (solution B): Mix 625 mM HCl in water (40.0 mL) and DMSO (10.0 mL) in an amber glass bottle.
Preparation of 5.0 or 20 mM RAE in DMSO: Dissolve RAE (25.3 mg) in DMSO (20.00 or 5.00 mL) in an amber vial. Store the solution at -20 °C.
Preparation of 800 µM RAE in 16% DMSO/EtOH: Dilute 5.0 mM RAE solution in DMSO (8.0 mL) with EtOH (42.0 mL) in an amber bottle. Store the solution at -20 °C.
Preparation of 250 ppm BHT in DMSO: Dissolve butylated hydroxytoluene (BHT; 12.5 mg) in DMSO (50 mL) in an amber bottle.
Preparation of 4.0 mM TFP in DMSO: Dissolve TFP (23.2 mg) in DMSO with 250 ppm BHT (25 mL) in an amber bottle. The solution can be stored at 24 °C for at least 6 months. All the TFP solutions in DMSO used in this study were stabilized by 250 ppm BHT.
Preparation of 800 µM TFP in DMSO: Dilute 4.0 mM TFP and 250 ppm BHT in DMSO (4.000 mL) with 250 ppm BHT in DMSO (16.00 mL) in an amber bottle. The solution can be stored at 24 °C for at least 6 months.
Preparation of 500 mM NH₄OAc in EtOH: Dissolve NH₄OAc (19.27 g) in EtOH to volume in a 500-mL volumetric cylinder.

Preparation of 393 and 380 mM NH₄OAc in EtOH: Dilute the 500 mM NH₄OAc in EtOH (39.3 or 38.0 mL) with EtOH (10.7 or 12.0 mL).

Preparation of 4.88, 9.77, 19.5, 39, 78, 156, 313, 625, 1250, and 2500 nM PdCl₂ in solution A: (1) Dilute the commercial 9.4 mM PdCl₂ (240 µL) with 0.7 M HCl in water (896 µL); [PdCl₂] = 2.0 mM. (2) Dilute the 2.0 mM PdCl₂ solution (200 µL) with 0.7 M HCl in water (3800 µL); [PdCl₂] = 100 µM. (3) Dilute the 100 µM PdCl₂ solution (200 µL) with solution A (1800 µL); [PdCl₂] = 10 µM. (4) Perform serial 2-fold dilutions of the PdCl₂ solution with solution A until [PdCl₂] = 4.88 nM.

2.0 mM PdCl₂ solutions were stored for 1 month. Less concentrated palladium solutions were prepared immediately prior to use (within 6 h).

Preparation of 2.67 M NaBH₄ in 10 M NaOH: Dissolve a NaBH₄ pellet (1.00 g; 26.4 mmol) with 10 M NaOH (9.91 mL) to prepare 2.67 M NaBH₄ in 10 M NaOH on ice. Keep this solution on ice for up to 8 h. This solution must be prepared within 8 h prior to use.

Preparation of 10 M NaBH₄ in 4 or 10 M NaOH: Dissolve two pellets of NaBH₄ (2.00 g) in 4 or 10 M NaOH (5.28 mL) in a 15-mL plastic centrifuge tube. The resulting solution can be stored on ice for 8 h.

Preparation of 400 mM NaBH₄ and 4 M NaOH in water: (1) Dissolve two pellets of NaBH₄ (2.00 g) in 4 M NaOH (5.28 mL) to prepare 10 M NaBH₄ and 4 M NaOH in water. (2) Add the solution from step 1 (300 µL) to 4 M NaOH in water (2700 µL) to prepare 1 M NaBH₄ and 4 M NaOH in water. (3) Add the solution from step 2 (2100 µL) to 4 M NaOH in water (3510 µL).

Preparation of 1 M NaBH₄ in 10 M NaOH: Dilute 10 M NaBH₄ in 10 M NaOH (300 µL) with 10 M NaOH (2.700 mL) in a 15-mL plastic centrifuge tube.

Preparation of 4 M NaBH₄ in 10 M NaOH: Dissolve a NaBH₄ pellet (1.00 g) in 10 M NaOH (6.60 mL) in a 15-mL plastic centrifuge tube. The resulting solution can be stored on ice for 8 h.

Procedure for Table 1, entry 2

Final reaction conditions: 2-1: 200 µM TFP, 30 µM RAE, 10 mM NaBH₄, 50 mM NaOH, 300 mM NH₄OAc, 6% DMSO in EtOH, 0 – 250 nM Pd, 24 °C, 16 min, n = 3. 2-2: 181 µM TFP, 27 µM RAE, 45 mM NaBH₄, 405 mM NaOH, 272 mM NH₄OAc, 6% DMSO in EtOH, 0 – 250 nM Pd, 24 °C, 16 min, n = 3. 2-3: 167 µM TFP, 23 µM RAE, 75 mM NaBH₄, 700 mM NaOH, 250 mM NH₄OAc, 6% DMSO in EtOH, 0 – 250 nM Pd, 24 °C, 16 min, n = 3.

Preparation of 34.2 µM RAE, 228 µM TFP, and 342 mM NH₄OAc in 1:9 v/v DMSO/EtOH
Add 380 mM NH₄OAc in EtOH (9.002 mL), 800 µM RAE in DMSO (428 µL), and 4.0 mM TFP in DMSO (570 µL) to a 20-mL vial.

Preparation of 33.3 µM RAE, 222 µM TFP, 333 mM NH₄OAc, 11.2 mM NaBH₄, and 112 mM NaOH in DMSO/water/EtOH
Add 400 mM NaBH₄ in 4 M NaOH (290 µL) to the 34.2 µM RAE, 228 µM TFP, and 342 mM NH₄OAc solution in 1:9 v/v DMSO/EtOH (10.063 mL) in a 15-mL plastic centrifuge tube.

Assay:
1) Add 33.3 μM RAE, 222 μM TFP, 333 mM NH₄OAc, 11.2 mM NaBH₄, and 112 mM NaOH in DMSO/water/EtOH (180 μL) to 33 wells (rows A-C, columns 1-11) in a colorless 96-well plate.

2) Treat the wells with either 0, 4.88, 9.77, 19.5, 39, 78, 156, 313, 625, 1250, or 2500 nM PdCl₂ solution (20 μL) to each column in rows A–C.

3) Measure fluorescence after 2, 4, 8, and 16 min. Take photographs.

4) Add 400 mM NaBH₄ in 4 M NaOH (20 μL) to all the wells. Measure fluorescence after 2, 4, 8, and 16 min. Take photographs.

5) Add 400 mM NaBH₄ in 4 M NaOH (20 μL) again to all the wells. Measure fluorescence after 2, 4, 8, and 16 min. Take photographs.

Procedure for Table 1, entry 3

**Final reaction conditions:** 200 μM TFP, 30 μM RAE, 50 mM NaBH₄, 450 mM NaOH, 300 mM NH₄OAc, 6.5% DMSO in EtOH, 0, 0.488, 0.977, 1.95, 3.9, 7.8, 15.6, 31.3, 62.5, 125, or 250 nM Pd, 24 °C, 16 min, n = 3.

**Preparation of 35.3 μM RAE, 235 μM TFP, 352 mM NH₄OAc in 6.6% DMSO/EtOH**

Add 800 μM RAE in 16% DMSO/EtOH (833 μL) and 4.0 mM TFP in DMSO (1.11 mL) to 393 mM NH₄OAc in EtOH (16.95 mL) in a 50-mL plastic centrifuge tube.

**Preparation of 222 μM TFP, 33.3 μM RAE, 55.5 mM NaBH₄, 500 mM NaOH, and 333 mM NH₄OAc**

Add 1 M NaBH₄ in 10 M NaOH (1.11 mL) to 35.3 μM RAE, 235 μM TFP, and 352 mM NH₄OAc in 6.6% DMSO/EtOH (18.903 mL).

**Assay**

1) Add 222 μM TFP, 33.3 μM RAE, 55.5 mM NaBH₄, 500 mM NaOH, and 333 mM NH₄OAc (180 μL) to 33 wells (rows A-C and columns 1-11) in a black 96-well plate.

2) Treat the wells with either 0, 4.88, 9.77, 19.5, 39, 78, 156, 313, 625, 1250, or 2500 nM PdCl₂ in solution A (20 μL) to each column in rows A–C.

3) Measure fluorescence after 2, 4, 8, and 16 min.

4) Transfer the assay solutions (150 μL) to a white 96-well plate to take a photograph of the plate.

Procedure for Table 1, entry 4

**Final reaction conditions:** 180 μM TFP, 30 μM RAE, 50 mM NaBH₄, 20, 45, 70, 120, 220, or 420 mM NaOH, 250 mM NH₄OAc, 6% DMSO and 20% water in EtOH, 0 or 30 nM Pd, 24 °C, 16 min, n = 3.

**Preparation of 351 NH₄OAc in EtOH**

Dilute the 393 mM NH₄OAc in EtOH (17.86 mL) with EtOH (2.14 mL) in a colorless glass bottle.

**Preparation of 4.0, 2.0, 1.0, 0.50, and 0.25 M NaOH in water**

Dilute 10 M NaOH in water (24.0 mL) with water (6.0 mL) in a 15-mL plastic centrifuge tube to prepare 8.0 M NaOH in water. Perform 2-fold serial dilutions with water.

**Preparation of 37.5 μM RAE, 225 μM TFP, 312 mM NH₄OAc, 62.5 mM NaBH₄, and 25 mM NaOH**
Add 351 mM NH₄OAc in EtOH (8.91 mL), 800 μM RAE in 16% DMSO/EtOH (469 μL), 4.0 mM TFP in DMSO (562 μL), and 10 M NaBH₄ in 4 M NaOH (62.5 μL) to a 15-mL plastic centrifuge tube.

**Assay**

1) Add 37.5 μM RAE, 225 μM TFP, 312 mM NH₄OAc, 62.5 mM NaBH₄, and 25 mM NaOH (160 μL) to 36 wells (columns C–H, rows 1–6) of a black 96-well plate

2) Add either 0 (column C), 0.25 (column D), 0.50 (column E), 1.0 (column F), 2.0 (column G), or 4.0 (column H) M NaOH (20 μL) to 6 wells in each row (rows 1–6).

3) Add solution A (20 μL) (0 nM Pd) to 18 wells (columns C–H, rows 1–3).

4) Add 300 nM Pd in solution A (20 μL) to 18 wells (columns C–H, rows 4–6).

5) Measure fluorescence after 2, 4, 8, and 16 min.

6) Transfer the assay solutions (150 μL) to a white 96-well plate to take a photograph of the plate.

**Procedure for Table 1, entry 5**

*Final conditions:* 180 μM TFP, 30 μM RAE, 50 mM NaBH₄, 70 mM NaOH, 0, 74, 111, 167, 250, or 375 mM NH₄OAc, 6% DMSO and 9% water in EtOH, 0 or 30 nM Pd, 24 °C, 16 min, n = 3.

**Preparation of 465, 310, 207, 138, and 92 mM NH₄OAc in EtOH**

Dilute 500 mM NH₄OAc in EtOH (46.5 mL) with EtOH (3.5 mL) in a 50-mL plastic centrifuge tube to prepare 465 mM NH₄OAc. Dilute this solution (20 mL) with EtOH (10 mL) to prepare 310 mM NH₄OAc. Perform 1.5-fold serial dilutions with EtOH to prepare 207, 138, and 92 mM NH₄OAc in EtOH.

**Preparation of 3000 and 300 nM Pd in solution A**

1) Dilute the 2.0 mM Pd solution (100 μL) with 0.7 M HCl (1900 μL) to prepare 100 μM Pd.

2) Dilute the 100 μM Pd solution (30 μL) with solution A (970 μL) to prepare 3000 nM Pd.

3) Dilute the 3000 nM Pd (400 μL) with solution A (3600 μL) to prepare 300 nM Pd.

**Preparation of cocktail**

Mix 800 μM RAE in 16% DMSO/EtOH (167 μL), 4.0 mM TFP in DMSO (200 μL), NH₄OAc at each corresponding concentration (3578 μL), and 4.0 M NaBH₄ in 10 M NaOH (55 μL).

**Assay**

1) Transfer the cocktail with either 0 (column C), 92 (column D), 138 (column E), 207 (column F), 310 (column G), 465 (column H) mM NH₄OAc (180 μL) to 36 wells (rows 1-6).

2) Add solution A (20 μL) (0 nM Pd) to 18 wells (columns C–H, rows 1-3).

3) Add 300 nM Pd in solution A (20 μL) to 18 wells (columns C–H, rows 4-6).

4) Measure fluorescence after 2, 4, 8, and 16 min.

5) Transfer the assay solutions (150 μL) to a white 96-well plate to take a photograph.
Procedure for Table 1, entry 6

**Final conditions:** 180 μM TFP, 30 μM RAE, 50 mM NaBH₄, 120 mM NaOH, 0, 74, 111, 167, 250, 375 mM NH₄OAc, 6% DMSO and 10% water in EtOH, 0 or 200 nM Pd, 24 °C, 16 min, n = 3.

**Preparation of NH₄OAc solutions in EtOH**

1) Dilute 500 mM NH₄OAc (46.5 mL) with EtOH (3.5 mL) to prepare 465 mM NH₄OAc.
2) Dilute 461 mM NH₄OAc (20 mL) with EtOH (10 mL) to prepare 310 mM NH₄OAc.
3) Dilute 308 mM NH₄OAc (20 mL) with EtOH (10 mL) to prepare 207 mM NH₄OAc.
4) Dilute 205 mM NH₄OAc (20 mL with EtOH (10 mL) to prepare 138 mM NH₄OAc.
5) Dilute 136 mM NH₄OAc (20 mL) with EtOH (10 mL) to prepare 92 mM NH₄OAc.

**Preparation of palladium solutions**

1) Dilute the 2.0 mM Pd solution (100 µL) with 0.7 M HCl (1900 µL) to prepare 100 µM Pd.
2) Dilute the 100 µM Pd solution (60 µL) with solution A (2940 µL) to prepare 2 µM Pd.

