



Abiotic reduction of ketones with silanes catalysed by carbonic anhydrase through an enzymatic zinc hydride

Pengfei Ji¹, Jeeyoung Park¹, Yang Gu¹, Douglas S. Clark^{1,2} and John F. Hartwig¹✉

Enzymatic reactions through mononuclear metal hydrides are unknown in nature, despite the prevalence of such intermediates in the reactions of synthetic transition-metal catalysts. If metalloenzymes could react through abiotic intermediates like these, then the scope of enzyme-catalysed reactions would expand. Here we show that zinc-containing carbonic anhydrase enzymes catalyse hydride transfers from silanes to ketones with high enantioselectivity. We report mechanistic data providing strong evidence that the process involves a mononuclear zinc hydride. This work shows that abiotic silanes can act as reducing equivalents in an enzyme-catalysed process and that monomeric hydrides of electropositive metals, which are typically unstable in protic environments, can be catalytic intermediates in enzymatic processes. Overall, this work bridges a gap between the types of transformation in molecular catalysis and biocatalysis.

The mechanisms of reactions catalysed by transition-metal complexes in the laboratory often differ from those in biology. If the intermediates of these abiotic reactions of synthetic systems could be transported to those of metalloenzymes, then the scope of enzymatic reactions could be broadened. Recently, interest in the application of enzymes to abiotic reactions has expanded^{1,2}, and directed evolution of natural enzymes and of artificial metalloenzymes has led to a series of abiotic group-transfer processes^{3–6}. However, the scope of reactions in organometallic chemistry that involve X-type ligands⁷ is broader than those of group transfers^{1,8,9}. In particular, monomeric, metal-hydride complexes are important intermediates in reactions catalysed by transition-metal complexes, but natural enzymes that react through such intermediates are unknown^{1,10}. Instead, enzymatic reactions involving monomeric hydrides have occurred with artificial metalloenzymes in which well-established molecular iridium, rhodium or ruthenium catalysts are linked to a host protein through covalent interactions or supramolecular non-covalent interactions^{11–14}. Natural enzymes that react through multimetallic metal-hydride intermediates, such as hydrogenases, are known but these enzymes contain dinuclear active sites, comprising a bridging hydride ligand, that are prone to undergo electron transfer instead of hydride transfer.

Zinc enzymes are among the most prevalent families of metalloenzymes in biological systems¹⁵. Carbonic anhydrases are particularly well-studied zinc enzymes¹⁶. They convert carbon dioxide to carbonic acid by a mechanism involving binding of CO₂ to the active site by hydrogen-bonding interactions (PDB ID 3D92) to place the electrophilic carbon centre of CO₂ in close proximity to the oxygen atom of the zinc hydroxide (Fig. 1a, left). Addition of the hydroxo group to CO₂ generates a bicarbonate complex and subsequent displacement of the bicarbonate with water forms carbonic acid and regenerates the zinc hydroxide. Thus, the enzyme functions by the cooperative action of a Lewis acid (the zinc ion) and a hydrogen-bonding donor (amide N–H of Thr199) to achieve the addition of a nucleophilic hydroxide to the electrophile.

We hypothesized that the reactions of synthetic zinc complexes could be translated into zinc enzymes, thereby creating pathways that are distinct from those of natural enzymes. Specifically, we reasoned that reductions mediated by zinc complexes could be catalysed by carbonic anhydrases if the zinc active site transferred hydride, instead of hydroxide, to unsaturated compounds (Fig. 1a, right). These reactions could occur with simple synthetic reducing agents not found in nature, rather than the nicotinamide adenine dinucleotide phosphate (NADPH) cofactor of natural systems (Fig. 1b). Because zinc enzymes can be produced in large quantities and possess earth-abundant metals, they could be valuable replacements for precious-metal catalysts with chiral ligands^{17,18}, and because such catalysts consist of a single component, rather than the combination of a protein and cofactor, abiotic reactions catalysed by such enzymes could be conducted readily with whole cells. Here, we report the catalytic enantioselective transfer of the hydride of a silane to a range of ketones to form chiral alcohols enantioselectively. Mechanistic studies strongly implicate the intermediacy of a protein-bound, mononuclear zinc hydride complex that transfers this hydride to the ketone during the catalytic cycle.

