Cofactor-Induced Refinement of Catalytic Antibody Activity: A Metal-Specific Allosteric Effect

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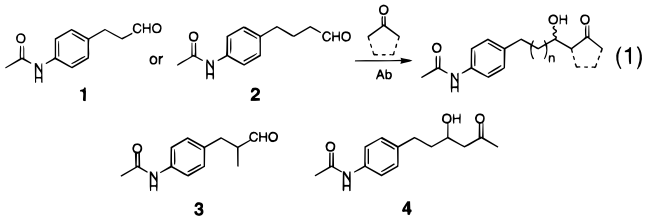
We recently described the development of the strategy of reactive immunization for the production of two catalytic antibodies for the aldol reaction.[1] These catalysts accept a tremendous variety of substrates in aldol addition, aldol condensation, and retro-aldol reactions.[2] They also catalyze a mechanistically related decarboxylation of β-keto acids.[3] A striking example of their effective promiscuity is the highly enantioselective intramolecular aldol condensation to give the Wieland−Miescher ketone, using a catalyst developed by immunization with a hapten only remotely similar to the reaction substrate.[4]

An X-ray crystal structure of one of these aldol catalysts (antibody 33F12) has revealed the presence of a single lysine side chain at the bottom of a large binding pocket lined with hydrophobic residues.[2a] This is consistent with evidence that catalysis occurs via enamine intermediates,[1,2] characteristic of class I aldolases.[5] Along with the ability of metal complexes to assist in the biocatalysis of the aldol reaction, the presence of potential metal-binding groups from several tyrosine and tryptophan residues in the putative antibody active site prompted us to screen the reactivities of these systems with a variety of added metal ions. We report here that "hard" metal ions typically utilized by class II aldolases do not perturb catalysis of the aldol reaction by our antibodies. In contrast, palladium(II) additives improve the rate and enantioselectivity of the process, but only for substrates closely resembling the hapten used for the generation of the antibody.

The reaction of aldehyde **1** with acetone (eq 1)[1] was used to screen the effect of added metal ion. Initial rates in the presence of the following species at 20, 50, and 150 μM were found to be indistinguishable (±5%) from the rates of the aldol reaction in the absence of additive under a standard set of conditions in the saturation regime of catalytic turnover (2 μM antibody;[6] 100 μM **1**; 5% (v/v) acetone; phosphate/saline (PBS) buffer at pH 7.4; 22 ± 1 °C): LiCl, $MgNO_3$, $CaSO_4$, $BaCO_3$, $ZrOCl_2$, $CrCl_2$, $CrCl_3$, $MnCl_2$, $FeSO_4$, $FeCl_3$, $RuCl_3$, $CoCl_2$, $NiCl_2$, K_2PtCl_4, $PtCl_4$, $CuCl_2$, $ZnCl_2$, $CdCl_2$, $HgCl_2$ $CeCl_3$, $LaCl_3$, $La(OTf)_3$, $Eu(OTf)_3$, $YbCl_3$, and $Yb(OTf)_3$. Visible precipitation of the antibodies, with concomitant reduction of the apparent reaction rate, often occurs at metal ion concentrations higher than 150 μM. In contrast,



(1)

$VOSO_4$ at relatively high concentration (100−300 μM relative to 2−5 μM antibody) was found to inhibit the aldol reactions of **1** and **2** with acetone in direct proportion to the amount of metal added,[7] without antibody precipitation.[8]

Palladium(II) is the only metal species tested to have a significant effect on the antibody system at low concentrations. Thus, the standard aldol reaction (**1** + acetone), catalyzed by both 38C2 and 33F12 (2 mM), is *accelerated* by a variety of Pd^{II} salts [K_2PdCl_4 and Na_2PdCl_4, Figure 1A; $Pd(OAc_2)$ and $PdSO_4$, data not shown] and palladium amine complexes [$Pd(en)Cl_2$ and *trans*-$Pd(NH_3)_2Cl_2$, Figure 1B,C]. The magnitude of the relative rate (k_{rel})[9] is roughly proportional to metal concentration below 20 mM, often remaining detectable with as little as a 2-fold excess of Pd with respect to the concentration of antibody active sites.[10] Higher concentrations of Pd^{II} lead to apparent reaction inhibition or attenuation of the acceleratory effect, which is attributed to removal of active antibody from the reaction mixture by increased precipitation as metal concentrations are raised.[11] $Pd(en)Cl_2$ (en = ethylenediamine) consistently induces much less observable precipitation than the other palladium species; accordingly, accelerated rates are observed at substantially higher concentrations of this complex than for other Pd sources. Pd-induced rate accelerations are also observed at higher antibody concentrations and in water instead of PBS buffer.[7]

Michaelis−Menten kinetic parameters measured for the 38C2-catalyzed reaction reveal an improvement in k_{cat} and a small increase in K_m in the presence of Pd^{II} (Table 1). Control experiments show that $Pd(en)Cl_2$−antibody binding is reversible[12] (dissociation constant ≈ 1 μM)[13] without causing functional