**Prepare RAE/TFP/NH₄OAc cocktail**

Mix 800 μM RAE in 16% DMSO/EtOH (167 μL), 4.0 mM TFP and 250 ppm in DMSO (200 μL), 0, 92, 138, 207, 310, or 465 mM NH₄OAc in EtOH (3578 μL), and 4.0 M NaBH₄ in 10 M NaOH (55 μL).

**Assay**

1) Transfer either 0 (column C), 82 (column D), 123 (column E), 185 (column F), 278 (column G), or 416 (column H) mM NH₄OAc, 200 μM TFP, and 33.3 μM RAE cocktail solution (180 μL) to 36 wells (rows 1–6).
2) Add solution A (20 μL) to 18 wells (columns C–H, rows 1–3)
3) Add 2 μM Pd (20 μL) to 18 wells (columns C–H, rows 4–6).
4) Measure fluorescence after 2, 4, 8, and 16 min.
5) Transfer the assay solutions (150 μL) to a colorless 96-well plate to take a photograph.

Procedure for Figure 4a (Combinatorial screening for NaOH, NH₄OAc, and NaBH₄)

**Final conditions:** 180 μM TFP, 30 μM RAE, 5, 10, 20, 40, or 80 mM NaBH₄, 160, 200, 400, 800, or 1600 mM NaOH, 32, 47, 71, 107, 160, 240, or 360 mM NH₄OAc, 6% DMSO in EtOH, 0 or 200 nM Pd, 24 °C, 16 min, n = 1.

**Preparation of 10 μM Pd in solution A**

1) Dilute the 2.0 mM Pd solution (100 µL) with 0.7 M HCl (1900 µL) to prepare 100 µM Pd.
2) Dilute the 100 µM Pd solution (400 µL) with solution A (3600 µL).

**Preparation of 80 μM RAE/481 μM TFP/534 nM Pd solution**

Mix EtOH (21.80 mL), 800 μM RAE in 16% DMSO/EtOH (3.00 mL), 4.0 mM TFP in DMSO (3.600 mL), and 10 μM Pd in solution A (1.600 mL).

**Preparation of 80 μM RAE/481 μM TFP solution**
Mix EtOH (21.80 mL), 800 μM RAE in 16% DMSO/EtOH (3.00 mL), 4.0 mM TFP in DMSO (3.600 mL), and solution A (1.600 mL).

**Preparation of 720, 480, 320, 214, 142, and 64 mM NH₄OAc solutions in EtOH**

1) Dissolve NH₄OAc (1.670 g) in EtOH (30.000 mL) to prepare 720 mM NH₄OAc.
2) Dilute the 720 mM NH₄OAc (20.000 mL) with EtOH (10.000 mL) to prepare 480 mM NH₄OAc.
3) Dilute the 480 mM NH₄OAc (20.000 mL) with EtOH (10.000 mL) to prepare 320 mM NH₄OAc.
4) Dilute the 320 mM NH₄OAc (20.000 mL) with EtOH (10.000 mL) to prepare 214 mM NH₄OAc.
5) Dilute the 214 mM NH₄OAc (20.000 mL) with EtOH (10.000 mL) to prepare 142 mM NH₄OAc.
6) Dilute the 94 mM NH₄OAc (10.000 mL) with EtOH (5.000 mL) to prepare 64 mM NH₄OAc.

**Preparation of 6.4, 3.2, 1.6, and 1.28 M NaOH**

1) Serially dilute 12.8 M NaOH (8.000 mL) with water (8.000 mL) to prepare 6.4, 3.2, and 1.6 M NaOH.
2) Dilute the 1.6 M NaOH (6.400 mL) with water (1.600 mL) to prepare 1.28 M NaOH.

**Preparation of 640, 320, 160, 80, and 40 mM NaBH₄ in 12.8 M NaOH**

1) Dissolve NaBH₄ (1.00 g, 26.4 mmol) in 12.8 M NaOH (4.13 mL) in a 50-mL plastic centrifuge tube to prepare 6.4 M NaBH₄.
2) Dilute 6.4 M NaBH₄ (400 μL) with 12.8 M NaOH (3600 μL) to prepare 640 mM NaBH₄.
3) Perform 2-fold serial dilutions of the 640 mM NaBH₄ (1.000 mL) with 12.8 M NaOH (1.000 mL) to prepare 320, 160, 80, and 40 mM NaBH₄.

**Preparation of 640, 320, 160, 80, and 40 mM NaBH₄ in 6.4 M NaOH**

1) Dilute 6.4 M NaBH₄ in 12.8 M NaOH (1.000 mL) with water (1.000 mL) to prepare 3.2 M NaBH₄ in 6.4 M NaOH.
2) Dilute 3.2 M NaBH₄ in 6.4 M NaOH (400 μL) with 6.4 M NaOH (1600 μL) to prepare 640 mM NaBH₄.
3) Perform 2-fold serial dilutions of the 640 mM NaBH₄ in 6.4 M NaOH (1.000 mL) with 6.4 M NaOH (1.000 mL) to prepare 320, 160, 80, and 40 mM NaBH₄.

**Preparation of 640, 320, 160, 80, and 40 mM NaBH₄ in 3.2 M NaOH**

1) Dilute 3.2 M NaBH₄ in 6.4 M NaOH (1.000 mL) with water (1.000 mL) to prepare 1.6 M NaBH₄.
2) Dilute 1.6 M NaBH₄ in 3.2 M NaOH (960 μL) with 3.2 M NaOH (1440 μL) to prepare 640 mM NaBH₄.
3) Perform 2-fold serial dilutions of the 640 mM NaBH₄ in 3.2 M NaOH (1.000 mL) with 3.2 M NaOH (1.000 mL) to prepare 320, 160, 80, and 40 mM NaBH₄.

**Preparation of 640, 320, 160, 80, and 40 mM NaBH₄ in 1.6 M NaOH**

1) Dilute 1.6 M NaBH₄ in 3.2 M NaOH (1.000 mL) with water (1.000 mL) to prepare 0.80 M NaBH₄.
2) Dilute the 0.80 M NaBH₄ in 1.6 M NaOH (1920 μL) with 1.6 M NaOH (480 μL) to prepare 640 mM NaBH₄.
3) Perform 2-fold serial dilutions of the 640 mM NaBH₄ in 1.6 M NaOH (1.000 mL) with 1.6 M NaOH (1.000 mL) to prepare 320, 160, 80, and 40 mM NaBH₄.

**Preparation of 640, 320, 160, 80, and 40 mM NaBH₄ in 1.28 M NaOH**

1) Dilute 800 mM NaBH₄ in 1.6 M NaOH (1280 μL) with water (320 μL) to prepare 640 mM NaBH₄.
2) Perform 2-fold serial dilutions of the 640 mM NaBH₄ (1.000 mL) with 1.28 M NaOH (1.000 mL) to prepare 320, 160, 80, and 40 mM NaBH₄.
Assay

1) Transfer the 80 µM RAE/481 µM TFP solution (75 µL) to 35 wells (columns 1-5, rows A-G) of a black 96-well plate.

2) Transfer the 80 µM RAE/481 µM TFP solution (75 µL) to 35 wells (columns 6-10, rows A-G) of the same plate.

3) Transfer 63, 95, 142, 213, 320, 480, and 720 mM NH₄OAc in EtOH (100 µL) to each row. Start with 63 mM NH₄OAc in row A and finish with 720 mM NH₄OAc in row G.

4) Transfer 40, 80, 160, 320, and 640 mM NaBH₄ in 12.8 M NaOH (25 µL) into the first half of columns. Start with 40 mM in column 1 and finish with 640 mM in column 5. Repeat with columns 6-10.

5) Measure the fluorescence after 2, 4, 8, and 16 min.

Assay

1) Repeat step 1.

2) Repeat step 2.

3) Repeat step 3.

4) Transfer 40, 80, 160, 320, and 640 mM NaBH₄ in 6.4 M NaOH (25 µL) into the first half of columns. Start with 40 mM in column 1 and finish with 640 mM in column 5. Repeat with columns 6-10.

5) Repeat step 5.

Assay

1) Repeat step 1.

2) Repeat step 2.

3) Repeat step 3.

4) Transfer 40, 80, 160, 320, and 640 mM NaBH₄ in 3.2 M NaOH (25 µL) into the first half of columns. Start with 40 mM in column 1 and finish with 640 mM in column 5. Repeat with columns 6-10.

5) Measure the fluorescence after 2, 4, 8, and 16 min.

Assay

1) Repeat step 1.

2) Repeat step 2.

3) Repeat step 3.

4) Transfer 40, 80, 160, 320, and 640 mM NaBH₄ in 1.6 M NaOH (25 µL) into the first half of columns. Start with 40 mM in column 1 and finish with 640 mM in column 5. Repeat with columns 6-10.

5) Repeat step 5.

Assay

1) Repeat step 1.

2) Repeat step 2.

3) Repeat step 3.

4) Transfer 40, 80, 160, 320, and 640 mM NaBH₄ in 1.28 M NaOH (25 µL) into the first half of columns. Start with 40 mM in column 1 and finish with 640 mM in column 5. Repeat with columns 6-10.

5) Repeat step 5.
Procedure for Figure 4b-d (Combinatorial screening for NaOH and NaBH₄ with 200 mM NH₄OAc)

**Final conditions:** 180 μM TFP, 30 μM RAE, 5, 10, 20, 40, or 80 mM NaBH₄, 160, 200, 400, 800, or 1600 mM NaOH, 200 mM NH₄OAc, 6% DMSO in EtOH, 0 or 200 nM Pd, 24 °C, 16 min, n = 1.

**Preparation of 10 μM Pd**

1) Dilute the 2.0 mM Pd solution (100 μL) with 0.7 M HCl (1900 μL) to prepare 100 μM Pd.

2) Dilute the 100 μM Pd solution (400 μL) with solution A (3600 μL) to prepare 10 μM Pd.

**Preparation of 60 μM RAE/360 μM TFP/400 nM Pd solution**

Mix EtOH (850 μL), 800 μM RAE in 16% DMSO/EtOH (750 μL), 800 μM RAE in 16% DMSO/EtOH (750 μL), 500 mM NH₄OAc in EtOH (8.000 mL), and 10 μM Pd in solution A (400 μL).

**Preparation of 60 μM RAE/360 μM TFP solution**

Mix EtOH (850 μL), 800 μM RAE in 16% DMSO/EtOH (750 μL), 800 μM RAE in 16% DMSO/EtOH (750 μL), 500 mM NH₄OAc in EtOH (8.000 mL), and solution A (400 μL).

**Preparation of 5.818, 2.909, 1.455, 0.727, and 0.592 M NaOH in water**

1) Dilute 10 M NaOH (14.55 mL) with water (10.45 mL) to prepare 5.818 M NaOH.

2) Perform 2-fold serial dilutions of 5.818 M NaOH (8.000 mL) with water (8.000 mL) to prepare 2.909, 1.455, and 0.727 M NaOH.

3) Dilute the 0.727 M NaOH (6.40 mL) with water (1.60 mL) to prepare 0.582 M NaOH.

**Preparation of 291, 146, 73, 36, and 18 mM NaBH₄ in 5.818 M NaOH**

1) Dissolve NaBH₄ (1.00 g, 26.4 mmol) in 5.818 M NaOH (9.08 mL) to prepare 2.91 M NaBH₄ in 5.818 M NaOH.

2) Dilute 2.91 M NaBH₄ in 5.818 M NaOH (0.600 mL) with 5.818 M NaOH (5.400 mL) to prepare 291 mM NaBH₄ in 5.818 M NaOH.

3) Perform 2-fold serial dilutions of 291 mM NaBH₄ in 5.818 M NaOH (1.000 mL) with 5.818 M NaOH (1.000 mL) to prepare 146, 73, 36, and 18 mM NaBH₄ in 5.818 M NaOH.

**Preparation of 291, 146, 73, 36, and 18 mM NaBH₄ in 2.909 M NaOH**

1) Dilute 2.91 M NaBH₄ in 5.818 M NaOH (2.00 mL) with water (2.00 mL) to prepare 1.46 M NaBH₄ in 2.909 M NaOH.

2) Prepare 291 mM NaBH₄ in 2.909 M NaOH (400 μL 1.46 M NaBH₄ in 2.909 M NaOH; 1600 μL 2.909 M NaOH).

3) Perform 2-fold serial dilutions of 291 mM NaBH₄ in 2.909 M NaOH (1.000 mL) with 2.909 M NaOH (1.000 mL) until 18 mM NaBH₄ in 2.909 M NaOH.

**Preparation of 291, 146, 73, 36, and 18 mM NaBH₄ in 1.455 M NaOH**

1) Dilute 1.46 M NaBH₄ in 2.909 M NaOH (2.00 mL) with water (2.00 mL) to prepare 0.73 M NaBH₄ in 1.455 M NaOH.

2) Dilute 0.73 M NaBH₄ in 1.455 M NaOH (800 mL) with 1.455 M NaOH (1.200 mL) to prepare 291 mM NaBH₄ in 1.455 M NaOH.
3) Perform 2-fold serial dilutions of 291 mM NaBH$_4$ in 1.455 M NaOH (1.000 mL) with 1.455 M NaOH (1.000 mL) to prepare 146, 73, 36, and 18 mM NaBH$_4$ in 1.455 M NaOH.

**Preparation of 291, 146, 73, 36, and 18 mM NaBH$_4$ in 0.727 M NaOH**

1) Dilute 0.73 M NaBH$_4$ in 1.455 M NaOH (2.00 mL) with water (2.00 mL) to prepare 0.365 M NaBH$_4$ in 0.727 M NaOH.

2) Dilute 0.365 M NaBH$_4$ in 0.727 M NaOH (1.920 mL) with 0.727 M NaOH (0.480 mL) to prepare 291 mM NaBH$_4$ in 0.727 M NaOH.

3) Perform 2-fold serial dilutions of 291 mM NaBH$_4$ in 0.727 M NaOH (1.000 mL) with 0.727 M NaOH (1.000 mL) to prepare 146, 73, 36, and 18 mM NaBH$_4$ in 0.727 M NaOH.