Results and discussion

Asymmetric reduction of ketones with silanes catalysed by human carbonic anhydrase II. Small molecular zinc alkoxide or fluoride complexes are known to react readily with silanes to produce zinc hydrides^{19–21}. On the basis of this reactivity of small molecules, we hypothesized that silanes could react with zinc hydroxide within carbonic anhydrase to form a zinc hydride. We tested phenylsilane, methylphenylsilane and dimethylphenylsilane as hydride donors for the reaction of 4-acetylpyridine in the presence of human carbonic anhydrase II (hCAII) as the catalyst (Table 1, entries 1–4). 4-Acetylpyridine was chosen as the model ketone because its solubility in water is greater than that of more typical ketones like acetophenone.

The identity of the silane was shown to influence the yield of alcohol. The reaction of 4-acetylpyridine with dimethylphenylsilane,

¹Department of Chemistry, University of California, Berkeley, CA, USA. ²Department of Chemical & Biomolecular Engineering, University of California, Berkeley, CA, USA. ✉e-mail: jhartwig@berkeley.edu

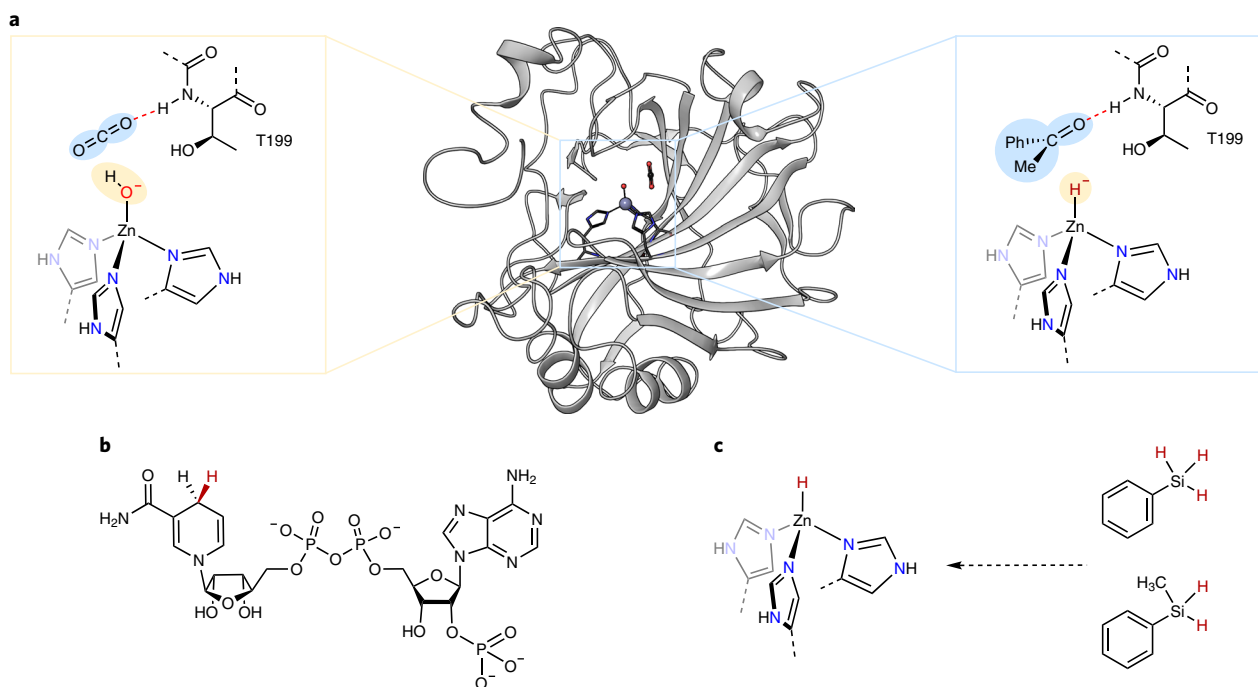


Fig. 1 | Structure and catalytic mechanism of human carbonic anhydrase. **a**, Mechanism for the conversion of CO₂ to bicarbonate catalysed by hCAII and the proposed zinc-hydride addition to ketones catalysed by the same hCAII. **b**, NADPH as the reductant for natural reactions catalysed by ketoreductases. **c**, Zinc hydride generated from silanes was used as the reductant in this work for abiotic enzyme-catalysed reductions of ketones.