(1) Wagner, J.; Lerner, R. A.; Barbas, C. F., III *Science* **1995**, *270*, 1797.
(2) (a) Barbas, C. F., III; Heine, A.; Zhong, G.; Hoffmann, T.; Gramatikova, S.; Björnestedt, R.; List, B.; Anderson, J.; Stura, E. A.; Wilson, E. A.; Lerner, R. A. *Science* **1997**, *278*, 2085. (b) Hoffmann, T.; Zhong, G.; List, B.; Shabat, D.; Anderson, J.; Gramatikova, S.; Lerner, R. A.; Barbas, C. F., III. *J. Am. Chem. Soc. J. Am. Chem. Soc.* **1998**, *120*, 2768. (c) List, B.; Shabat, D.; Barbas, C. F., III; Lerner, R. H. *Chem. Eur. J.* In press.
(3) Björnestedt, R.; Zhong, G.; Lerner, R. A.; Barbas, C. F., III *J. Am. Chem. Soc.* **1996**, *118*, 11720.
(4) Zhong, G.; Hoffmann, T.; Lerner, R. A.; Danishefsky, S.; Barbas, C. F., III. *J. Am. Chem. Soc.* **1997**, *119*, 8131.
(5) Rutter, W. J. *Fed. Proc. Am. Soc. Exp. Biol.* **1964**, *23*, 1248. Hill, H. A. O.; Lobb, R. R.; Sharp, S. L.; Stokes, A. M.; Harris, J. I.; Jack, R. S. *Biochem. J.* **1976**, *153*, 551. Morris, A. J.; Tolan, D. R. *Biochemistry* **1994**, *33*, 12291 and references therein.
(6) All antibody concentrations are reported as the concentration of active sites; an intact IgG antibody molecule contains two active sites.
(7) See the Supporting Information for details.
(8) Removal of small amounts of antibody from the reaction by precipitation, which could account for the diminished rates, was ruled out by the observation that antibody samples treated with $VOSO_4$, filtered, and then subsequently dialyzed to remove the metal operated with undiminished catalytic efficiency. Inhibition by vanadyl ion is on the order of 10−50% for $VOSO_4$ concentrations of 100−400 μM, in the presence of catalytic antibodies at 2.0 and 5.0 μM.
(9) Relative rate is defined as the ratio of the initial reaction rates measured in the presence and absence of the additive under otherwise identical conditions.
(10) No independent metal-mediated aldol reaction occurs in the absence of antibody, even at concentrations of Pd^{II} compounds (500 μM) much higher than those used in the presence of antibody. The pH of the reaction mixtures in PBS buffer are unchanged by the addition of Pd^{II} salts at the concentrations indicated.
(11) Na_2PdCl_4, $Pd(OAc)_2$, and *trans*-$Pd(NH_3)_2Cl_2$ cause visible precipitation of catalytic antibodies 38C2 and 33F12 (2.5 μM) at ≥50 μM Pd. As might be expected, the metal concentration corresponding to the onset of observable precipitation varies with antibody concentration and also varies slightly between different batches of antibody samples.
(12) Incubation of antibody 38C2 with a 2-fold molar excess of $Pd(en)Cl_2$ (relative to antibody active sites) followed by dialysis of the mixture against PBS buffer gives an antibody preparation that mediates aldol condensation at a rate identical to that of a control sample not treated with metal. Both dialyzed samples then respond to the addition of Pd^{II} with increased catalytic rates in the usual fashion.
(13) (a) Measurement of tryptophan fluorescence at 340 nm (for 280-nm excitation, see: Crowder, M. W.; Stewart, J. D.; Roberts, V. A.; Bender, C. J.; Tevelrakh, E.; Peisach, J.; Getzoff, E. D.; Baffney, B. J.; Benkovic, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 5627) showed a loss of 20% of the fluorescence signal of the catalytic antibodies upon the addition of 3 equiv of Pd with respect to antibody active sites, whereas very little quenching occurs for the addition of Pd to bovine serum albumin or for the addition of K_2PtCl_4 to antibodies 38C2 and 33F12. (b) MALDI mass spectral analysis of the 33F12 F_{ab} fragment in water, in the absence and presence of Pd^{II} compounds, revealed an increase in molecular weight corresponding to 1−2 mol of metal complex

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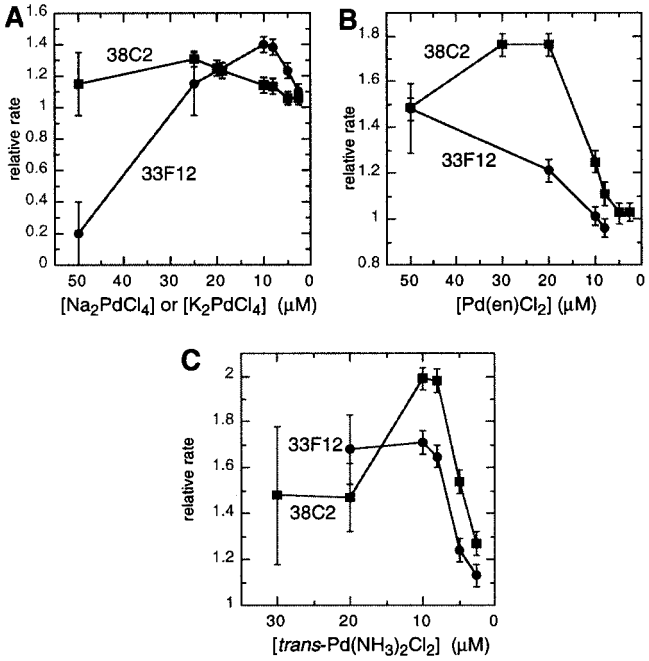

Figure 1. Rates of aldol condensation reactions of **1** + acetone in PBS buffer, in the presence of 2 μM antibody and the following additives, relative to reactions in the absence of additive: A = Na_2PdCl_4 or K_2PdCl_4; B = $Pd(en)Cl_2$; C = *trans*-$Pd(NH_3)_2Cl_2$.