**Preparation of 291, 146, 73, 36, and 18 mM NaBH$_4$ in 0.582 M NaOH**

1) Dilute 0.365 M NaBH$_4$ in 0.727 M NaOH (1.200 mL) with water (0.300 mL) to prepare 291 mM NaBH$_4$ in 0.582 M NaOH.

2) Perform 2-fold serial dilutions of 291 mM NaBH$_4$ in 0.582 M NaOH (1.00 mL) with 0.582 M NaOH (1.00 mL) to prepare 146, 73, 36, and 18 mM NaBH$_4$ in 0.582 M NaOH.

**Preparation of TFP-NaBH$_4$-NaOH 2-fold dilution stock solutions**

Mix 800 µM TFP in DMSO (450 µL) and each NaBH$_4$/NaOH solution to be tested (550 µL) in a 1-mL-per-well 96-well-plate.

a. Add 291 mM NaBH$_4$ in 5.818, 2.909, 1.455, 0.727, and 0.582 M NaOH to prepare 360 µM TFP, 160 mM NaBH$_4$ in 3.2, 1.6, 0.8, 0.40, and 0.32 M NaOH

b. Add 145 mM NaBH$_4$ in 5.818, 2.909, 1.455, 0.727, and 0.582 M NaOH to prepare 360 µM TFP, 80 mM NaBH$_4$ in 3.2, 1.6, 0.8, 0.40, and 0.32 M NaOH

c. Add 73 mM NaBH$_4$ in 5.818, 2.909, 1.455, 0.727, and 0.582 M NaOH to prepare 360 µM TFP, 40 mM NaBH$_4$ in 3.2, 1.6, 0.8, 0.40, and 0.32 M NaOH

d. Add 36 mM NaBH$_4$ in 5.818, 2.909, 1.455, 0.727, and 0.582 M NaOH to prepare 360 µM TFP, 20 mM NaBH$_4$ in 3.2, 1.6, 0.8, 0.40, and 0.32 M NaOH

e. Add 18 mM NaBH$_4$ in 5.818, 2.909, 1.455, 0.727, and 0.582 M NaOH to prepare 360 µM TFP, 10 mM NaBH$_4$ in 3.2, 1.6, 0.8, 0.40, and 0.32 M NaOH

**Assay**

1) Add 60 µM RAE/360 µM TFP/400 nM Pd solution (100 µL) to 25 wells (columns 1-5, rows A-E)

2) Add 60 µM RAE/360 µM TFP solution (100 µL) to 25 wells (columns 6-10, rows A-E)

3) Add each TFP-NaBH$_4$-NaOH 2x stock solution (100 µL) to the wells.

4) Measure the fluorescence after 2, 4, 8, and 16 min.

**Procedure for Figure 5a and Figure 5b ([NaBH$_4$]-dependent autonomous stalling)**

*Final conditions: 180 µM TFP, 30 µM RAE, 0, 0.625, 1.25, 2.5, 5, 10, 20, or 40 mM NaBH$_4$, 150 mM NaOH, 200 mM NH$_4$OAc, 6% DMSO and 15% water in EtOH, 0 or 31 nM Pd, 24 °C, 60 min (read fluorescence every 3 min), n = 2. 150 mM NaOH becomes 100 mM NaOH upon the addition of 10x Pd solution in 500 mM HCl.*
Preparation of 0 and 312.5 nM Pd in solution A

1) Dilute the 2.0 mM Pd solution (200 µL) with 0.7 M HCl (3800 µL) to prepare 100 µM Pd.
2) Dilute the 100 µM Pd solution (200 µL) with solution A (1800 µL) to prepare 10 µM Pd.
3) Perform 2-fold serial dilutions of the 10 µM Pd (0.500 mL) with solution A (0.500 mL) to prepare 5000, 2500, 1250, 625, and 312.5 nM Pd.
4) Transfer an aliquot (120 µL) of 312.5 nM Pd to row A, columns 1-8 of a black 96-well plate. Transfer an aliquot (120 µL) of 0 nM Pd (i.e., solution A) to row B, columns 1-8 of the same plate.

Preparation of 42.9 μM RAE/286 mM NH₄OAc in 5.4%DMSO/EtOH

Mix 800 μM RAE in DMSO (1.125 mL), 500 mM NH₄OAc in EtOH (12.0 mL), and EtOH (7.875 mL) at 24 °C in an amber vial. Keep the solution at -20 °C for storage.

Preparation of 900 µM TFP/200, 100, 50, 25, 12.5, 6.25, 3.125, or 0 mM NaBH₄/750 mM NaOH in 6% DMSO/44% EtOH/water

1) Prepare 500 ppm BHT (50 mg) in DMSO (100 mL) in an amber bottle.
2) Dissolve TFP (69.7 mg, 300 µmol) in 500 ppm BHT in DMSO (10.0 mL) to prepare 30 mM TFP in DMSO in an amber vial. Store the solution at 24 °C for future use.
3) Dilute the 30 mM TFP in DMSO with the equal volume of 500 ppm BHT in DMSO to prepare 15 mM TFP in DMSO in a PCR tube. Cover the tube with aluminum foil to avoid light.
4) Dissolve a NaBH₄ pellet (1.00 g; 26.4 mmol) with 10 M NaOH (9.91 mL) to prepare 2.67 M NaBH₄ in 10 M NaOH.
5) Immediately before step 2 of the assay, dilute the 2.67 M NaBH₄ in 10 M NaOH (1.80 mL) with water (10.20 mL) in a 15-mL plastic centrifuge tube to prepare 400 mM NaBH₄ in 1.5 M NaOH on ice. CAUTION: NaBH₄ in diluted NaOH solution degrades rapidly.
6) Serially dilute the 400 mM NaBH₄ solution with 1.5 M NaOH to prepare 200, 100, 50, 25, 12.5, and 6.25 mM NaBH₄ in 1.5 M NaOH. Separately, prepare NaBH₄-free 1.5 M NaOH.
7) Immediately before the assay starts (i.e., after step 3 in assay), add EtOH (88 µL), 15 mM TFP in DMSO (12 µL), and either 0, 6.25, 12.5, 25, 50, 100, or 200 mM NaBH₄ in 1.5 M NaOH (100 µL) in row C of a 96 well plate on ice. CAUTION: A 8-channel pipet must be used to add NaBH₄ because timing is critical.

Assay

1) Add 42.9 μM RAE/286 mM NH₄OAc in 5.4%DMSO/EtOH (140 µL per well) to row D-G, column 1-8 (32 wells).
2) Transfer the Pd solutions (20 µL per well) from row A to rows D and E of the corresponding column.
3) Transfer Pd-free solution A (20 µL per well) from row B to row F and G.
4) Add 900 µM TFP/0, 6.25, 12.5, 25, 50, 100, or 200 mM NaBH₄/750 mM NaOH in 6% DMSO/44% EtOH/water (40 µL per well) from row C to rows D-G columns 1-8. CAUTION: A 8-channel pipet must be used to add NaBH₄ because timing is critical.
5) Measure fluorescence every 3 min for 1.5 h.

Data analysis
Plot $F_{40\text{min}/31\text{nM}} - F_{40\text{min}/0\text{nM}}$ as the y-axis vs. NaBH$_4$ concentration as the x-axis.

**Procedure for Figure 5c (Combinatorial screening for TFP and DMSO)**

*Final conditions:* 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, or 300 μM TFP, 30 μM RAE, 1.0 mM NaBH$_4$, 150 mM NaOH, 200 mM NH$_4$OAc, 5, 7.5, 10, 12.5, 15, or 17.5% DMSO and 20% water in EtOH, 0 or 30 nM Pd, 24 °C, 60 min, $n = 1$. 150 mM NaOH becomes 100 mM NaOH upon the addition of 0 or 30 nM Pd solution in 500 mM HCl.

*Preparation of 300 nM Pd in aqueous HCl (no DMSO)*

1) Dilute the 2.0 mM Pd solution (300 μL) with 0.5 M HCl (1700 μL) to prepare 300 μM Pd.
2) Perform 3 rounds of 10-fold serial dilution with 0.5 M HCl to prepare 300 nM Pd (2 mL).

*Preparation of 60 μM RAE/400 mM NH$_4$OAc in 1.2%DMSO/EtOH*

Mix 800 μM RAE in 16% DMSO/EtOH (2.40 mL), 500 mM NH$_4$OAc in EtOH (25.60 mL), and EtOH (4.00 mL) at 24 °C.

*Preparation of 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1500 μM TFP/5.0 mM NaBH$_4$/750 mM NaOH in 5% DMSO/44% EtOH/water*

1) Dissolve TFP (69.6 mg, 280 μmol) in 250 ppm BHT/DMSO (10 mL) to prepare 30 mM TFP and 250 ppm BHT in DMSO in an amber vial.
2) Dilute the 30 mM TFP (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 μL) with EtOH (300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, or 150 μL) to prepare 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, 11, 12, 13, 14, or 15 mM TFP in 1:1 DMSO/EtOH (300 μL) on a 96-well plate.
3) Dilute 10 N NaOH (3.75 mL) with water (21.25 mL) to prepare 1.5 M NaOH.
4) Dissolve a NaBH$_4$ pellet (1.00 g; 26.4 mmol) with 10 M NaOH (9.91 mL) to prepare 2.67 M NaBH$_4$ in 10 M NaOH. Keep this solution on ice. CAUTION: Do not store the solution in a refrigerator due to the evolution of hydrogen gas.
5) Immediately after step 3 of the assay, dilute the 2.67 M NaBH$_4$ in 10 M NaOH (1.80 mL) with cold water (10.20 mL) in a 15-mL plastic centrifuge tube to prepare 400 mM NaBH$_4$ in 1.5 M NaOH (12 mL) on ice.
6) Dilute the 400 mM NaBH$_4$ solution (0.300 mL) with cold 1.5 M NaOH (11.7 mL) to prepare 10.0 mM NaBH$_4$ in 1.5 M NaOH (12 mL) on ice.
7) Mix EtOH (400 μL), 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, 11, 12, 13, 14, or 15 mM TFP in DMSO (100 μL), and 10.0 mM NaBH$_4$ in 1.5 M NaOH (500 μL) in row A of a deep 96-well on ice.

*Preparation of 17, 30, 42, 55, 67, and 80% DMSO/EtOH*

Mix DMSO (510, 885, 1260, 1635, 2010, or 2385 μL) and EtOH (2490, 2115, 1740, 1365, 990, or 615 μL) in vials.

*Assay*

1) Add 60 μM RAE/400 mM NH$_4$OAc in 5.4%DMSO/EtOH (100 μL per well) from a reservoir to all wells in a black 96-well plate (i.e., 96 wells).
2) Add 17, 30, 42, 55, 67, or 80% DMSO/EtOH solution (40 µL per well) to rows A–H columns 1&7, 2&8, 3&9, 4&10, 5&11, or 6&12.

3) Transfer the Pd solution (20 µL per well) from a reservoir to all wells.

4) Add 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1500 µM TFP/5.0 mM NaBH₄/750 mM NaOH in 5% DMSO/44% EtOH/water (40 µL per well) from row A of the deep-well 96-well plate to row A column 1-6, row B column 1-6, row C column 1-6, row D column 1-6, row E column 1-6, row F column 1-6, row G column 1-6, row H column 1-6, row A column 7-12, row B column 7-12, row C column 7-12, row D column 7-12, row E column 7-12, row F column 7-12, row G column 7-12, or row H column 7-12.

5) Measure fluorescence after 1 h.

Data analysis

Plot F₆₀/₃₀ as the z-axis, TFP concentration as the x-axis, and DMSO concentration as the y-axis in a heatmap format. Fₓᵧ means X min/Y nM Pd.

Procedure for Figure 6a (Metal selectivity)

Final conditions: 180 µM TFP, 30 µM RAE, 1.0 mM NaBH₄, 150 mM NaOH, 200 mM NH₄OAc, 5% DMSO and 17% water in EtOH, 30 nM (no metal), Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Ru, Rh, Pd, Ag, Cd, Re, Ir, Pt, Au, Hg, Pb, 24 °C, 60 min, n = 3. 150 mM NaOH becomes 100 mM NaOH upon the addition of 10x metal solution in 500 mM HCl

Preparation of 1.0 mM metal solutions

1) Dissolve AlCl₃, Ca(OAc)₂, Sc(OTf)₃, GaCl₃, InCl₃, Cp₂HfCl₂, WCl₆, ReCl₃, and OsO₄ in solution B to prepare 1.0 mM solutions.

2) Dilute the following commercially available metal solutions with solution B to prepare 1.0 mM solutions: 1000 ppm (41.1 mM) magnesium standard in 2.46% HNO₃ (Fisher), 1000 ppm (20.89 mM) titanium standard in 2% HNO₃ (Fisher), 1000 ppm (19.63 mM) vanadium standard in 2% HNO₃ (Fisher), 1000 ppm (19.23 mM) chromium standard in dilute HNO₃ (Fisher), 1000 ppm (18.20 mM) manganese standard in 2–5% HNO₃ (Fisher), 1000 ppm (17.91 mM) iron standard in 3% HCl (Fisher), 1000 ppm (16.97 mM) cobalt standard in 2% HNO₃ (Fisher), 1000 ppm (17.04 mM) nickel standard in 2% HNO₃ (Fisher), 1000 ppm (15.74 mM) copper standard in 2% HNO₃ (Fisher), 1000 ppm (15.30 mM) zinc standard in 2% HCl (Fisher), 1000 ppm (10.96 mM) zirconium standard in 2% HNO₃ (Fisher), 1000 ppm (10.42 mM) molybdenum standard in 5% HNO₃ (Crescent), 1000 ppm (9.89 mM) ruthenium standard in 20% HCl (Fisher), 1000 ppm (9.72 mM) rhodium standard (Fisher), 1000 ppm (9.27 mM) silver standard in 0.5 N HNO₃ (Fisher), 1000 ppm (8.90 mM) cadmium standard (Fisher), 1000 ppm (7.28 mM) barium standard in 3% HCl (Fisher), 1000 ppm (5.20 mM) iridium standard in 5% HCl (Fisher), 1000 ppm (5.13 mM) platinum standard in 10% HCl (Fisher), 1000 ppm (5.08 mM) gold standard in 5% HCl (Sigma-Aldrich), 1000 ppm (4.99 mM) 4.83 mM mercury standard in 10% HNO₃ (Fisher), 1000 ppm lead reference standard solution (Fisher).