Table 1 | Reduction of 4-acetylpyridine with silanes catalysed by wild-type hCAII

Reaction scheme: 4-acetylpyridine (1) is reduced to 4-(1-hydroxyethyl)pyridine (2) using 0.2 mol% hCAII and PhSiH₃ (3.0 equiv.) in Tris buffer (50 mM, pH 8) at 20 °C for 3 hours.

Entry	Variation from the standard conditions	Yield (%)	e.e. (%)
1	None	99	98
2	3.0 equiv. PhMeSiH ₂	78	98
3	3.0 equiv. PhMe ₂ SiH	0	-
4	10.0 equiv. PhMe ₂ SiH	0	-
5	3.0 equiv. Et ₃ SiH	0	-
6	3.0 equiv. (Me ₃ SiO) ₂ MeSiH	0	-
7	3.0 equiv. (iPr) ₂ SiH ₂	0	-
8	No enzyme	0	-
9	0.2 mol% hCAVII	67	97
10	0.2 mol% hCAXIII	21	89

Standard reaction conditions: 0.1 μmol of hCAII, 1.0 ml of buffer, 50.0 μmol of ketone, 150.0 μmol of PhSiH₃ (3.0 equiv. with respect to ketone). Yields were determined by ¹H NMR, e.e. values were determined by HPLC or supercritical fluid chromatography.

the most hindered and least hydridic silane, did not occur, but the reactions with methylphenylsilane and phenylsilane produced the alcohol in 78% yield and 99% yield, respectively. PhSiMe₂H has been computed to be less hydridic than PhSiH₃, by 11 kcal mol⁻¹. Although several factors could cause the differences in reactivity between the silanes, the lower hydricity is consistent with the lack of reaction of PhSiMe₂H (ref. 22). We also tested various alkylsilanes

and siloxysilanes for these reductions of ketones, but none of them gave detectable amounts of alcohol (Table 1, entries 5–7).

In addition to hCAII, we tested the activity of human carbonic anhydrase VII (hCAVII) and human carbonic anhydrase XIII (hCAXIII) as catalysts for the asymmetric reduction of ketones. Both hCAVII and hCAXIII are homologous to hCAII and contain the same zinc active site and CO₂ binding site. Both hCAVII and hCAXIII catalysed the reaction of phenylsilane with 4-acetylpyridine under the same conditions as the reactions catalysed by hCAII but with lower activity or enantioselectivity, or both (Table 1, entries 9–10).

In practice, biocatalytic processes on a large scale are typically performed with *Escherichia coli* whole cells or cell lysates to avoid expensive and labour-intensive protein purification steps. We found that hCAII in wet whole cells catalysed the asymmetric reduction of 4-acetylpyridine in 95% yield and 98% e.e. at the same loading of enzyme as that used for reactions with purified hCAII; this yield and enantioselectivity are almost identical to those of the purified hCAII (Fig. 2a). Although wet whole cells lost part of their catalytic activity after storing for three days, a dry powder formed by lyophilizing freshly cultured whole cells catalysed the reduction of the ketone as well after three months of storage as when freshly prepared. Therefore, lyophilized whole cells were used for our studies on substrate scope.

We performed deuterium labelling experiments to test if the whole-cell reactions occurred with the silane catalysed by hCAII or if they occurred with NAD(P)H catalysed by another enzyme^{23,24}. 4-Acetylpyridine was reduced in the presence of the *E. coli* cells to the chiral alcohol in quantitative yield and 98% e.e. with PhSiD₃ as the reductant. The same level of incorporation of deuterium was observed in the alcohol formed by reduction of acetophenone. These yields and e.e. values are similar to those achieved with PhSiH₃ (Fig. 2b). Most importantly, the percentage of deuterium incorporation, which was analysed by ¹H NMR spectroscopy, was 99% in both cases. This experiment confirmed that the terminal reductant with whole cells, as in vitro, is the silane, not NAD(P)H.