Table 1. Kinetic Parameters for the Reaction of **1** with Acetone Catalyzed by Antibody 38C2 (2.6 μM) in PBS Buffer in the Presence of the Indicated Additives

additive	concn (μM)	k_{cat} (min^{-1})	K_m (μM)
none		4.3×10^{-3}	18.0
$VOSO_4$	100	3.1×10^{-3}	11.6
Na_2PdCl_4	15	6.1×10^{-3}	27.7
$Pd(en)Cl_2$	15	6.6×10^{-3}	28.4

degradation of the protein.[14] The aldol reaction is strongly inhibited by 2,4-pentanedione in both the presence and absence of metal, supporting the assumption that the active-site lysine residue is important to the catalyzed process.[3] The Fab fragment of antibody 33F12 shows the same metal-dependent behavior as the intact antibody; in no case is precipitation observed. As might therefore be expected, the greatest rate enhancement factor (2.3−2.6) is observed with this system.[7]

The enantioselectivity of the antibody-catalyzed aldol reaction is also affected by Pd^{II} additives. The two catalytically active antibodies, which exhibit similar absolute rates, substrate tolerances, and often extraordinarily high levels of asymmetric induction,[2b] give different results in the reaction of aldehyde **1** with acetone (38C2, 53% ee; 33F12, 77% ee). Furthermore, while the reaction rate increases with added Pd^{II} in both cases, the enantioselectivity improves with 38C2 but diminishes with 33F12 (Figure 2). For the reaction of acetone with substrate **2**, the 38C2-catalyzed process is accelerated, but the 33F12-catalyzed reaction is inhibited, by added Pd^{II}.[7,15]

Antibody-catalyzed aldol reactions involving the following components are all inhibited by Pd^{II} over the same concentration

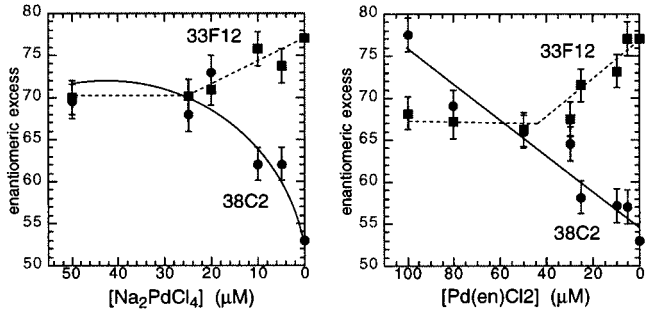

Figure 2. Enantiomeric excess (ee) vs Pd^{II} concentration for the reaction of **1** with acetone, catalyzed by the indicated antibodies at 2.0 μM. Similar results are observed at antibody concentrations of 5 and 10 μM with proportional increases in metal concentration.

range as the **1** + acetone reaction is accelerated: (1) **1** + cyclopentanone, (2) **1** + hydroxyacetone, (3) branched aldehyde **3** + acetone, and (4) the self-aldol condensation reaction of propionaldehyde catalyzed by the same antibodies.[2b,16] The antibody-catalyzed retro-aldol conversion of compound **4** into **1** + acetone was found to be inhibited by $VOSO_4$, but is accelerated only mildly by Pd^{II} compounds (relative rates $\leq$1.4 in the presence of Na_2PdCl_4 or $Pd(en)Cl_2$). A complete kinetic study of this process was not performed. Simple Pt^{II} amine complexes, which are close structural analogues to their Pd^{II} congeners,[17] exhibit a very weak form of the Pd^{II} aldol effect.[7]

Our results show that the antibody-catalyzed aldol reaction responds to Pd^{II} additives by an improvement in rate and selectivity only in a highly specific sense—for the substrate that most closely resembles the hapten to which the antibody was educated. It is unlikely that Pd^{II} binds in the active site, since one would expect much more dramatic effects on the aldol process in that event. Instead, the effect appears to be allosteric, where Pd binding induces a conformational change that promotes a closer fit of the active site to the transition state structure for the hapten-like case and a poorer fit for substrates that differ from the hapten. The technique of reactive immunization is expected to produce antibodies of a "permissive" nature, since the evolution of multiple noncovalent contacts to the hapten (constructing a "lock" for the "key") is not required to achieve the tight binding that is the goal of the immune response. The results reported here suggest that noncovalent interactions may play a limited role in the reactive immunization process, that cofactors can be found to amplify these interactions, and that such amplification may be expected to make promiscuous catalysts more specific. It may therefore be advantageous when addressing particular problems of catalysis to couple reactive immunization (or other techniques of combinatorial catalyst development) with cofactor screening.

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Supporting Information Available: Experimental procedures for kinetics and spectroscopic experiments (8 pages). See any current masthead page for ordering information and Web access instructions.