Preparation of 300 nM metal in solution B

1) Add 1.0 mM metal solutions (200 µL) to row A of two white 96-well plates.

2) Add solution B (200 µL) to rows B, C, and D.
3) Perform 3 rounds of 14.4-fold serial dilution to prepare 300 nM metal in row D. Specifically, transfer an aliquot (14.9 µL) from row A to B, mix the solutions thoroughly, transfer the resulting solutions (14.9 µL) from row B to C, mix the solutions thoroughly, and transfer the resulting solutions (14.9 µL) from row C to D.

**Preparation of 42.9 µM RAE/286 mM NH₄OAc in 2.6%DMSO/EtOH**

Mix 800 µM RAE in 16% DMSO/EtOH (2.250 mL), 500 mM NH₄OAc in EtOH (24.0 mL), and EtOH (15.75 mL) at 24 °C in an amber vial. Store the solution at -20 °C after sealing the cap tightly.

**Preparation of 900 µM TFP/5 mM mM NaBH₄/750 mM NaOH in 6% DMSO/44% EtOH/water**

1) Dissolve a NaBH₄ pellet (1.00 g; 26.4 mmol) with 10 M NaOH (9.91 mL) to prepare 2.67 M NaBH₄ in 10 M NaOH on ice. Keep this solution on ice.

2) After step 2 of the assay, dilute the above solution (1.80 mL) with water (10.20 mL) in a 15-mL plastic centrifuge tube to prepare 400 mM NaBH₄ in 1.5 M NaOH on ice.

3) Dilute the 400 mM NaBH₄ in 1.5 M NaOH (0.100 mL) with cold 1.5 M NaOH (3.900 mL) to prepare 10 mM NaBH₄ in 1.5 M NaOH on ice.

4) Mix cold EtOH (2.64 mL), 15 mM TFP in DMSO (360 µL), and 10 mM NaBH₄ in 1.5 M NaOH (3.00 mL) in a reservoir (6 mL).

**Assay**

1) Transfer 42.9 µM RAE/286 mM NH₄OAc in 2.6%DMSO/EtOH from a reservoir to rows A–C on two black 96-well plates (140 µL per well).

2) Transfer the 300 nM metal solution (20 µL per well) from row D of the white plates to rows A–C of the black plates.

3) Transfer 900 µM TFP/5 mM NaBH₄/750 mM NaOH in 6% DMSO/44% EtOH/water (40 µL per well) from a reservoir to rows A–C of the black plates.

4) Read fluorescence after 1 h.

**Procedure for Figure 6b and Figure 6c (Metal interference)**

**Final conditions:** 180 µM TFP, 30 µM RAE, 1.0 mM NaBH₄, 150 mM NaOH, 200 mM NH₄OAc, 5% DMSO and 17% water in EtOH, 30 nM Pd and 345 nM of either no metal, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Ru, Rh, Ag, Cd, , Re, , Ir, Pt, Au, Hg, or Pb, 24 °C, 60 min, n = 3. 150 mM NaOH becomes 100 mM NaOH upon the addition of 10x metal solution in 500 mM HCl.

**Preparation of 300 nM Pd and 3450 nM of either Mg, Al, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Zr, Mo, Ru, Rh, Ag, Cd, In, Ba, Hf, W, Re, Os, Ir, Pt, Au, Hg, or Pb in solution B**

1) Dilute the 2.0 mM Pd solution (600 µL) with 3% HCl (1400 µL) to prepare 600 µM Pd.

2) Perform 2 rounds of 10-fold serial dilution with 3% HCl to prepare 6 µM Pd (2 mL).

3) Dilute the 6 µM Pd (1.00 mL) with DMSO (1.80 mL) and 3% HCl in water (7.20 mL) to prepare 600 nM Pd.

4) Transfer the 69 µM metal solution (100 µL) from row B of the previous experiment (Metal selectivity) to row F of the same plate.
5) Add 600 nM Pd (100 µL) from a reservoir to the wells in row F containing 69 µM metal to prepare 300 nM Pd + 3450 nM metal or 300 nM Pd with no other metal in column 1 of the white plate #1.

Preparation of 42.9 µM RAE/286 mM NH₄OAc in 2.6%DMSO/EtOH

See the procedure for Figure 6a (Metal selectivity).

Preparation of 900 µM TFP/5 mM mM NaBH₄/750 mM NaOH in 6% DMSO/44% EtOH/water

See the procedure for Figure 6a (Metal selectivity).

Assay

1) Transfer 42.9 µM RAE/286 mM NH₄OAc in 2.6%DMSO/EtOH from a reservoir to rows F–H on two black 96-well plates (140 µL per well).

2) Transfer the 300 nM Pd + 3450 nM metal solution (20 µL per well) from row F of the white plates to rows F-H of the black plates.

3) Transfer 900 µM TFP/5 mM NaBH₄/750 mM NaOH in 6% DMSO/44% EtOH/water (40 µL per well) from a reservoir to rows F–H of the black plates.

4) Read fluorescence and absorbance (560 nm) after 1 h.

Procedure for Figure 7a (Correlation between palladium concentration and fluorescence intensity)

Final conditions: 180 µM TFP, 30 µM RAE, 1.0 mM NaBH₄, 150 mM NaOH, 200 mM NH₄OAc, 5% DMSO and 17% water in EtOH, 0–2000 nM Pd, 24 °C, 1 h, n = 3. 150 mM NaOH becomes 100 mM NaOH upon the addition of Pd solution in 500 mM HCl.

Preparation of 0, 19.6, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000, 10000, 20000 nM Pd solutions

1) Dilute 2.0 mM Pd in 3% trace metal HCl (100 µL) with solution B (900 µL) to prepare 200 µM Pd.

2) Dilute the 200 µM Pd (100 µL) with solution B (900 µL) in row H column 12 in a deep 96-well plate to prepare 20 µM Pd.

3) Add solution B (500 µL) to row H columns 1-11.

4) Serially dilute 2-fold in a deep 96-well plate until 19.6 nM in column 2 row H in a deep 96-well. Suction slowly and mix extensively each time. Add Pd-free solution B to column 1 row H. Do not add Pd to row H column 1.

5) Transfer an aliquot (150 µL) to row A columns 1-12 of the first black 96-well plate with clear bottom.

Preparation of 900 µM TFP/5 mM NaBH₄/750 mM NaOH in 6% DMSO/44% EtOH/water

1) After step 2 of the assay, dilute the 2.67 M NaBH₄ in 10 M NaOH (1.80 mL) with water (10.20 mL) in a 15-mL plastic centrifuge tube to prepare 400 mM NaBH₄ in 1.5 M NaOH on ice.

2) Dilute the 400 mM NaBH₄ in 1.5 M NaOH (0.100 mL) with cold 1.5 M NaOH (3.900 mL) to prepare 10 mM NaBH₄ in 1.5 M NaOH on ice.

3) Mix cold EtOH (2.64 mL), 15 mM TFP in DMSO (360 µL) and 10 mM NaBH₄ in 1.5 M NaOH (3.00 mL) in a reservoir.

Assay
1) Transfer 42.9 μM RAE/286 mM NH₄OAc in 2.6% DMSO/EtOH from a reservoir to a black 96-well plate (140 μL per well) with clear bottom.

2) Add 0, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000, 10000, and 20000 nM Pd in solution B (20 μL).

3) Add 900 μM TFP/5 mM NaBH₄/750 mM NaOH in 6% DMSO/44% EtOH/water (40 μL per well).

4) Measure fluorescence after 1 h.

**Procedure for Figure 7b (Correlation between palladium concentration and fluorescence intensity)**

*Final conditions:* 180 μM TFP, 30 μM RAE, 5 mM NaBH₄, 75 mM NaOH, 200 mM NH₄OAc, 5% DMSO and 17% water in EtOH, 0-2000 nM or unknown Pd, 24 °C, 40 min, n = 4. 75 mM NaOH becomes 25 mM NaOH upon the addition of 10x metal solution in 500 mM HCl. Then add more NaBH₄.

*Assay*

1) Add 42.9 μM RAE/286 mM NH₄OAc in 2.6% DMSO/EtOH to a black 96-well plate (140 μL per well) with clear bottom.

2) Add 0, 19.6, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000, 10000, 20000 nM Pd in solution B (20 μL).

3) Add 900 μM TFP/2.5 mM NaBH₄/375 mM NaOH in 6% DMSO/44% EtOH/water (40 μL per well).

4) Measure fluorescence at t = 10, 20, 30 min.

5) Dilute the 400 mM NaBH₄/1.5 M NaOH (3 mL) with water (3 mL) and add the resulting 200 mM NaBH₄/750 mM NaOH (20 μL).

6) Measure fluorescence after 90 min.

**Procedure for Figure 7c (Correlation between palladium concentration and fluorescence intensity)**

*Final conditions:* 180 μM TFP, 60 μM RAE, 20 mM NaBH₄, 75 mM NaOH, 200 mM NH₄OAc, 8.7% DMSO and 17% water in EtOH, 0-2000 nM Pd, 24 °C, 60 min, n = 3.

*Assay*

1) Add 85.7 μM RAE/286 mM NH₄OAc in 10.7% DMSO/EtOH (140 μL) to a black 96-well plate with clear bottom.

2) Add 0, 19.6, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000, 10000, 20000 nM Pd in solution B (20 μL).

3) Add 900 μM TFP/100 mM NaBH₄/375 mM NaOH in 6% DMSO/44% EtOH/water (40 μL).

4) Measure fluorescence every 5 min for 60 min.

**Procedure for Figure 7d (Quantification of palladium with mock API samples and synthetic samples)**

*Final conditions:* 180 μM TFP, 60 μM RAE, 20 mM NaBH₄, 75 mM NaOH, 200 mM NH₄OAc, 8.7% DMSO and 17% water in EtOH, 0-2000 nM Pd, 24 °C, 30 min, n = 3.

*Preparation of 85.7 μM RAE/286 mM NH₄OAc in 10.7% DMSO/EtOH*

Mix 800 μM RAE in DMSO (32.14 mL), 500 mM NH₄OAc (171.60 mL), and EtOH (96.26 mL).
Preparation of mock API samples contaminated with Pd

1) Dilute 1000 ppm Pd standard (121.5 μL) to 121.5 ppm with 10% HCl (878.5 μL).

2) Perform 3-fold serial dilutions starting with 121.5 ppm (100 μL) in 10% HCl (200 μL) to prepare 40.5, 13.5, 4.5, 1.5, and 0.5 ppm Pd. Separately, prepare 0 ppm Pd in 10% HCl (300 μL).

3) Add 121.5, 40.5, 13.5, 4.5, 1.5, or 0 ppm Pd (5 μL) to 5.26 mg/mL API stock solution in DMSO (95 μL) to prepare 5 mg/mL samples with 6075, 2025, 675, 225, 75, 25, or 0 ppb Pd in solutions.

4) Dilute 5 mg/mL spiked samples (100 μL) with 1.75 HCl in water (400 μL) to prepare 1.0 mg/mL with 1215, 405, 135, 45, 15, 5, or 0 ppb Pd. X ppb Pd in the solution corresponds to X ppm in the original solid state of API.

Sample IDs and their palladium content (ppm in their solid states or ppb in solution)

| Compound       | A  | B  | C  |
|----------------|----|----|----|
| 6-bromoindole  | 45 | 405| 0  |
| tyrosine       | 15 | 5  | 45 |
| shikimic acid  | 15 | 405| 1215|
| uridine        | 0  | 1215| 45 |
| biotin         | 15 | 405| 5  |
| brucine        | 0  | 135| 45 |
| yohimbine      | 1215| 135| 5  |
| progesterone   | 45 | 135| 5  |
| Imatinib       | 5  | 135| 405|

Preparation of 0, 19.6, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000, 10000, 20000 nM Pd solutions for calibration curve

See the procedure for Figure 7a

Samples: All of the synthetic compounds contain a heterocycle with a nitrogen or oxygen atom, or both.

- Palladium-catalyzed hydrogenation products, crude (RKB9101 crude, RKB9102 crude), after column chromatography (RKB9101 after CC), after resin treatment (RKB9102 after resin), after filter paper (PLM 3108A, PLM 3109A), and after filter paper and Celite (PLM 3108B, PLM 3109B)

- Crude product sample from Negishi coupling with Pd(PPh₃)₂Cl₂ (BMK1030)

Preparation of synthetic samples in solution B (1.0 mg/mL)

1) Add DMSO to a solid synthetic sample to prepare 5.0 mg/mL sample in DMSO. Specifically, dissolve BMK1030 (45 mg) in 9.00 mL, RKB9101 crude (18.4 mg) in 3.68 mL, RKB9101 after CC (11.3 mg) in 2.26 mL, RKB9102 impure (6.4 mg) in 1.28 mL, RKB9102 after resin (12.2 mg) in 2.44 mL, PLM
3108A (81.4 mg) in 16.3 mL, PLM 3108B (91.5 mg) in 18.3 mL, PLM 3109A (126 mg) in 25.2 mL, PLM 3109B (92.7 mg) in 18.5 mL, JAB7140 (24.3 mg) in 4.86 mL, JAB7141 (25.2 mg) in 5.04 mL.

2) Dilute the 5 mg/mL samples (20 μL) in row D columns 1-12 of a third black well plate with a clear bottom with 625 mM HCl (80 μL) of the well plate to prepare 1 mg/mL. Dilute the remaining sample in row E column 1 of a fourth black well plate.

3) Dilute the 1 mg/mL samples (5 μL) in row A columns 1-12 of the fourth black plate with solution B (95 μL) to prepare 0.05 mg/mL samples and seal the wells with scotch tape to prevent evaporation while not being used. Dilute the remaining sample in row E column 2 of the fourth black plate.