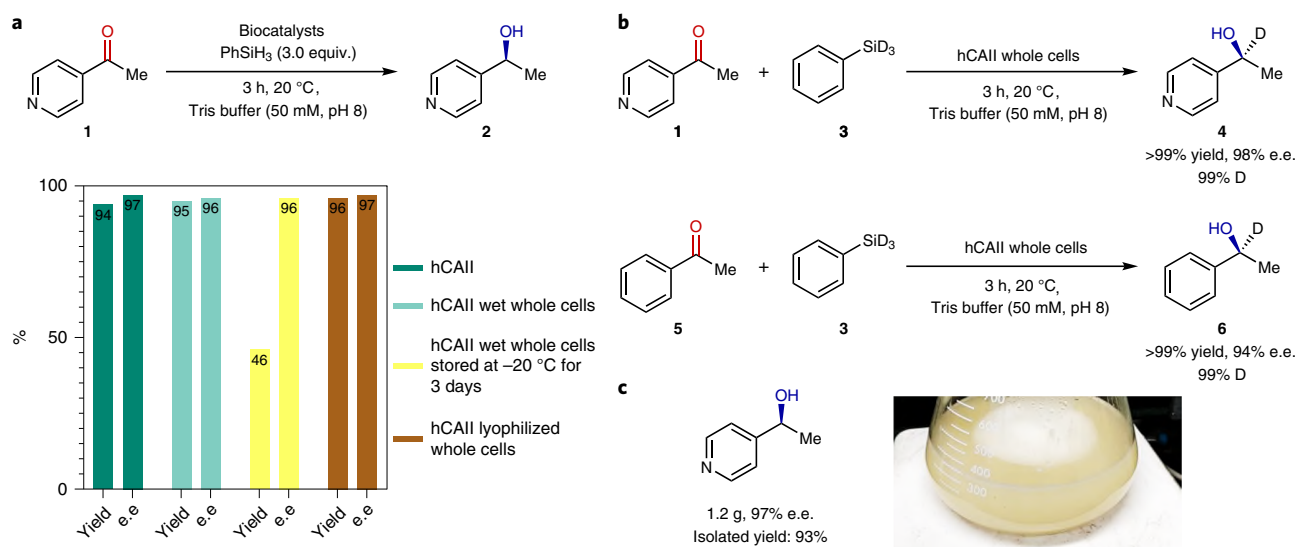


Fig. 2 | Catalytic reduction of ketones with hCAII in whole cells. a, Catalytic activity of purified protein (green), wet whole cells (cyan), stored wet whole cells (yellow) and lyophilized whole cells (brown). **b**, Enantioselective reduction of 4-acetylpyridine and acetophenone by deuterated phenylsilane. **c**, The yield and enantioselectivity of reaction product from whole-cell reduction of 4-acetylpyridine on gram scale. The picture is a snapshot of the gram-scale reaction.

Substrate scope of ketones and gram-scale reaction. With optimized reaction conditions in hand, we explored the scope of whole-cell asymmetric reductions of ketones with phenylsilane catalysed by hCAII in *E. coli*. This system catalysed the reduction of a large scope of ketones. Acetophenone was reduced in 89% yield and 94% e.e. at 0.2 mol% loading of hCAII in the presence of 3.0 equiv. of phenylsilane. Reaction of 4-chloroacetophenone occurred in quantitative yield and e.e. of >99%. 4-Bromoacetophenone also reacted with a high 81% conversion with undetectable amounts of the minor enantiomer (Table 2, 7–9). Acetophenones containing electron-donating groups, such as 4-methyl, 4-methoxy and 4-thiomethoxy groups reacted with lower conversions (51–81%), but all of the ketones reacted with e.e. values above 99% (Table 2, 10–12). We presume that the electron-donating groups lead to lower yields because the lower electrophilicity of the carbonyl group reduces the rate of nucleophilic addition of the zinc hydride relative to the reaction of the hydride with water. Acetophenones containing substituents at the 2 and 3 positions, including 3-bromo, 2-methoxy and 2-chloro groups, were also reduced in high yields and enantioselectivities (Table 2, 13–15). Sterically hindered ketones that contain di-substituted and tri-substituted aryl groups were also converted to the corresponding chiral alcohols in high yields and enantioselectivities (Table 2, 16 and 17). However, methyl β -naphthyl ketone (Table 2, 18) was reduced in only 51% yield, presumably due to a mismatch between the size of the naphthyl group and that of the binding site within hCAII.