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bound per F_{ab} polypeptide when incubated with 5 equiv of Pd precursor. (c) The Pd^{II}−catalyzed ring closure of an *o*-allylphenol is inhibited by both catalytic antibodies at the micromolar level, demonstrating the ability of the antibody to sequester the metal center. See the Supporting Information for details.

(14) Palladium salts can mediate hydrolysis of oligopeptide amide bonds adjacent to the site of metal binding: Parac, T. N.; Kostic, N. M. *J. Am. Chem. Soc.* **1996**, *118*, 51.

(15) Asymmetric induction is poor for substrate **2** with both antibodies (15−25% ee) and is diminished further in each case by the addition of Pd^{II} compounds.

(16) Reactions 1−4 were not as thoroughly examined as the acceleratory reactions described above. However, the inhibitory effect of Pd^{II} is clear in each case, is proportional to metal concentration, and reaches a maximum of approximately 50−60% of the nonadditive rate at approximately 40 μM Pd^{II}.

(17) (a) Pettit, L. D.; Bezer, M. *Coord. Chem. Rev.* **1985**, *61*, 97−114. (b) Potentiometric titration of $Pd(en)Cl_2$ under conditions of high NaCl concentration very close to those used here [Tercero-Moreno, J. M.; Matilla-Hernández, A. M.; González-García, S.; Niclós-Gutiérrez, J. *Inorg. Chim. Acta* **1996**, *253*, 23] show that the major species present at micromolar total Pd levels are the following: $Pd(en)Cl_2$ (~85%), Pd(en)Cl(OH) (~12%), and $Pd(en)(Cl)(H_2O)^+$ (~3%). Indeed, $[Pd(en)(H_2O)_2]^{2+}$ and [*cis*-$Pd(NH_3)_2(H_2O)_2]^{2+}$, generated by treatment of $Pd(en)Cl_2$ and *trans*-$Pd(NH_3)_2Cl_2$ with $AgNO_3$ in H_2O, gave the same aldol response as their chloride precursors in antibody-catalyzed reactions performed in PBS buffer.

SUPPORTING INFORMATION

Cofactor Induced Refinement of Catalytic Antibody Activity: A Metal-Specific Allosteric Effect

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A. Plots of kinetics data described in text.

Inhibition by $VOSO_4$

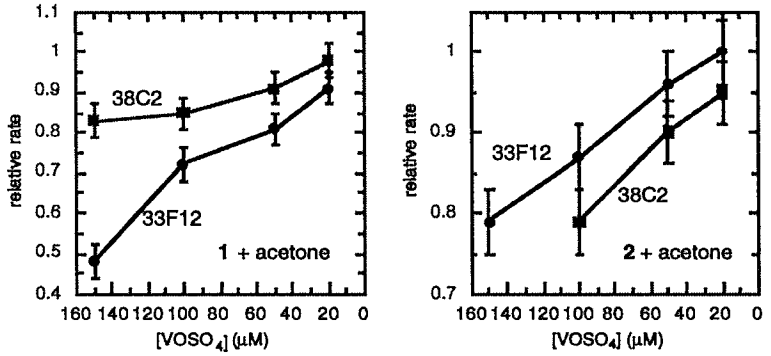


Figure S1. Rates of aldol condensation of acetone with the indicated aldehyde in the presence of $VOSO_4$, relative to reactions in the absence of additive.

Rate Enhancement by Pd(II) at higher antibody concentrations and in water instead of buffer

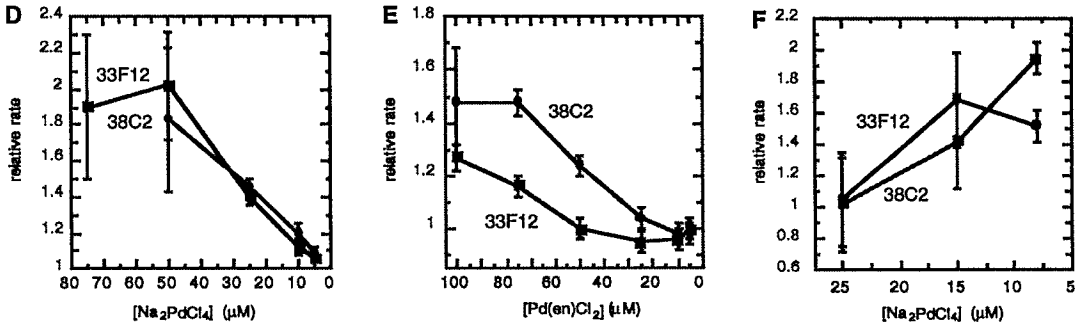


Figure S2. Rates of aldol condensation reactions of **1** with acetone under the following conditions, relative to reactions in the absence of additive: **D** = 10 μM antibody, PBS buffer, Na_2PdCl_4; **E** = 10 μM antibody, PBS buffer, $Pd(en)Cl_2$; **F** = 2 μM antibody, unbuffered water, Na_2PdCl_4.

Note: In water, the reaction rate appears to vary inversely with [Pd] throughout the concentration range, due to the greater propensity of the antibody to precipitate from water (vs. PBS buffer) in the presence of even low concentrations of Pd(II).