Preparation of 900 μM TFP/100 mM NaBH₄/375 mM NaOH in 6% DMSO/44% EtOH/water

1) After step 5 of the assay, dilute the 2.67 M NaBH₄ in 1 M NaOH (1.80 mL) with water (10.20 mL) in a 15-mL Corning tube to prepare 400 mM NaBH₄ in 1.5 M NaOH on ice.

2) Mix cold EtOH (5.28 mL), 15 mM TFP in DMSO (720 μL), 400 mM NaBH₄ in 1.5 M NaOH (3 mL), and water (3 mL) in a reservoir.

Assay

1) Transfer 85.7 μM RAE/286 mM NH₄OAc in 10.7%MDSO/EtOH (140 μL per well) from a reservoir to rows B-H, columns 1-12 on the first black 96-well plate with clear bottom. Transfer the same solution (140 μL per well) to rows A-H of the second black well plate with clear bottom. Transfer the same solution (140 μL per well) to rows A-C and F-H columns 1-12 of the third black well plate with clear bottom. Transfer again (140 μL per well) to rows B-D 1-12 and F-H 1 and 2 of the fourth plate.

2) Transfer 0, 19.6, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000, 10000, 20000 nM Pd (20 µL) from row A to row B-E of the first plate

3) Transfer 1 mg/mL mock API samples (20 µL) to row F-H of the first plate. Finish transferring 1 mg/mL mock API samples (20 µL) to row A-C columns 1-12 of the second plate.

4) Transfer 0.05 mg/mL mock API samples (20 μL) to row F-H of the second plate. Finish transferring 0.05 mg/mL mock API samples (20 μL) to row A-C columns 1-12 of the third plate.

5) Transfer 1 mg/mL synthetic samples (20 μL) from row E of the third plate to rows B-H. Transfer 0.05 mg/mL synthetic samples (20 μL) from row A of the fourth plate to rows B-D of the same plate.

6) Transfer 900 μM TFP/100 mM NaBH₄/375 mM NaOH in 6% DMSO/44% EtOH/water (40 μL per well) from a reservoir to all wells.

7) Measure fluorescence after 30 min

Data analysis

One phase decay and allosteric models fit well for the broad range of palladium concentrations. With both Prism GraphPad and Microsoft Excel, we also used \( Y = A \ln [Pd] + B \) to solve for [Pd]. We also used \( Y = V_{\text{max}}*[Pd]^h/(K' + [Pd]^h) \).

\[ Y = \text{fluorescence intensity, [Pd] = palladium concentration in nM in wells.} \]

When the allosteric sigmoidal was used, \([Pd] = \exp(F*K'/h*(F_{\text{max}} - F))\)
\[ [\text{Pd}] = \frac{1}{h} \times (\exp\left(F \times K' / (F_{\text{max}} - F)\right)) \]

For the 0-500 nM graph, \( F_{\text{max}} = 46680, K' = 17.68, h = 0.6315 \)

\[ [\text{Pd}] = \exp\left(1.584 \times \ln 17.68 \times F / (466080 - F)\right) \]

\( Y = V_{\text{max}} \times [\text{Pd}]^h / (K' + [\text{Pd}]^h) \)

\( V_{\text{max}} = 461558, h = 0.6456, K' = 18.45 \)
| Compound            | Average Fluorescence with 1 mg/mL sample | Calculated [Pd] (nM) With 1 mg/mL sample | Ppm in solid determined with 1 mg/mL sample | Average Fluorescence with 0.1 mg/mL sample | Calculated [Pd] (nM) With 0.1 mg/mL sample | Ppm in solid determined with 0.1 mg/mL sample | Theoretical ppm in solid |
|---------------------|------------------------------------------|------------------------------------------|---------------------------------------------|--------------------------------------------|--------------------------------------------|---------------------------------------------|--------------------------|
| 6-bromoindole A     | 26,847                                    | NQ                                       | NQ                                         | 82,398                                    | 8.9                                       | 95                                         | 45                       |
| 6-bromoindole B     | 200,719                                   | 61                                       | 65                                         | 397,496                                   | NQ                                        | NQ                                         | 405                      |
| 6-bromoindole C     | 21,329                                    | NQ                                       | NQ                                         | 17,201                                    | 0                                         | 0                                          | 0                        |
| tyrosine A          | 58,743                                    | 4.4                                      | 4.7                                        | 48,758                                    | 1.8                                       | 19                                         | 15                       |
| tyrosine B          | 37,452                                    | 0                                        | 0                                          | 38,545                                    | 0                                         | 0                                          | 5                        |
| tyrosine C          | 113,033                                   | 17.3                                     | 18                                         | 73,286                                    | 6.8                                       | 73                                         | 45                       |
| shikimic acid A     | 51,537                                    | 2.3                                      | 2                                          | 54,998                                    | 3.0                                       | 32                                         | 15                       |
| shikimic acid B     | 272,363                                   | 162                                      | 172                                        | 384,437                                   | NQ                                        | NQ                                         | 405                      |
| shikimic acid C     | 371,154                                   | 820                                      | 871                                        | 435,532                                   | NQ                                        | NQ                                         | 1215                     |
| uridine A           | 19,128                                    | 0                                        | 0                                          | 21,873                                    | 0                                         | 0                                          | 0                        |
| uridine B           | 413,897                                   | NQ                                       | NQ                                         | 420,263                                   | NQ                                        | NQ                                         | 1215                     |
| uridine C           | 94,571                                    | 12.0                                     | 13                                         | 82,592                                    | 9.0                                       | 96                                         | 45                       |
| biotin A            | 502,667                                   | NQ!                                      | NQ                                         | 52,387                                    | 2.5                                       | 26                                         | 15                       |
| biotin B            | 374,779                                   | 885                                      | 941                                        | 282,094                                   | 186                                       | 1979                                        | 405                      |
| biotin C            | 39,346                                    | NQ                                       | NQ                                         | 39,222                                    | NQ                                        | NQ                                         | 5                        |
| brucine A           | 22,979                                    | NQ                                       | NQ                                         | 21,446                                    | NQ                                        | NQ                                         | 0                        |
| brucine B           | 289,030                                   | 205                                      | 219                                        | 148,341                                   | 30.8                                      | 328                                        | 135                      |
| brucine C           | 186,849                                   | 50                                       | 53                                         | 103,716                                   | 14.5                                      | 154                                        | 45                       |
| yohimbine A         | 413,107                                   | NQ                                       | NQ                                         | 400,248                                   | NQ                                        | NQ                                         | 1215                     |
| yohimbine B         | 243,196                                   | 109                                      | 116                                        | 172,197                                   | 45.0                                      | 478                                        | 135                      |
| yohimbine C         | 42,391                                    | 2.5                                      | 2.6                                        | 47,749                                    | 1.6                                       | 17                                         | 5                        |
| progesterone A      | 149,166                                   | 31.2                                     | 33                                         | 101,893                                   | 14.0                                      | 149                                        | 45                       |
| progesterone B      | 167,719                                   | 41.8                                     | 44                                         | 219,278                                   | 78                                        | 830                                        | 135                      |
| progesterone C      | 42,191                                    | 0.5                                      | 1                                          | 45,477                                    | 1.1                                       | 12                                         | 5                        |
| Imatinib A          | 38,689                                    | NQ                                       | NQ                                         | 28,539                                    | NQ                                        | NQ                                         | 5                        |
| Imatinib B          | 256,956                                   | 131                                      | 140                                        | 162,552                                   | 38.5                                      | 409                                        | 135                      |
| Imatinib C          | 385,891                                   | 1138                                     | 1210                                       | 340,725                                   | NQ                                        | NQ                                         | 405                      |

NQ = not quantified because the fluorescence range is outside of the palladium standard curve.

Correlation between theoretical Pd and measured Pd conc.
|                  | Average Fluorescence with 1 mg/mL sample | Calculated [Pd] (nM) with 1 mg/mL sample | Ppm in solid with 1 mg/mL sample | Average Fluorescence with 0.1 mg/mL sample | Calculated [Pd] (nM) with 0.1 mg/mL sample | Ppm in solid with 0.1 mg/mL sample |
|------------------|-----------------------------------------|----------------------------------------|---------------------------------|----------------------------------------|----------------------------------------|----------------------------------|
| BMK1030          | 419,382                                 | NQ                                     | NQ                              | 282,298                                | 187                                    | 1984                             |
| RKB9101 crude    | 389,039                                 | NQ                                     | NQ                              | 345,459                                | 500                                    | 5322                             |
| RKB9101 after CC | 354,876                                 | NQ                                     | NQ                              | 174,104                                | 42                                     | 443                              |
| RKB9102 impure   | 402,049                                 | NQ                                     | NQ                              | 369,354                                | 789                                    | 8393                             |
| RKB9102 after resin | 366,114                               | NQ                                     | NQ                              | 211,317                                | 70                                     | 748                              |
| PLM 3108A        | 66,335                                  | 5.5                                    | 6                               | 46,734                                  | NQ                                     | NQ                               |
| PLM 3108B        | 72,445                                  | 6.5                                    | 7                               | 48,040                                  | NQ                                     | NQ                               |
| PLM 3109A        | 84,119                                  | 8.6                                    | 9                               | 47,645                                  | NQ                                     | NQ                               |
| PLM 3109B        | 79,770                                  | 7.8                                    | 8                               | 43,570                                  | NQ                                     | NQ                               |
| RKB9019          | 367,647                                 | NQ                                     | NQ                              | 123,829                                | 19                                     | 201                              |
| JAB7140          | 420,706                                 | NQ                                     | NQ                              | 350,323                                | 546                                    | 5808                             |
| JAB7141          | 398,091                                 | NQ                                     | NQ                              | 375,119                                | 891                                    | 9480                             |
| JAB7140 purified | 394,048                                 | NQ                                     | NQ                              | 116,076                                | 16                                     | 175                              |
| JAB7141 purified | 322,234                                 | NQ                                     | NQ                              | 93,833                                 | 11                                     | 113                              |

Correlation between ICP-MS and current method in synthetic samples

Procedure for the experiment to repeat of Figure 7d

**Final conditions:** See the procedure for Figure 7d

**Preparation of Pd solutions for calibration curve**

See the procedure for Figure 7d

**Preparation of 900 μM TFP/100 mM NaBH₄/375 mM NaOH in 6% DMSO/44% EtOH/water**

See the procedure for Figure 7d

**Assay**
1) Transfer 85.7 μM RAE/286 mM NH₄OAc in 10.7%DMSO/EtOH (140 μL per well) from a reservoir to rows B-H, columns 1-12 on the first black 96-well plate with clear bottom. Transfer again (140 μL per well) to rows A-C of the second black well plate with clear bottom.

2) Transfer 0, 19.6, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000, 10000, 20000 nM Pd in solution B (20 μL) from row A to row B-E of the first plate.

3) Transfer 1 mg/mL mock API samples (20 μL) to row F-H of the first plate.

4) Transfer 0.1 mg/mL mock API samples (20 μL) to row A-C of the second plate.

5) Transfer 900 μM TFP/100 mM NaBH₄/375 mM NaOH in 6% DMSO/44% EtOH/water (40 μL per well) from a reservoir to all wells with the reaction mixture.

6) Measure fluorescence after 30 min.

---

**Procedure for reproducing the experiment for the above experiment with freshly prepared mock API samples**

*Final conditions:* 180 μM TFP, 60 μM RAE, 20 mM NaBH₄, 75 mM NaOH, 200 mM NH₄OAc, 8.7% DMSO and 17% water in EtOH, 0-2000 nM Pd, 24 °C, 30 min, n = 3.

**Preparation of Pd solutions for calibration curve**

See the procedure for Figure 7d.

**Preparation of mock Pd samples**

1) Prepare spiked 1 mg/mL mock API samples (95 μL shikimic acid or biotin stock 5.26 mg/mL; 5 μL of corresponding Pd sample; 400 μL 1.75% HCl)

2) Prepare spiked 0.1 mg/mL mock API samples (85.5 μL DMSO; 9.5 μL shikimic acid or biotin stock 5.26 mg/mL; 400 μL 1.75% HCl)

**Preparation of 900 μM TFP/100 mM NaBH₄/375 mM NaOH in 6% DMSO/44% EtOH/water**

See the procedure for Figure 7d.

**Assay**

1) Transfer 85.7 μM RAE/286 mM NH₄OAc in 10.7%DMSO/EtOH (140 μL per well) from a reservoir to rows B-H, columns 1-12 on a black 96-well plate with clear bottom.
2) Transfer 0, 19.6, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000, 10000, 20000 nM Pd in solution B (20 µL) from row A to row B-E.

3) Transfer 1.0 mg/mL samples (20 µL) to row F-H columns 1-6 of the same plate.

4) Transfer 0.1 mg/mL samples (20 µL) to row F-H columns 7-12 of the same plate.

5) Transfer 900 µM TFP/100 mM NaBH₄/375 mM NaOH in 6% DMSO/44% EtOH/water (40 µL per well) from a reservoir to all wells with the reaction mixture.