In addition to aryl methyl ketones, aryl ethyl ketones and aryl fluoromethyl ketones were readily reduced by phenylsilane in high yields and enantioselectivities when catalysed by hCAII in whole cells (Table 2, 19–22). Various heteroaryl ketones, including 2- and 3-acetylpyridine, 3-acetylpyrazine and 1-acetylfuran were readily reduced in high yields and exceptionally high enantioselectivities (Table 2, 23–27). It is striking that the electron pair of the pyridyl, pyrazyl and furanyl groups did not disrupt the hydrogen bonding of the carbonyl group in the binding site of hCAII (see below). hCAII also catalysed the 1,2-reduction of an α,β -unsaturated ketone to form the corresponding allylic alcohol (Table 2, 28) in high yield and enantioselectivity.

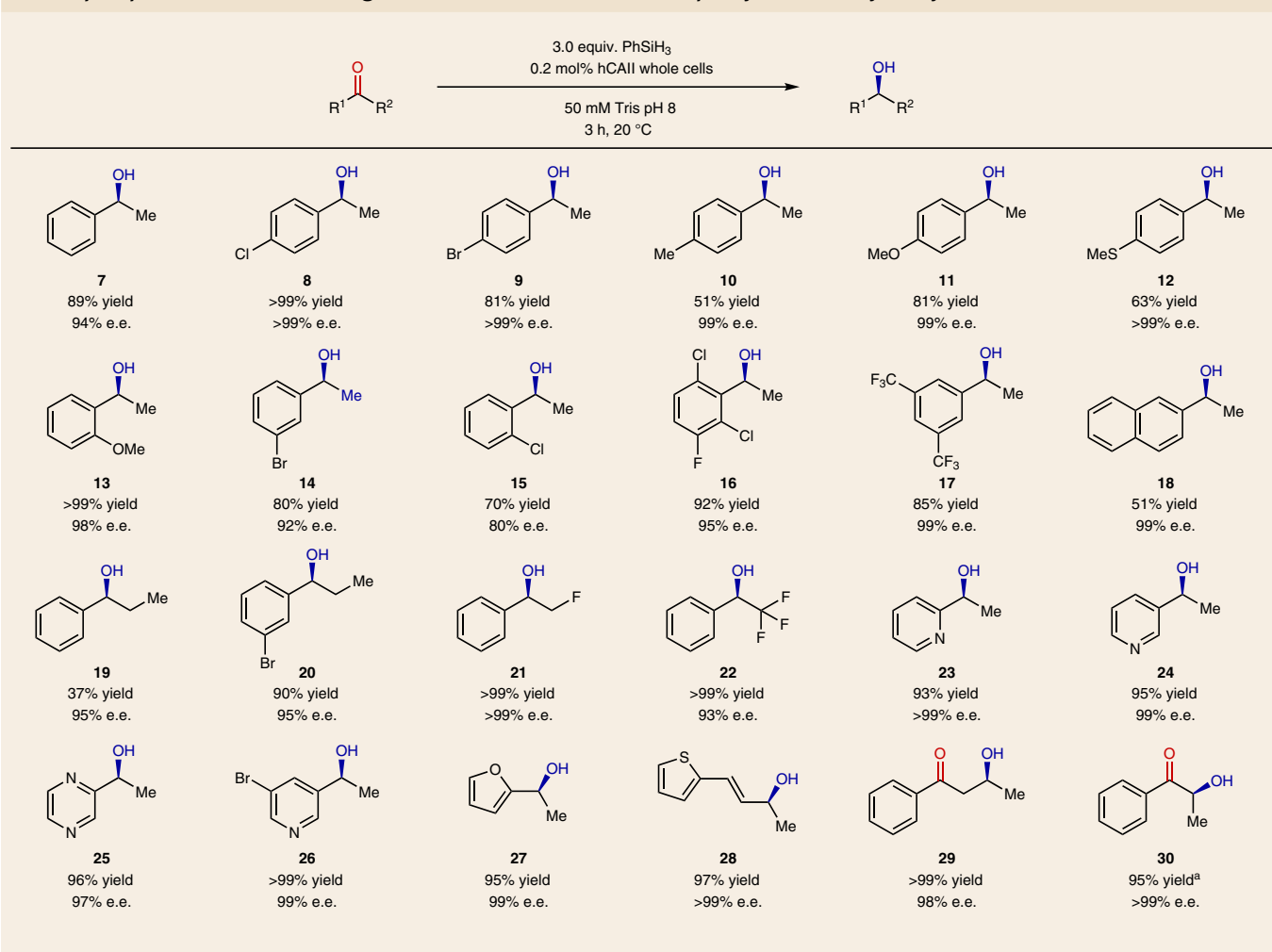
Encouraged by the high enantioselectivity of hCAII towards a broad scope of substrates, we tested the regioselectivity of the