Rate enhancements for antibody 33F12 F_{ab} fragment with addition of Pd(II).

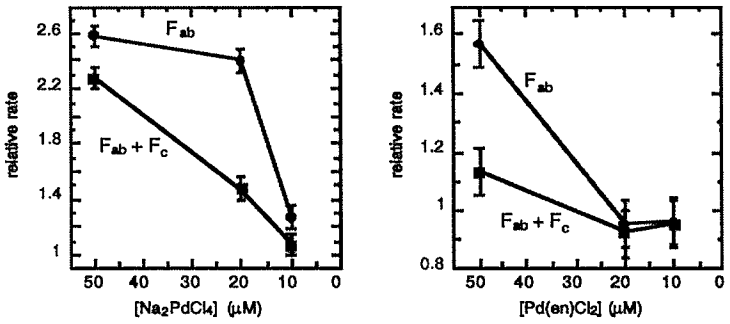


Figure S3. Rates of aldol condensation reactions of **1** with acetone catalyzed by 33F12 F_{ab} (4.0 μM) and {F_{ab} (4.0 μM) + F_c (2.0 μM)} antibody fragments in the presence of Na_2PdCl_4 (left) and Pd(en)Cl_2 (right) in PBS buffer, relative to reactions in the absence of additive.

Control experiments show that the F_c fragment is not an active catalyst, and that a 2:1 combination of F_{ab} and F_c fragments operates with the same efficiency as the F_{ab} fragment alone.

*Effects of Pd(II) on the aldol reaction of **2** + acetone.*

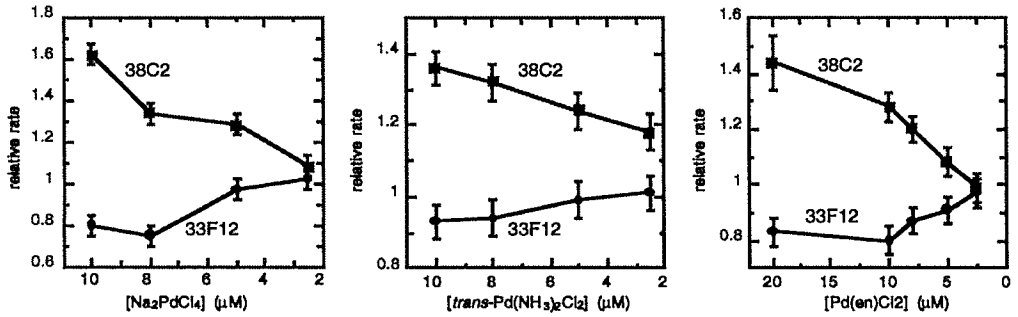


Figure S4. Rates of antibody-catalyzed aldol condensation reactions of **2** with acetone in the presence of Na_2PdCl_4 (left), *trans*-Pd(NH_3)$_2$$Cl_2$ (middle), and Pd(en)Cl_2 (right) in PBS buffer, relative to reactions in the absence of additive; antibody concentration = 2.5 μM.

Platinum(II) effect on antibody-catalyzed aldol reactions

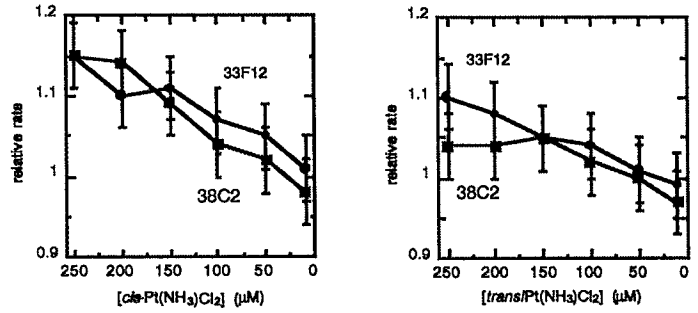


Figure S5. Rates of aldol condensation reactions of **1** with acetone in the presence *cis*-Pt(NH_3)$_2$$Cl_2$ (left) and *trans*-Pt(NH_3)$_2$$Cl_2$ (right) in PBS buffer, relative to reactions in the absence of additive; antibody concentration = 2.5 μM.

Michaelis-Menten kinetics.