6) Measure fluorescence after 30 min.

| Sample           | Average Fluorescence (nM) | Calculated [Pd] (nM) | Ppm in solid with 1 mg/mL | Average Fluorescence (nM) | Calculated [Pd] (nM) | Ppm in solid (mg/mL) | Theoretical ppm in solid |
|------------------|---------------------------|---------------------|---------------------|---------------------------|---------------------|---------------------|-------------------------|
| Shikimic acid A  | 9.03E+04                  | 10                  | 10                  | 9.12E+04                  | 10                  | 212                 | 15                      |
| Shikimic acid B  | 3.82E+05                  | 175                 | 186                 | 3.99E+05                  | NQ                  | NQ                  | 405                     |
| Shikimic acid C  | 4.12E+05                  | NQ                  | NQ                  | 4.03E+05                  | NQ                  | NQ                  | 1215                    |
| Biotin A         | 4.08E+04                  | 4                   | 4.1                 | 3.55E+04                  | 2.5                 | 53                  | 5                       |
| Biotin B         | 8.83E+04                  | 9                   | 9.9                 | 8.51E+04                  | 8                   | 170                 | 15                      |
| Biotin C         | 3.87E+05                  | 230                 | 245                 | 3.82E+05                  | 225                 | 4787                | 405                     |

\[
Y = V_{\max} \times \frac{X}{(K_m + X)}
\]

\[
F = \frac{457919 \times [Pd] \times (38.97 + [Pd])}{[Pd] = 38.97 \times F / (457917 - F)}
\]

\[
Y = (Y_0 - \text{Plateau}) \times \exp(-K \times X) + \text{Plateau}
\]

\[
[Pd] = -38.21 \times \ln(347185 - F) / 333437
\]
Summary of method evaluation

|                  | Ppm in solid determined with 1 mg/mL sample | Ppm in solid determined with 0.1 mg/mL sample | Theoretical ppm in solid |
|------------------|--------------------------------------------|---------------------------------------------|--------------------------|
| 6-bromoindole A  | 95                                         | 45                                          |                          |
| 6-bromoindole B  | 85                                         | 405                                         |                          |
| 6-bromoindole C  | <1                                         | 0                                           |                          |
| tyrosine A       | 19                                         | 15                                          |                          |
| tyrosine B       | 4                                          | 5                                           |                          |
| tyrosine C       | 73                                         | 45                                          |                          |
| shikimic acid A  | 10                                         | 15                                          |                          |
| shikimic acid B  | 209                                        | 405                                         |                          |
| shikimic acid C  | 871                                        | 1215                                        |                          |
| uridine A        | <1                                         | 0                                           |                          |
| uridine B        | >2000                                      | 1215                                        |                          |
| uridine C        | 96                                         | 45                                          |                          |
| biotin A         | 26                                         | 15                                          |                          |
| biotin B         | 941                                        | 405                                         |                          |
| biotin C         | <1                                         | 5                                           |                          |
| brucine A        | <1                                         | 0                                           |                          |
| brucine B        | 219                                        | 135                                         |                          |
| brucine C        | 53                                         | 45                                          |                          |
| yohimbine A      | >2000                                      | 1215                                        |                          |
| yohimbine B      | 116                                        | 135                                         |                          |
| yohimbine C      | 3                                          | 5                                           |                          |
| progesterone A   | 33                                         | 45                                          |                          |
| progesterone B   | 44                                         | 135                                         |                          |
| progesterone C   | 12                                         | 5                                           |                          |
| Imatinib A       | <1                                         | 5                                           |                          |
| Imatinib B       | 140                                        | 135                                         |                          |
| Imatinib C       | 1210                                       | 405                                         |                          |
Procedure for Figure 8 (Michaelis-Menten kinetics)

Final conditions: 180 μM TFP, 7.5, 15, 30, 60, 120, 240, 480, 960 μM RAE, 0.5 mM NaBH₄, 75 mM NaOH, 200 mM NH₄OAc, 8.7% DMSO and 17% water in EtOH, 0, 15.6, 31.3 nM Pd, 24 °C, 0, 4, 8, 12, 16, 20 min, n = 3.

Preparation of 286 mM NH₄OAc in 1:9 DMSO/EtOH

Mix 500 mM NH₄OAc in EtOH (17.16 mL), EtOH (9.84 mL), and DMSO (3.00 mL) in a 50-mL plastic centrifuge tube.

Preparation of 10.7, 21.4, 42.9, 85.7, 171.4, 342.9, 685.7, and 1371 μM RAE/286 mM NH₄OAc in 10% DMSO/EtOH

1) Mix 20 mM RAE in DMSO (274 μL), DMSO (226 μL), 500 mM NH₄OAc in EtOH (2.002 mL), and EtOH (1.4998 mL) to prepare 1371 μM RAE/286 mM NH₄OAc in 1:9 DMSO/EtOH (5.00 mL) in an amber vial.

2) Serially dilute the above solution (2.00 mL) with 286 mM NH₄OAc in 1:9 DMSO/EtOH (2.00 mL) up to 10.7 μM RAE.

Preparation of 313 and 156 nM Pd in solution B

1) Dilute 2.0 mM Pd in (3% trace metal) M HCl (100 μL) with solution B (900 μL) to prepare 200 μM Pd.

2) Dilute the 200 μM Pd (50 μL) with solution B (950 μL) in row F column 12 in a deep 96-well plate to prepare 10 μM Pd.

3) Add solution B (500 μL) to row F columns 5-11.

4) Serially dilute 2-fold in a deep 96-well plate until 156 nM in column 6 in a deep 96-well. NOTE: Suction slowly and mix extensively each time. Add Pd-free solution B to column 35 row F. Do not add Pd to row F column 5.

5) Seal the wells with Scotch tape till the end of the day.

Preparation of 900 μM TFP/2.5 mM NaBH₄/375 mM NaOH in 6% DMSO/44% EtOH/water

1) After step 2 of the assay, dilute the 2.67 M NaBH₄ in 10 M NaOH (1.80 mL) with water (10.20 mL) in a 15-mL plastic centrifuge tube to prepare 400 mM NaBH₄ in 1.5 M NaOH on ice.

2) Dilute 400 mM NaBH₄ in NaOH (100 μL) with 1.5 M NaOH (3.900 mL) to prepare 10 mM NaBH₄ in 1.5 NaOH

3) Mix cold EtOH (5.28 mL), 15 mM TFP in DMSO (720 μL), 10 mM NaBH₄ in 1.5 M NaOH (3.00 mL), and water (3.00 mL) in a reservoir (12 mL).

Assay

1) Transfer each corresponding RAE solution (140 μL) to columns 1-8, rows A-F of a 96 well black plate with clear bottom. Begin with 10.7 μM RAE in column 1 and finish with 1371 μM RAE in column 8

2) Transfer Pd free solution B (20 μL) to rows A-C of the first plate. Transfer 156 nM Pd solution (20 μL) to rows D-F of the same plate.

3) Measure fluorescence with a green filter. If the standard deviation is too large, pipette again onto a different plate.
4) Transfer 900 µM TFP/10 mM NaBH₄/375 mM NaOH in 6% DMSO/44% EtOH/water (40 µL) to all the wells.

5) Measure fluorescence with a green filter immediately and every 3 minutes until t = 12 min. Stop the reaction when the highest yield exceeds 10% yield.

Procedure for Scheme 2

A 50-mL round-bottomed flask equipped with a Teflon-coated magnetic stir bar containing RAE (5.8 mg, 0.023 mmol) was treated with EtOH (10.0 mL), DMSO (0.60 mL), water (2.0 mL), and 16 M NaOH (25 µL). Dark red reaction mixture turned dark purple. EtOH was removed in vacuo, then the resulting mixture was neutralized with 1 M HCl to pH 7. Dark purple reaction mixture turned dark red. The mixture was extracted with CH₂Cl₂ (2 × 10 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated in vacuo to afford dark red solid.

Spectroscopic data for this product matches that of RAE as shown in the literature.¹⁹
**Table S1.** Raw data for Table 1 and Figure 2a (fluorescence of resorufin as a function of palladium concentration at 16 minutes).

| Pd (nM) | 10.0 mM NaBH₄ | 45.0 mM NaBH₄ |
|---------|----------------|----------------|
| 0.000   | 4,697          | 4,750          |
| 0.490   | 5,131          | 10,418         |
| 0.980   | 5,055          | 9,747          |
| 1.95    | 5,026          | 11,979         |
| 3.90    | 5,214          | 14,888         |
| 7.80    | 5,329          | 19,034         |
| 15.6    | 5,740          | 19,979         |
| 31.3    | 5,570          | 47,399         |
| 62.5    | 7,661          | 61,236         |
| 125     | 11,711         | 77,176         |
| 250     | 12,788         | 92,274         |

**Table S2.** Raw data for Table 1 and Figure 2a and Figure 2b (fluorescence of resorufin as a function of palladium concentration at 16 minutes).

| Pd (nM) | 50.0 mM NaBH₄ | 75.0 mM NaBH₄ |
|---------|----------------|----------------|
| 0.000   | 6,342          | 6,591          |
| 0.490   | 6,244          | 13,015         |
| 0.980   | 7,938          | 11,948         |
| 1.95    | 8,564          | 13,163         |
| 3.90    | 9,191          | 17,218         |
| 7.80    | 10,461         | 23,197         |
| 15.6    | 11,531         | 41,026         |
| 31.3    | 11,677         | 75,258         |
| 62.5    | 20,121         | 84,584         |
| 125     | 29,823         | 108,508        |
| 250     | 50,922         | 29,823         |

**Table S3.** Raw data for Table 1 and Figure 3a (effect of NaOH concentration on fluorescence).

| NaOH (mM) | no Pd | 30.0 nM Pd |
|-----------|-------|------------|
| 20.0      | 6,318 | 6,094      |
| 45.0      | 6,830 | 7,084      |
| 70.0      | 7,074 | 8,335      |
| 120       | 6,806 | 16,280     |
| 220       | 6,613 | 14,533     |
| 420       | 6,255 | 10,646     |
Table S4. Raw data table for Table 1 and Figure 3b (effect of NH₄OAc concentration on fluorescence).

| NH₄OAc (mM) | No Pd and 70.0 mM NaOH | 30.0 nM Pd and 70.0 mM NaOH |
|-------------|------------------------|----------------------------|
| 0.000       | 34,204 31,436 32,972   | 53,323 41,113 48,578       |
| 74.0        | 16,781 14,581 13,516   | 28,823 44,127 35,408       |
| 111         | 5,037 4,885 5,071      | 5,088 5,144 5,259          |
| 167         | 6,581 6,609 6,490      | 7,047 7,543 7,978          |
| 250         | 6,628 6,452 6,420      | 6,829 7,042 6,293          |
| 375         | 7,728 7,339 7,236      | 7,557 7,585 8,041          |

Table S5. Raw data table for Table 1 and Figure 3c (effect of NH₄OAc concentration on fluorescence).

| NH₄OAc (mM) | No Pd and 120 mM NaOH | 200 nM Pd and 120 mM NaOH |
|-------------|-----------------------|---------------------------|
| 0.0         | 13,547 12,411 15,244  | 128,847 135,578 138,502  |
| 74.0        | 7,539 6,384 5,938     | 8,008 9,191 9,813         |
| 111         | 6,884 5,583 5,190     | 8,081 8,219 12,502        |
| 167         | 5,092 4,963 4,658     | 5,781 5,675 6,046         |
| 250         | 7,320 5,965 6,582     | 21,187 18,748 36,458      |
| 375         | 6,009 5,953 5,938     | 12,082 11,261 17,774      |
Table S6. Raw data table for Figure 4a (combinatorial screening for NaOH, NH₄OAc, and NaBH₄).

no Pd

| NH₄OAc (mM) | NaOH (M) | NaBH₄ (mM) | 5.00  | 10.0  | 20.0  | 40.0  | 80.0  |
|-------------|-----------|------------|-------|-------|-------|-------|-------|
| 32          |           |            | 68,881| 66,324| 66,715| 65,906| 69,869|
| 47          |           |            | 65,561| 61,847| 64,457| 63,964| 66,473|
| 71          |           |            | 61,899| 61,368| 61,600| 62,164| 64,095|
| 107         |           |            | 61,179| 61,102| 64,645| 64,257| 63,386|
| 160         |           |            | 60,989| 64,297| 59,727| 60,414| 63,214|
| 240         |           |            | 62,280| 62,284| 59,761| 55,566| 61,080|
| 360         |           |            | 61,370| 60,593| 63,759| 58,997| 59,712|

| NH₄OAc (mM) | NaOH (M) | NaBH₄ (mM) | 0.800 | 0.400 | 0.200 | 0.160 |
|-------------|-----------|------------|-------|-------|-------|-------|
| 32          |           |            | 75,988| 80,973| 53,076| 83,303| 83,936| 82,111| 82,913| 82,058|
| 47          |           |            | 71,186| 80,502| 78,029| 79,937| 83,936| 84,807| 83,168| 81,446|
| 71          |           |            | 71,218| 78,769| 75,631| 75,860| 71,455| 79,363| 82,061| 79,133| 78,667|
| 107         |           |            | 67,048| 72,947| 74,363| 66,878| 71,335| 75,189| 75,285| 78,699| 74,365| 72,666|
| 160         |           |            | 66,199| 72,771| 74,380| 69,946| 70,510| 75,607| 80,255| 80,684| 72,127| 72,923|
| 240         |           |            | 66,441| 70,363| 67,262| 63,773| 70,188| 76,520| 79,740| 77,092| 73,032| 73,646|
| 360         |           |            | 62,807| 72,884| 69,198| 69,369| 72,908| 76,694| 78,617| 75,709| 78,397| 77,989|

| NH₄OAc (mM) | NaOH (M) | NaBH₄ (mM) | 0.200 | 0.160 |
|-------------|-----------|------------|-------|-------|
| 32          |           |            | 79,082| 76,148| 82,610| 77,150| 93,288| 87,635| 86,517| 90,601| 79,082| 76,148|
| 47          |           |            | 76,735| 75,725| 82,661| 81,757| 91,467| 83,991| 86,679| 77,778| 76,735| 75,725|
| 71          |           |            | 76,612| 74,209| 79,033| 80,737| 94,155| 82,621| 83,403| 80,589| 76,612| 74,209|
| 107         |           |            | 73,778| 72,497| 75,928| 73,792| 97,636| 83,478| 79,717| 80,766| 73,778| 72,497|
| 160         |           |            | 71,368| 70,976| 72,998| 74,166| 91,008| 78,989| 72,683| 76,753| 71,368| 70,976|
| 240         |           |            | 66,999| 63,299| 74,078| 71,932| 84,154| 78,533| 76,477| 74,376| 66,999| 63,299|
| 360         |           |            | 68,161| 70,892| 71,503| 76,144| 85,944| 79,775| 78,942| 73,659| 68,161| 70,892|
200 nM Pd