enzyme for the reduction of diketones. The selective reduction of one ketone within a diketone is a challenge for molecular catalysts^{25,26}. The reaction of 1-phenylbutane-1,3-dione with phenylsilane catalysed by hCAII occurred at the terminal acetyl group in >99% yield and 98% e.e., leading to the *S* enantiomer (Table 2, 29). 1-Phenylpropane-1,2-dione is a more challenging substrate for semi-reduction than 1-phenylbutane-1,3-dione because the internal ketone is sterically more hindered but electronically more activated. Again, hCAII selectively reduced the terminal ketone. The terminal carbonyl group was selectively reduced in this case with 0.4 equiv. of silane, showing that multiple silane hydrogens can be transferred in the reduction process. The corresponding α -hydroxyketone was obtained in 95% yield and >99% e.e. (Table 2, 30).

To assess the ability of hCAII in *E. coli* to catalyse reactions on a large scale, we conducted the asymmetric reduction of 4-acetylpyridine on a gram scale with lyophilized cell powder. The reaction was run in Tris buffer at ambient conditions for 3 h, leading to complete conversion of the starting ketone to the corresponding chiral alcohol. The alcohol was isolated simply by extraction and evaporation of the solvent in 93% yield (1.2 g) and 97% e.e. (Fig. 2c).

Mechanistic experiments indicating the intermediacy of zinc hydride.

Control experiments with mutants of hCAII were conducted to confirm that the catalyst for the whole-cell reactions was hCAII rather than another cell component and to determine if the reaction occurred in the same binding site as the natural one for addition of hydroxide to CO₂. We prepared ten mutants in which four different residues in the enzyme active site were modified to various non-polar or polar residues (Supplementary Table 1). Based on our computational models (see below), all four non-polar residues of wild-type hCAII (V121, V143, L198 and W209) interact with the aryl group of the bound ketones. Changing the Val to the larger non-polar residue Ile significantly reduced the activity of the enzyme for the reaction of phenylsilane with acetophenone, while changing Val to much larger non-polar residues, including Phe and Trp, completely eliminated the activity of the enzyme. Mutants in which any of these non-polar residues had been changed to polar ones eliminated the enzyme activity of hCAII for the reaction of silane with acetophenone. These results show clearly that the reactions in whole cells containing hCAII are catalysed by this enzyme

Table 2 | Scope of ketones that undergo enantioselective reduction with phenylsilane catalysed by hCAII

Standard reaction conditions: 0.1 μmol of hCAII, 1.0 ml of buffer, 50.0 μmol of ketone, 150.0 μmol of PhSiH_3 (3.0 equiv. with respect to ketone). Yield was determined by ^1H NMR spectroscopy, e.e. was determined by chiral HPLC or supercritical fluid chromatography. ^a0.1 μmol of hCAII, 1.0 ml of buffer, 50.0 μmol of ketone, 20.0 μmol of PhSiH_3 (0.4 equiv. with respect to ketone).

and not by an NAD(P)H-dependent alcohol dehydrogenase, other classes of zinc enzyme, or other components of these cells, and the effect of mutations in the native active site are consistent with reductions by the silane occurring in the same site of hCAII.

A series of experiments provided strong evidence that this abiotic reaction occurred through a monomeric zinc hydride in this active site. Reductions of ketones with silanes catalysed by synthetic molecular zinc catalysts occur by one of two classes of mechanism²⁷. In the first, the carbonyl group is activated by zinc acting as a Lewis acid. In the second, the hydride of the silane is activated. This activation of the hydride can occur by the association of the silane with a basic group or by transfer of the hydride to zinc.