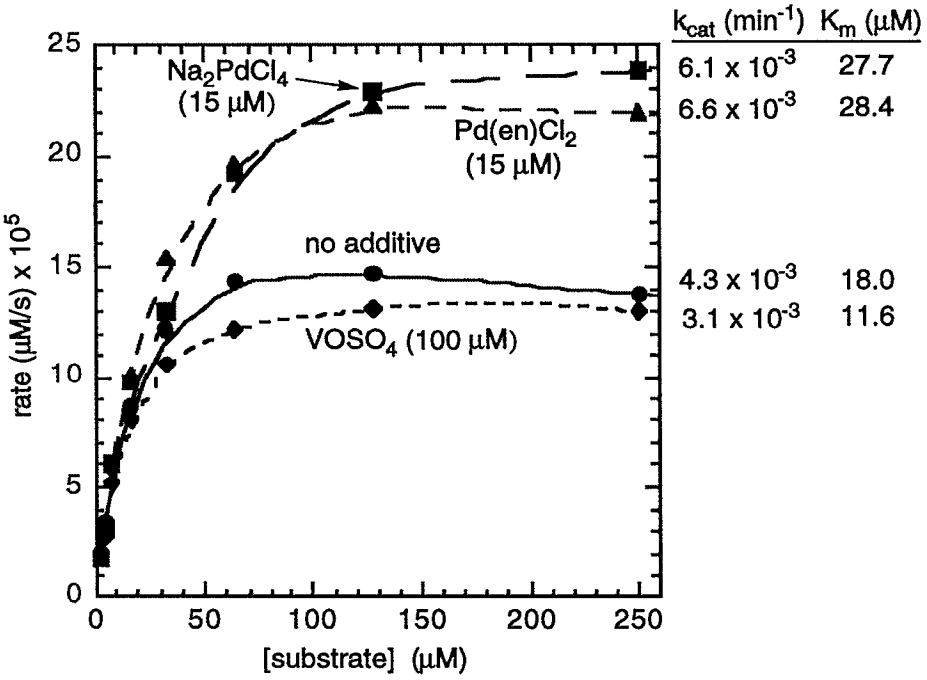


Figure S6 Rate vs. substrate concentration for the reaction of **1** with acetone catalyzed by antibody 38C2 (2.6 μM) in PBS buffer in the presence of the indicated additives, and derived Michaelis-Menten kinetic parameters.

Fluorescence quenching

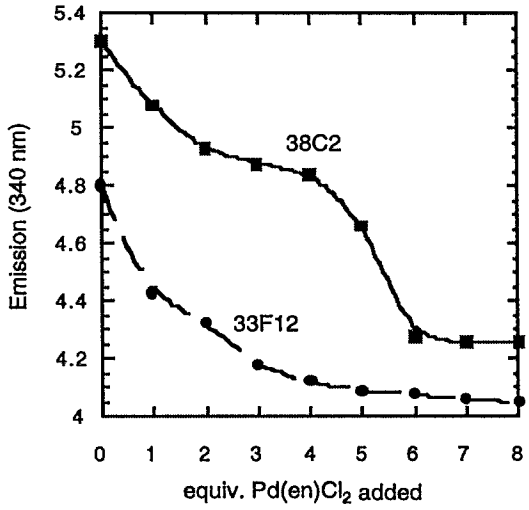


Figure S7. Fluorescence titration of $Pd(en)Cl_2$ binding to catalytic antibodies; the concentration of antibody molecules, not active sites, is 167 nM in PBS buffer.

Fluorescence quenching (continued).

Most of the total spectroscopic change occurs over the course of adding six equivalents of Pd(II) with respect to antibody, or three equivalents per active site. Because no independent measurement of free Pd(II) concentration was available, this value was calculated by assuming that there exist two Pd binding sites per antibody molecule; the resulting Scatchard plots are shown below. If one assumes more than two binding sites per antibody, the derived binding constants are apparently higher, but the early data points in the titration are fit with poor precision. Figure S7 above clearly shows two different binding regiemes for 38C2; a similar break is barely visible for 33F12. The fact that the spectroscopic change is complete with the addition of so little $Pd(en)Cl_2$ shows that the binding constants are very high and that there are few binding sites available that perturb the fluorescence spectrum. The estimate provided by the Scatchard plots below is in the range of 0.08 – 1.7 μM Beyond this, the determination of a firm value for dissociation constant must await additional measurements.

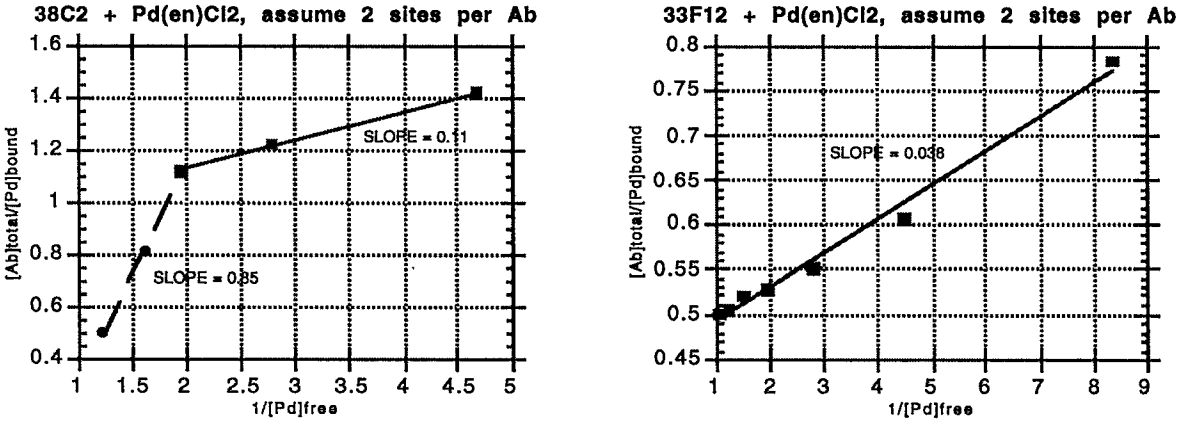


Figure S8. Scatchard plots of data shown in Figure S7, assuming that two binding sites for Pd(II) exist for each antibody molecule. The slope of each line is equal to $K_d/2$, where K_d = dissociation constant. Units of concentration are μM.