| NH₄OAc (mM) | NaOH (M) | 1.60 |
|------------|----------|------|
|            | NaBH₄ (mM) | 5.00 | 10.0 | 20.0 | 40.0 | 80.0 |
| 32         | 22,766    | 26,005 | 34,745 | 49,704 | 127,950 |
| 47         | 20,820    | 23,968 | 34,183 | 51,929 | 118,669 |
| 71         | 21,309    | 24,141 | 36,086 | 60,482 | 116,172 |
| 107        | 16,798    | 23,325 | 34,360 | 49,884 | 105,033 |
| 160        | 20,765    | 24,231 | 34,639 | 57,444 | 123,943 |
| 240        | 17,972    | 22,449 | 30,147 | 52,701 | 100,035 |
| 360        | 19,980    | 21,813 | 31,954 | 53,931 | 92,179 |

| Fluorescence (A.U.) | NaOH (M) | 0.800 | 0.400 |
|---------------------|----------|-------|-------|
|                     | NH₄OAc (mM) | NaBH₄ (mM) | 5.00 | 10.0 | 20.0 | 40.0 | 80.0 | 5.00 | 10.0 | 20.0 | 40.0 | 80.0 |
| 32                   | 37,419   | 81,260 | 93,169 | 122,731 | 109,558 | 42,967 | 53,461 | 63,112 | 102,048 | 22,766 |
| 47                   | 43,348   | 69,862 | 83,371 | 103,114 | 112,040 | 48,734 | 55,710 | 73,016 | 86,681 | 20,820 |
| 71                   | 40,439   | 66,410 | 85,718 | 111,084 | 47,472 | 59,876 | 62,647 | 16,798 | 21,309 |
| 107                  | 42,458   | 64,079 | 77,379 | 82,903 | 91,563 | 44,017 | 55,478 | 67,819 | 82,206 | 16,798 |
| 160                  | 45,611   | 60,140 | 79,109 | 82,588 | 113,622 | 53,551 | 55,371 | 71,384 | 81,447 | 20,765 |
| 240                  | 38,548   | 53,430 | 79,280 | 90,648 | 96,703 | 45,164 | 60,771 | 69,833 | 84,822 | 17,972 |
| 360                  | 39,086   | 49,509 | 66,406 | 87,633 | 85,407 | 41,071 | 60,336 | 67,626 | 88,046 | 19,980 |

| Fluorescence (A.U.) | NaOH (M) | 0.200 | 0.160 |
|---------------------|----------|-------|-------|
|                     | NH₄OAc (mM) | NaBH₄ (mM) | 5.00 | 10.0 | 200.0 | 40.0 | 80.0 | 5.00 | 10.0 | 200.0 | 40.0 | 80.0 |
| 32                   | 94,058   | 47,592 | 62,129 | 68,362 | 96,137 | 100,000 | 94,058 | 47,592 | 62,129 | 68,362 |
| 47                   | 87,102   | 57,514 | 64,280 | 78,850 | 91,204 | 99,566 | 87,102 | 57,514 | 64,280 | 78,850 |
| 71                   | 107,765  | 63,887 | 74,693 | 76,591 | 86,833 | 104,020 | 107,765 | 63,887 | 74,693 | 76,591 |
| 107                  | 84,434   | 59,836 | 66,236 | 73,363 | 84,458 | 96,063 | 84,434 | 59,836 | 66,236 | 73,363 |
| 160                  | 101,838  | 64,059 | 62,709 | 78,236 | 85,625 | 100,257 | 101,838 | 64,059 | 62,709 | 78,236 |
| 240                  | 90,169   | 59,933 | 65,691 | 75,179 | 90,453 | 91,314 | 90,169 | 59,933 | 65,691 | 75,179 |
| 360                  | 98,572   | 60,071 | 76,448 | 76,528 | 98,455 | 93,499 | 98,572 | 60,071 | 76,448 | 76,528 |
### Table S7. Raw data for Figure 4b (NaOH and NaBH₄ screening).

| NH₄OAc (mM) | NaBH₄ (mM) | Fluorescence (A.U.) | 200 nM Pd | no Pd |
|-------------|------------|---------------------|-----------|-------|
|             |            |                     | 5 | 10 | 20 | 40 | 80 | 5 | 10 | 20 | 40 | 80 |
| 32          |            |                     | 78,037 | 106,179 | 132,923 | 137,266 | 175,848 | 5,275 | 5,380 | 6,179 | 7,809 | 8,924 |
| 47          |            |                     | 73,555 | 90,517 | 142,854 | 170,027 | 172,850 | 5,652 | 6,072 | 7,058 | 7,206 | 9,101 |
| 71          |            |                     | 24,427 | 53,425 | 119,802 | 199,550 | 209,722 | 3,518 | 4,020 | 4,369 | 5,418 | 7,589 |
| 107         |            |                     | 5,481 | 15,143 | 38,069 | 72,222 | 187,427 | 2,446 | 2,552 | 2,559 | 2,955 | 4,959 |
| 160         |            |                     | 4,254 | 11,327 | 25,704 | 66,958 | 197,269 | 1,802 | 2,355 | 2,603 | 5,515 | 2,479 |
| 240         |            |                     | 78,037 | 106,179 | 132,923 | 137,266 | 175,848 | 5,275 | 5,380 | 6,179 | 7,809 | 8,924 |
| 360         |            |                     | 73,555 | 90,517 | 142,854 | 170,027 | 172,850 | 5,652 | 6,072 | 7,058 | 7,206 | 9,101 |

### Table S8. Raw data for Figure 5a ([NaBH₄]-dependent fluorescence and autonomous stalling).

#### 31.0 nM Pd

| Time (min) | 0 mM NaBH₄ | 0.625 mM NaBH₄ | 1.25 mM NaBH₄ | 2.5 mM NaBH₄ |
|------------|------------|----------------|----------------|--------------|
| 0          | -460 216   | 3,037 3,456    | 5,332 2,414    | 6,519 4,433  |
| 3          | -317 284   | 6,089 6,928    | 9,578 4,106    | 12,444 9,146 |
| 6          | -533 138   | 8,586 9,227    | 12,739 5,237   | 16,815 12,784|
| 9          | -652 340   | 9,459 10,603   | 14,141 6,786   | 19,971 15,672|
| 12         | -569 633   | 10,945 12,999  | 15,646 7,777   | 20,793 18,248|
| 15         | -374 552   | 11,546 12,824  | 16,348 8,983   | 20,099 20,457|
| 18         | -273 147   | 12,158 13,460  | 17,095 9,599   | 24,493 20,018|
| 21         | -151 169   | 13,040 14,224  | 18,883 11,027  | 25,205 24,919|
| 24         | -267 93    | 13,899 15,556  | 19,632 12,642  | 27,325 24,825|
| 27         | -254 17    | 15,788 16,775  | 21,581 13,493  | 28,102 27,565|
| 30         | -453 182   | 16,833 17,734  | 22,018 15,014  | 28,935 29,104|
| 33         | -812 233   | 17,402 19,276  | 23,385 15,604  | 29,594 30,242|
| 36         | -788 137   | 18,971 21,232  | 24,064 15,834  | 31,867 31,685|
| 39         | -837 -74   | 20,084 22,303  | 24,603 17,024  | 33,522 34,737|
| 42         | -592 175   | 21,853 22,962  | 26,689 18,047  | 34,143 35,512|
| 45         | -904 229   | 23,765 23,278  | 28,831 19,473  | 36,016 36,451|
| 48         | -1098 203  | 23,591 23,490  | 30,148 20,204  | 37,348 37,577|
| 51         | -1011 83   | 22,465 23,503  | 31,527 21,790  | 39,164 38,969|
| 54         | -927 23    | 21,876 23,415  | 32,880 22,746  | 40,642 39,712|
| 57         | -847 -34   | 21,707 23,328  | 36,304 21,593  | 42,577 41,144|
| 60         | -798 -74   | 21,549 23,266  | 40,161 20,429  | 44,119 42,496|
| 63         | -863 -125  | 21,447 23,206  | 44,852 19,726  | 46,234 44,910|
| 66         | -916 -175  | 21,373 23,119  | 45,686 19,210  | 48,082 46,469|
| 69         | -974 -226  | 21,306 23,014  | 44,844 18,423  | 50,311 45,573|
| 72         | -1,023 -278| 21,182 22,945  | 43,857 17,277  | 52,115 45,214|
| 75         | -1,079 -333| 21,058 22,940  | 43,518 15,691  | 53,491 44,377|
| 78         | -1,140 -381| 20,907 22,933  | 43,396 13,663  | 54,938 43,362|
| 81         | -1,196 -418| 20,843 22,968  | 43,316 11,246  | 53,809 42,093|
| 84         | -1,251 -429| 20,810 22,974  | 43,202 8,566   | 52,844 40,939|
| 87         | -1,296 -406| 20,719 22,942  | 43,092 5,707   | 52,488 39,273|
Table S9. Raw data for Figure 5b (background corrected [NaBH₄]-dependent fluorescence is shown at 40 min point).

31.0 nM Pd

| [NaBH₄] (mM) | F₄₀₀-F₀   |
|--------------|-----------|
| 0.000        | -837 -74  |
| 0.625        | 20,084 22,303 |
| 1.25         | 24,603 17,024 |
| 2.50         | 33,522 34,737 |
| 5.00         | 48,002 41,801 |
| 10.0         | 95,651 81,155 |
| 20.0         | 94,391 66,369 |
| 40.0         | 110,161 79,821 |

Table S10. Raw data for Figure 5c (TFP and DMSO optimization).

31.0 nM Pd

| TFP (μM)  | 5.00 | 7.50 | 10.0 | 12.5 | 15.0 | 17.5 |
|-----------|------|------|------|------|------|------|
| DMSO % (v/v) | 5.00 | 28,039 | 37,542 | 34,164 | 21,845 | 11,100 |
| 7.50      | 20,155 | 33,734 | 30,413 | 19,666 | 14,882 | 11,071 |
| 10.0      | 44,991 | 43,668 | 45,787 | 23,428 | 24,241 | 15,976 |
| 12.5      | 40.002 | 41,801 | 34,037 | 21,599 | 17,020 | 15,034 |
| 15.0      | 67,495 | 64,063 | 50,104 | 34,527 | 26,000 | 16,113 |
| 17.5      | 60,583 | 57,629 | 44,140 | 36,317 | 28,694 | 15,034 |

| TFP (μM)  | 5.00 | 7.50 | 10.0 | 12.5 | 15.0 | 17.5 |
|-----------|------|------|------|------|------|------|
| DMSO % (v/v) | 5.00 | 28,039 | 37,542 | 34,164 | 21,845 | 11,100 |
| 7.50      | 20,155 | 33,734 | 30,413 | 19,666 | 14,882 | 11,071 |
| 10.0      | 44,991 | 43,668 | 45,787 | 23,428 | 24,241 | 15,976 |
| 12.5      | 40.002 | 41,801 | 34,037 | 21,599 | 17,020 | 15,034 |
| 15.0      | 67,495 | 64,063 | 50,104 | 34,527 | 26,000 | 16,113 |
| 17.5      | 60,583 | 57,629 | 44,140 | 36,317 | 28,694 | 15,034 |

S31
| metal | Fluorescence (A.U.) |
|-------|---------------------|
| none  | 6,072 5,537 5,424 |
| Ag    | 5,834 5,859 5,427 |
| Au    | 6,126 5,975 5,601 |
| Cd    | 6,185 6,094 6,230 |
| Co    | 5,933 5,534 5,802 |
| Cr    | 5,795 5,796 5,650 |
| Cu    | 5,797 6,031 5,702 |
| Fe    | 5,942 5,858 5,802 |
| Hg    | 6,163 5,764 5,862 |
| Ir    | 7,268 5,888 5,726 |
| Mn    | 6,040 5,555 5,498 |
| Mo    | 5,797 5,760 5,624 |
| Ni    | 6,045 5,836 5,680 |
| Pb    | 5,840 5,742 5,639 |
| Pd    | 41,151 41,895 42,384 |
| Pt    | 8,961 8,904 9,862 |
| Re    | 5,898 5,825 5,603 |
| Rh    | 5,782 5,526 5,597 |
| Ru    | 5,717 5,633 5,516 |
| Ti    | 5,839 5,682 5,475 |
| V     | 5,693 5,691 5,411 |
| Zn    | 5,467 5,508 5,336 |
Table S12. Raw data for Figure 6b (metal interference).

| metal | Fluorescence (A.U.) |
|-------|---------------------|
| none  | 63,664 64,452 59,132 |
| Ag    | 9,725 9,715 9,595 |
| Au    | 64,426 66,644 69,297 |
| Cd    | 48,651 46,782 47,740 |
| Co    | 65,285 64,448 62,823 |
| Cr    | 65,130 65,697 59,387 |
| Cu    | 56,928 53,799 52,661 |
| Fe    | 65,815 64,481 60,928 |
| Hg    | 61,795 59,571 57,840 |
| Ir    | 62,458 65,461 60,467 |
| Mn    | 60,200 62,244 60,809 |
| Mo    | 60,853 63,935 59,044 |
| Ni    | 49,576 64,180 29,281 |
| Pb    | 49,704 61,599 34,807 |
| Pt    | 187,114 185,634 210,699 |
| Re    | 49,859 61,594 31,202 |
| Rh    | 58,986 68,689 35,342 |
| Ru    | 58,822 65,097 31,041 |
| Ti    | 38,110 44,878 28,584 |
| V     | 58,908 48,529 47,854 |
| Zn    | 65,500 55,397 47,700 |
Table S13. Raw data for Figure 6c (metal selectivity represented by absorbance).

| Metal | Absorbance  |
|-------|-------------|
| none  | 0.175313 0.176785 0.173232 |
| Ag    | 0.090077 0.090809 0.088071 |
| Au    | 0.190002 0.194609 0.204780 |
| Cd    | 0.156756 0.153170 0.159575 |
| Co    | 0.183185 0.182555 0.180978 |
| Cr    | 0.180438 0.181104 0.174438 |
| Cu    | 0.169814 0.166928 0.164996 |
| Fe    | 0.184518 0.182410 0.179330 |
| Hg    | 0.176286 0.177170 0.172978 |
| Ir    | 0.177201 0.183777 0.175588 |
| Mn    | 0.176372 0.183283 0.178441 |
| Mo    | 0.174992 0.183838 0.176276 |
| Ni    | 0.178084 0.205063 0.138170 |
| Pb    | 0.170566 0.194517 0.143136 |
| Pt    | 0.442797 0.451461 0.496279 |
| Re    | 0.173110 0.196287 0.136984 |
| Rh    | 0.194709 0.215271 0.145860 |
| Ru    | 0.206530 0.214584 0.148926 |
| Ti    | 0.160911 0.178591 0.135747 |
| V     | 0.196633 0.177054 0.173847 |
| Zn    | 0.227130 0.198099 0.182748 |

Table S14. Raw data for Figure 7a (fluorescence as a function of palladium concentration after 60 min).

| Pd (nM) | Fluorescence Intensity (A.U.) |
|---------|------------------------------|
| 0.00    | 13,853 12,076 12,685 |
| 3.91    | 25,548 19,962 19,785 |
| 7.81    | 35,678 26,495 27,088 |
| 15.6    | 52,344 37,460 42,481 |
| 31.3    | 77,634 60,785 67,805 |
| 62.5    | 111,809 95,829 100,347 |
| 125     | 135,712 125,884 130,989 |
| 250     | 156,351 155,600 152,334 |
| 500     | 164,869 165,422 153,549 |
| 1000    | 180,068 196,994 169,691 |
| 2000    | 194,411 202,883 186,366 |
Table S15. Raw data for Figure 7b (fluorescence as a function of palladium concentration at various time points).