To begin to distinguish between these pathways, we conducted the reactions of silane with hCAII in the absence of substrate and in D_2O . If the reaction occurred with zinc hydride formed from the reaction of a zinc hydroxide or alkoxide with silane, then hydrogen would form in the absence of substrate and, if the reaction was conducted in D_2O , then HD, rather than H_2 or D_2 , would form. Indeed, the reaction of silane alone without ketone evolved gas. This gas was determined to be H_2 by dissolving the headspace gases in benzene- d_6 and analysing the resulting solution by ^1H NMR spectroscopy. A resonance at 4.47 ppm corresponding to H_2 was observed (Fig. 3a, top). A similar evolution of gas was observed in the catalytic reaction, indicating that generation of H_2 competes

with reduction of the ketone. The same reaction in the absence of ketone in D_2O formed HD (87% versus H_2), as characterized by the distinct ^1H NMR signal of HD at 4.43 ppm and the H–D coupling constant of 43 Hz (Fig. 3a, bottom).

The two pathways that involve activation of a hydride can be distinguished by the effect of the silane on enantioselectivity. The first pathway occurs by the formation of a ternary complex, in which a zinc hydroxide is associated with the silane (Fig. 3b, left). The silane in this complex would be more hydridic, thereby enabling transfer of the hydride to the ketone. The second pathway is a stepwise mechanism involving the formation of a zinc hydride from the reaction of zinc hydroxide with phenylsilane and transfer of the zinc-bound hydride to the ketone (Fig. 3b, right).

If the mechanism involves the ternary complex, then the identity of the silane should influence the enantioselectivity of the reduction of the ketone. However, if the reaction occurs by formation of a zinc hydride, then the silane is absent in the step that involves reaction with the ketone, and the enantioselectivity should be independent of the identity of the silane. To test the effect of the silane, the reactions of 4-acetylpyridine with phenylsilane and methylphenylsilane were performed under identical reaction conditions, in triplicate (Fig. 3c). The reaction of phenylsilane as the reductant led to 99% yield and $98 \pm 0.7\%$ e.e., while the reaction of methylphenylsilane led to 78% yield of the alcohol with an identical $98 \pm 0.4\%$