B. Other Data

MALDI Mass Spectrometry of F_{ab} fragments + Pd additives

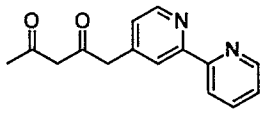
Purified 33F12 F_{ab} polypeptide was dialyzed into pure water to a concentration of 24±2 μM. Samples were incubated with varying amounts of $Pd(en)Cl_2$ or K_2PdCl_4 in aqueous solution for one hour at room temperature, and then were stored at 4°C overnight before being analyzed by MALDI (Matrix-Assisted Laser Desorption Ionization) mass spectrometry. Several samples were also incubated with diketones known to strongly bind to the antibody active site, in order to check the viability and resolution of the mass spectral method. Peak widths at half-height were measured,

and the peak centroids reported as the midpoints of the resulting mass ranges; this method gave the most reproducible values. Table S1 summarizes the experiments and results.

Table S1. Results of MALDI mass spectrometry analysis of 33F12 F_{ab}–additive mixtures.

additive	equivs added[a]	centroid mass	peak width[b]	difference[c]	equivs bound[d]
None	0.0	48052	236	n/a	n/a
Na_2PdCl_4	0.5	48101	324	49	0.3
Na_2PdCl_4	1.0	48097	265	45	0.3
Na_2PdCl_4	2.0	48119	284	67	0.4
Na_2PdCl_4	5.1	48392	511	340	1.9
$Pd(en)Cl_2$	0.5	48114	420	62	0.3
$Pd(en)Cl_2$	1.0	48124	365	72	0.3
$Pd(en)Cl_2$	2.0	48247	462	195	0.8
$Pd(en)Cl_2$	5.1	48435	520	383	1.6
trans-$Pd(NH_3)_2Cl_2$	0.5	48138	342	86	0.4
trans-$Pd(NH_3)_2Cl_2$	1.0	48092	326	40	0.2
trans-$Pd(NH_3)_2Cl_2$	2.0	48182	448	130	0.6
trans-$Pd(NH_3)_2Cl_2$	5.1	48441	499	389	1.8
2-acetylcyclohexanone	5.1	48156	376	104	0.7
bipyridyl diketone[e]	5.1	48313	361	261	1.0

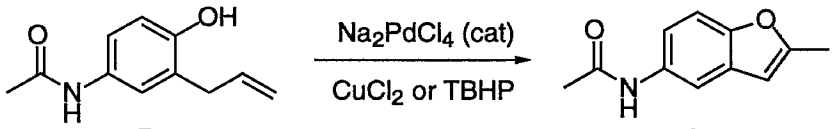
(a) molar equivalents of additive with respect to F_{ab} concentration
(b) peak width (amu) at half-height
(c) difference (amu) from mass value in the absence of additive
(d) difference/(MW of the additive)

(e) bipyridyl diketone = 

A reproducible and significant increase in mass and peak broadening occurs upon addition of Pd(II) compounds and diketones. Specific and high-affinity binding of each diketone to the active site lysine residue has been characterized by the appearance of a band at 325 nm for the derived vinylogous amide. While accurate binding constant values cannot be extracted from this data, the mass spectral analysis supports the notion that a strong Pd(II)-antibody interaction occurs. As expected, the mass spectrum of the antibody F_c fragment shows two pieces of approximately 25,000 daltons each, the masses of which do not shift upon addition of Pd(II).

Inhibition of Pd(II)-Catalyzed Wacker Reaction

The ability of a Pd-antibody complex to catalyze a metal-based transformation was also tested. Phenol **5** was found to undergo facile ring closure to **6** at room temperature in PBS buffer in the presence of catalytic amounts of Na_2PdCl_4 and stoichiometric quantities of $CuCl_2$ or *tert*-butyl hydroperoxide as reoxidants, a well-known variant of the Wacker reaction.[1] We hoped that an antibody-palladium complex could mediate the same transformation, but the antibodies instead function as inhibitors of the process (Table S1). Antibody 33F12 completely stops the cyclization reaction even when Pd(II) is present at more than four times the concentration of antibody active sites. Antibody 38C2 is a less potent inhibitor, allowing slow cyclization to occur with Pd(II) present in the same concentration range.



5 **6**

Table S2. Rates of cyclization of phenol **8** (80 μM) in the presence of $CuCl_2$ (160 μM), Na_2PdCl_4, and antibodies in PBS buffer, relative to the reaction performed with 15 μM Na_2PdCl_4 in the absence of antibody.

Ab	$[Na_2PdCl_4]$	k_{rel}
3312 (11 μM)	4	0
3312 (11 μM)	8	0
3312 (11 μM)	12	0
3312 (11 μM)	24	0
3312 (11 μM)	48	0
38C2 (8 μM)	4	0
38C2 (8 μM)	8	0
38C2 (8 μM)	12	0.03
38C2 (8 μM)	16	0.06
38C2 (8 μM)	24	0.2
38C2 (8 μM)	48	0.7

[1] (a) Hegedus, L.S. "Transition Metals in the Synthesis of Complex Organic Molecules", University Science Books: Mill Valley, CA, 1994; Chapter 7. (b) Hosokawa, T.; Murahashi, S.-I. *Accounts Chem. Res.* **1990**, *23*, 49-54.