10 minutes

| Pd (nM) | Fluorescence (A.U.) |
|---------|---------------------|
| 0.00    | 6,684 6,521 6,536 7,439 |
| 1.96    | 7,690 6,942 7,688 8,599 |
| 3.91    | 8,461 7,528 9,196 9,144 |
| 7.81    | 9,877 8,924 10,766 11,360 |
| 15.6    | 12,349 10,654 14,727 15,313 |
| 31.3    | 18,274 13,012 21,053 19,967 |
| 62.5    | 23,541 15,743 27,164 24,615 |
| 125     | 32,244 24,673 35,131 34,815 |
| 250     | 32,479 28,845 42,712 43,180 |
| 500     | 47,304 47,874 59,231 63,922 |
| 1000    | 70,818 60,902 81,707 82,705 |
| 2000    | 103,129 110,472 108,566 108,339 |

20 minutes

| Pd (nM) | Fluorescence (A.U.) |
|---------|---------------------|
| 0.00    | 6,666 6,388 6,439 7,575 |
| 1.96    | 7,825 6,811 7,656 9,118 |
| 3.91    | 8,484 7,438 10,033 9,903 |
| 7.81    | 10,059 8,922 11,023 12,053 |
| 15.6    | 12,295 10,615 15,864 17,041 |
| 31.3    | 18,774 12,964 22,060 20,412 |
| 62.5    | 24,011 15,769 28,829 25,019 |
| 125     | 33,786 25,792 36,199 36,072 |
| 250     | 33,035 29,594 61,202 60,662 |
| 500     | 49,058 50,104 79,925 84,836 |
| 1000    | 76,346 64,402 100,652 100,195 |
| 2000    | 112,046 120,793 124,302 119,516 |
### 30 minutes

| Pd (nM) | Fluorescence (A.U.) |
|---------|---------------------|
| 0.00    | 6,569               |
|         | 6,276               |
|         | 6,333               |
|         | 7,587               |
| 1.96    | 7,693               |
|         | 6,732               |
|         | 7,646               |
|         | 9,165               |
| 3.91    | 8,396               |
|         | 7,421               |
|         | 10,055              |
|         | 9,973               |
| 7.81    | 9,979               |
|         | 8,922               |
|         | 11,017              |
|         | 12,080              |
| 15.6    | 12,310              |
|         | 10,605              |
|         | 15,916              |
|         | 17,172              |
| 31.3    | 18,962              |
|         | 13,010              |
|         | 22,157              |
|         | 20,449              |
| 62.5    | 24,347              |
|         | 15,906              |
|         | 29,103              |
|         | 25,186              |
| 125     | 34,247              |
|         | 26,211              |
|         | 36,687              |
|         | 36,379              |
| 250     | 33,465              |
|         | 29,909              |
|         | 70,713              |
|         | 66,558              |
| 500     | 49,912              |
|         | 50,999              |
|         | 87,973              |
|         | 92,509              |
| 1000    | 78,942              |
|         | 66,314              |
|         | 106,875             |
|         | 106,950             |
| 2000    | 117,989             |
|         | 127,151             |
|         | 132,367             |
|         | 125,996             |

### 90 minutes

| Pd (nM) | Fluorescence (A.U.) |
|---------|---------------------|
| 0.00    | 33,440              |
|         | 29,798              |
|         | 33,224              |
|         | 37,095              |
| 1.96    | 45,752              |
|         | 45,464              |
|         | 49,419              |
|         | 50,259              |
| 3.91    | 58,048              |
|         | 54,470              |
|         | 57,989              |
|         | 57,727              |
| 7.81    | 72,121              |
|         | 70,716              |
|         | 74,831              |
|         | 73,076              |
| 15.6    | 98,902              |
|         | 97,011              |
|         | 100,480             |
|         | 97,960              |
| 31.3    | 132,615             |
|         | 128,500             |
|         | 133,123             |
|         | 128,366             |
| 62.5    | 164,737             |
|         | 155,018             |
|         | 159,658             |
|         | 156,115             |
| 125     | 203,971             |
|         | 183,073             |
|         | 198,262             |
|         | 186,877             |
| 250     | 225,206             |
|         | 216,725             |
|         | 218,249             |
|         | 217,700             |
| 500     | 261,418             |
|         | 245,789             |
|         | 248,175             |
|         | 241,100             |
| 1000    | 276,279             |
|         | 250,822             |
|         | 241,906             |
|         | 253,475             |
| 2000    | 294,047             |
|         | 297,507             |
|         | 291,311             |
|         | 290,476             |
Table S16. Raw data for Figure 7c (fluorescence as a function of palladium concentration at various time points).

5 minutes

| Pd (nM) | Fluorescence (A.U.) |
|---------|---------------------|
| 0.00    | 14,902 15,815 16,780 |
| 1.96    | 18,666 23,170 24,015 |
| 3.91    | 26,990 23,041 28,110 |
| 7.81    | 27,173 34,656 36,434 |
| 15.6    | 35,057 50,204 46,419 |
| 31.3    | 53,640 60,238 75,552 |
| 62.5    | 81,883 100,353 93,083 |
| 125     | 105,435 113,422 139,411 |
| 250     | 215,548 171,796 193,027 |
| 500     | 236,401 185,308 197,387 |
| 1000    | 226,477 213,937 237,050 |
| 2000    | 280,189 242,687 235,541 |

10 minutes

| Pd (nM) | Fluorescence (A.U.) |
|---------|---------------------|
| 0.00    | 16,844 20,889 19,924 |
| 1.96    | 24,070 30,784 30,610 |
| 3.91    | 37,441 34,175 37,678 |
| 7.81    | 36,949 49,513 52,387 |
| 15.6    | 49,687 75,775 71,264 |
| 31.3    | 78,186 97,136 115,589 |
| 62.5    | 119,359 149,486 143,503 |
| 125     | 147,606 166,545 207,959 |
| 250     | 311,809 267,319 284,576 |
| 500     | 332,816 276,313 285,173 |
| 1000    | 311,040 302,712 296,422 |
| 2000    | 326,822 318,921 311,355 |

15 minutes

| Pd (nM) | Fluorescence (A.U.) |
|---------|---------------------|
| 0.00    | 19,090 21,902 22,586 |
| 1.96    | 29,464 35,502 34,565 |
| 3.91    | 43,343 41,222 44,657 |
| 7.81    | 44,711 59,439 61,745 |
| 15.6    | 67,083 92,286 87,279 |
| 31.3    | 99,760 118,752 141,780 |
| 62.5    | 146,057 185,546 181,734 |
| 125     | 168,961 207,671 250,192 |
| 250     | 350,785 314,007 328,429 |
| 500     | 374,250 317,122 329,063 |
| 1000    | 350,390 346,607 320,618 |
| 2000    | 370,100 360,828 349,071 |
| Pd (nM) | Fluorescence (A.U.) | Fluorescence (A.U.) | Fluorescence (A.U.) |
|---------|---------------------|---------------------|---------------------|
| 0.00    | 20,170              | 23,644              | 22,776              |
| 1.96    | 32,599              | 37,154              | 36,369              |
| 3.91    | 45,588              | 46,083              | 49,118              |
| 7.81    | 54,344              | 65,342              | 67,745              |
| 15.6    | 79,261              | 101,220             | 96,072              |
| 31.3    | 115,954             | 134,555             | 155,959             |
| 62.5    | 165,958             | 208,100             | 197,052             |
| 125     | 187,801             | 231,192             | 276,124             |
| 250     | 364,159             | 336,283             | 349,083             |
| 500     | 398,821             | 342,755             | 345,813             |
| 1000    | 368,087             | 369,837             | 337,854             |
| 2000    | 400,544             | 394,992             | 374,215             |

| Pd (nM) | Fluorescence (A.U.) | Fluorescence (A.U.) | Fluorescence (A.U.) |
|---------|---------------------|---------------------|---------------------|
| 0.00    | 20,821              | 23,958              | 22,861              |
| 1.96    | 33,420              | 36,426              | 36,153              |
| 3.91    | 48,355              | 48,340              | 48,041              |
| 7.81    | 60,847              | 66,495              | 71,636              |
| 15.6    | 89,736              | 107,343             | 101,216             |
| 31.3    | 132,961             | 140,042             | 165,312             |
| 62.5    | 183,222             | 226,735             | 212,313             |
| 125     | 216,747             | 249,991             | 292,047             |
| 250     | 372,101             | 355,258             | 361,570             |
| 500     | 414,755             | 360,992             | 362,411             |
| 1000    | 381,404             | 391,133             | 346,786             |
| 2000    | 432,874             | 428,934             | 398,538             |

| Pd (nM) | Fluorescence (A.U.) | Fluorescence (A.U.) | Fluorescence (A.U.) |
|---------|---------------------|---------------------|---------------------|
| 0.00    | 20,504              | 23,620              | 22,014              |
| 1.96    | 33,021              | 34,833              | 34,431              |
| 3.91    | 47,423              | 48,507              | 47,436              |
| 7.81    | 60,303              | 66,110              | 71,920              |
| 15.6    | 89,226              | 109,007             | 105,065             |
| 31.3    | 139,813             | 142,604             | 165,212             |
| 62.5    | 189,173             | 222,776             | 212,131             |
| 125     | 228,082             | 251,057             | 292,877             |
| 250     | 366,978             | 358,157             | 362,116             |
| 500     | 410,781             | 364,891             | 355,468             |
| 1000    | 375,846             | 389,500             | 342,435             |
| 2000    | 433,451             | 430,699             | 398,796             |
### 50 minutes

| Pd (nM) | Fluorescence (A.U.) |
|---------|---------------------|
| 0.00    | 19,878 22,929 21,108 |
| 1.96    | 31,653 33,344 32,285 |
| 3.91    | 46,742 46,269 45,869 |
| 7.81    | 59,609 65,603 70,332 |
| 15.6    | 88,747 107,494 101,827 |
| 31.3    | 136,702 149,102 164,587 |
| 62.5    | 182,019 218,338 214,050 |
| 125     | 226,527 252,824 288,568 |
| 250     | 356,300 352,260 354,972 |
| 500     | 402,585 355,243 349,142 |
| 1000    | 364,736 384,327 336,900 |
| 2000    | 426,988 424,283 394,563 |

### 60 minutes

| Pd (nM) | Fluorescence (A.U.) |
|---------|---------------------|
| 0.00    | 18,867 22,078 20,084 |
| 1.96    | 30,442 31,823 29,565 |
| 3.91    | 45,487 45,226 44,204 |
| 7.81    | 58,637 62,796 67,710 |
| 15.6    | 87,605 105,273 100,559 |
| 31.3    | 135,138 140,475 161,914 |
| 62.5    | 179,842 218,042 212,334 |
| 125     | 226,527 252,824 286,426 |
| 250     | 356,300 352,260 354,972 |
| 500     | 402,585 355,243 349,142 |
| 1000    | 364,736 384,327 336,900 |
| 2000    | 426,988 424,283 394,563 |
**Table S17.** Raw data for Figure 7d (fluorescence as a function of palladium concentration at 30 minutes and method comparison).

| Pd (nM) | Fluorescence Intensity (A.U.) |
|---------|------------------------------|
| 0.00    | 12,818 13,739 14,332 16,535 |
| 1.96    | 31,006  27,094  28,613  29,875 |
| 3.91    | 49,080  48,497  45,412  45,246 |
| 7.81    | 79,939  71,884  77,023  70,907 |
| 15.6    | 122,206 128,167 129,672 123,090 |
| 31.3    | 214,278 195,160 194,486 196,558 |
| 62.5    | 270,793 299,944 298,938 297,058 |
| 125     | 340,893 356,781 350,714 339,304 |
| 250     | 382,389 390,245 395,692 392,439 |
| 500     | 425,237 425,734 434,023 429,331 |
| 1000    | 442,838 440,916 441,275 438,565 |
| 2000    | 439,466 446,306 447,313 443,343 |

**Table S18.** Raw data table for Figure 8 (saturation kinetics).

| RAE (μM) | Fluorescence Intensity (A.U.) increase per min during the first 5 min |
|----------|---------------------------------------------------------------------|
| 7.5      | 998                                                                 |
| 15       | 4,036                                                               |
| 30       | 7,427                                                               |
| 60       | 12,276                                                              |
| 120      | 15,326                                                              |
| 240      | 17,549                                                              |
| 480      | 14,228                                                              |