B. Experimental section

Monoclonal antibodies 38C2 and 33F12 were obtained as previously described. Unless otherwise indicated, all reactions were performed in PBS buffer (10 mM Na_2HPO_4 + 0.16 M NaCl; pH 7.4) at room temperature (22±1°C). Metal complex stock solutions (1 mM) were freshly prepared before use. With one exception, all metal complexes were obtained from commercial sources and used as received; $Pd(en)Cl_2$ was prepared by a literature method[2] in 67% yield as a yellow microcrystalline solid.

Aldol reaction rates were measured by following the conversion of starting aldehyde to aldol product by direct injection of reaction aliquots on reverse-phase HPLC (analytical C_{18} column; CH_3CN/H_2O mixtures containing 0.025% trifluoroacetic acid), with peak monitoring at 243 nm. Authentic samples of starting materials and aldol products in each case showed very similar λ_{max} and ε_{max} values, since the chromophoric residue is the same. Thus, the total peak intensities (starting material + product) remained constant throughout each run, demonstrating that the conversion is clean and eliminating the need for an internal standard. Plots of percent product formation (product peak intensity divided by total integrated intensity) vs. time were linear over at least the first 20% of reaction; the slope of the line provides the reaction rate. All plots show correlation coefficients (R) 0.99. Deviations from linearity at later stages was shown to be due to the slight but significant evaporation of acetone through the piecered septa capping the reaction vials, rather than from product inhibition or degredation of the antibody. Relative rates were determined by comparison to the average rate of 2-4 no-additive runs performed at the same time from the same mixed batch of reagents. In most cases, a stock mixture of antibody, acetone, and aldehyde was freshly prepared, and equal volumes of this mixture then immediately distributed among reaction tubes containing metal complexes in PBS buffer, such that the total volume in each reaction mixture was identical. Relative rate values are reported in the text as the average of at least two independent runs of this kind; the larger errors observed at higher metal concentrations reflect varying amounts of antibody precipitation.

The F_{ab} fragment of 33F12 was prepared by papain digest of the antibody followed by chromatography over a protein A column (ImmunoPure Fab Preparation Kit, Pierce).

Dialyses of antibody samples were performed by centrifugation in Centricon-10 concentrator tubes (Amicon) over five refill cycles. Concentrations of antibody in the resulting solutions were quantitatively determined by bicinchononinic acid (BCA) assay (Micro BCA Protein Assay Reagent Kit, Pierce), using antibodies 38C2 and 33F12 as concentration standards. The presence of $Pd(en)Cl_2$ at 20 times antibody concentration had no effect on the calibration results. Relative concentrations can be determined by simple A_{280} absorbance measurement.

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[2] Hohmann, H.; Van Eldik, R. *Inorg. Chim. Acta.* **1990**, *174*, 87-92.

Fluorescence measurements were performed in PBS buffer on a SPF-500C spectrofluorimeter (SLM-Aminco), by monitoring the quenching of antibody emission at 340 nm upon excitation of tryptophan residues at 280 nm. $Pd(en)Cl_2$ was employed to minimize the possibility that antibody samples would precipitate. Control measurements (addition of buffer to antibody samples) demonstrated negligible (2%) antibody denaturation over the course of the titration runs, and inner filter effects were likewise determined (by titration of tryptophan) to be insignificant at the concentration ranges used.[3] Antibody samples (200 μL) were employed at concentrations of 167 nM of antibody (= 333 nM active sites); freshly-prepared stock solutions of palladium compounds (50-80 μM) were then added and allowed to equilibrate for at least 10 minutes before recording of the fluorescence spectrum.

Asymmetric induction in the aldol condensations of **1** and **2** with acetone was determined by extraction of each reaction mixture with an equal volume of ethyl acetate, passage of the organic layer through a small plug of anhydrous $MgSO_4$, and direct analysis of the resulting solution (in some cases after partial evaporation of solvent) on a chiral analytical HPLC column (for **1**, Daicel Chiralcel OD, 20% isopropanol in hexane; for **2**, Daicel Chiralpak AD, 11% isopropanol in hexane; both columns from Chiral Technologies, Inc.).

Allylphenol **5** was prepared from 4-acetamidophenol by allylation (allyl bromide, K_2CO_3, DMF, room temperature), followed by Claisen rearrangement (neat, 190°C for 36 h in a sealed tube; recrystallized from hot 10% ethyl acetate in toluene; 80-85% for two steps). For **5**: ^{1}H NMR ($CDCl_3$, δ) 10.5 (br s, 1H), 7.08 (d, J = 8.4 Hz, 1H); 7.05 (s, 1H), 6.60 (d, J = 8.4 Hz, 1H), 5.88 (m, 1H), 4.88 (m, 2H), 3.20 (d, J = 6.3 Hz, 2H), 1.94 (s, 3H). Palladium-catalyzed conversion of **5** to **6** in PBS buffer at room temperature occurred without formation of byproducts detectable by reverse-phase HPLC.

[3] Crowder, M.W.; Stewart, J.D.; Roberts, V.A.; Bender, C.J.; Tevelrakh, E.; Peisach, J.; Getzoff, E.D.; Baffney, B.J.; Benkovic, S.J. *J. Am. Chem. Soc.* **1995**, *117*, 5627-5634.