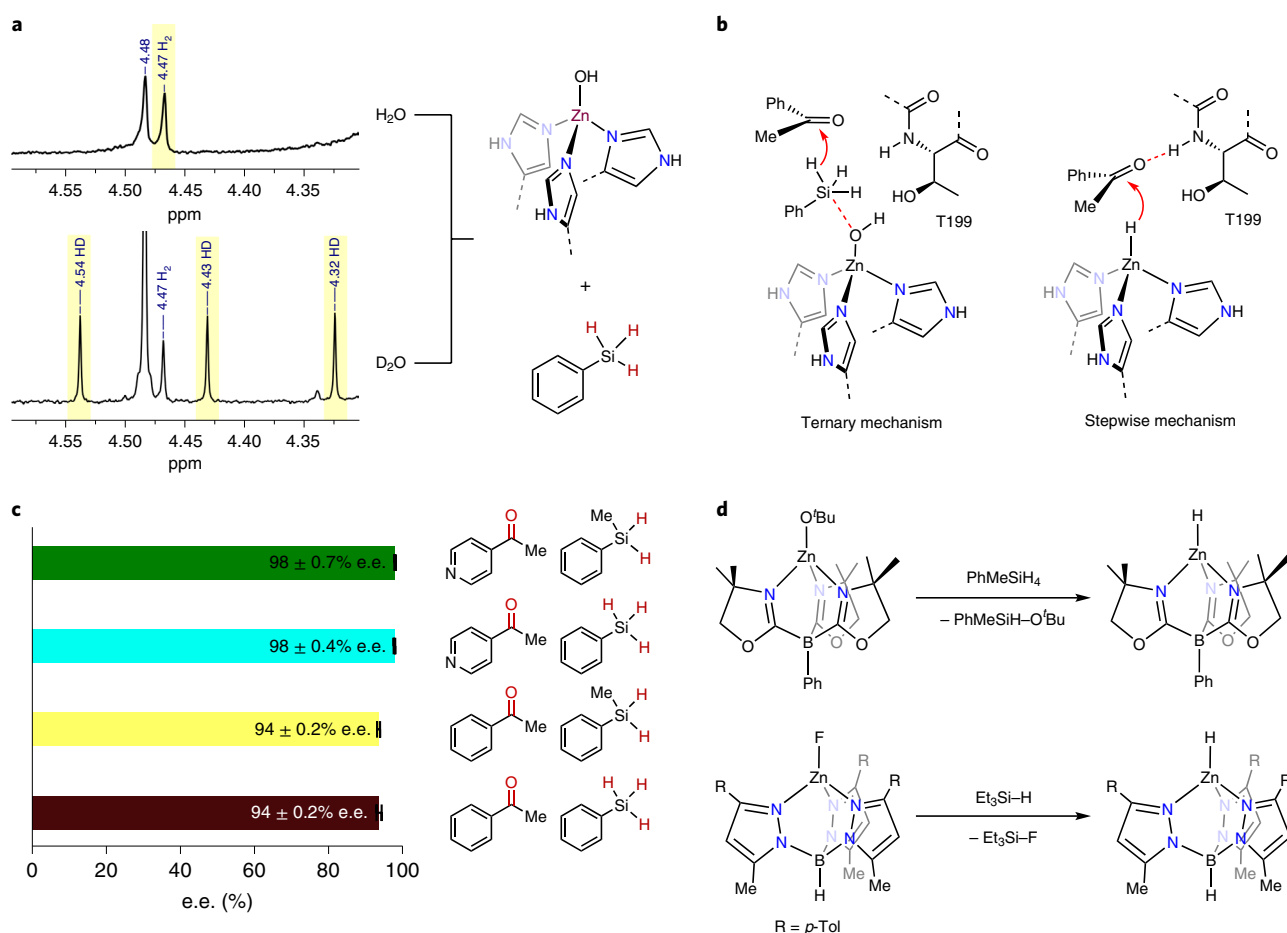


Fig. 3 | Mechanistic study of the reduction of ketone catalysed by carbonic anhydrase. **a**, ^1H NMR spectra of H_2 and HD generated by reaction of hCAII with phenylsilane in H_2O and D_2O . The NMR signal at 4.48 ppm is from residual PhSiH_3 . **b**, Two potential mechanisms for reduction of the ketone with PhSiH_3 , including a ternary mechanism (left) and a stepwise mechanism (right). **c**, hCAII-catalysed reduction of 4-acetylpyridine (green and cyan) and acetophenone (yellow and brown) by different silanes. Data are presented as average values, and error bars are calculated as the standard deviation. $n=3$ independent experiments. Individual data points are shown as grey circles. **d**, Formation of a zinc hydride from the reaction of zinc alkoxide (top) and zinc fluoride (bottom) with silanes.

e.e. Likewise, the reaction of acetophenone with phenylsilane occurred with the same enantioselectivity as the reaction of methylphenylsilane ($94 \pm 0.2\%$ and $94 \pm 0.2\%$ e.e.). These results are inconsistent with the intermediacy of the ternary complex. Instead, they are consistent with formation of a zinc hydride in the first step, followed by transfer of the hydride to the ketone in the absence of a silicon-containing unit in a second step. The slightly lower reactivity of methylphenylsilane than that of phenylsilane is consistent with slower formation of the zinc hydride in the rate-determining step.

The formation of zinc hydrides from silanes is well established in molecular systems. In many molecular complexes, zinc ions are coordinated to tridentate nitrogen-donor ligands, which were initially designed as model compounds to study the mechanisms of the reactions of zinc enzymes. Metathesis reactions of zinc alkoxides with silanes were shown to form zinc hydrides and silyl ethers^{19,28}. Similarly, silane rapidly converts zinc fluorides to zinc hydrides while forming a silyl fluoride as the side product (Fig. 3d)^{20,29}. The formation of zinc hydride from the metathesis of zinc hydroxide with boron hydride has been observed even in protic solvents^{30,31}. We propose that a similar reaction occurs with the zinc hydroxide inside the active site of carbonic anhydrase. In our proposed mechanism, silanes abstract the hydroxide from ZnOH through an intermediate or transition state containing a pentacoordinate silicon and

the resulting zinc hydride reacts with the ketone to form a zinc alkoxide that hydrolyses to the alcohol and the starting zinc hydroxide.

Computational study of the formation and transfer of zinc hydride. To assess the feasibility of a mechanism involving a zinc hydride and the potential origins of enantioselectivity, we conducted hybrid quantum mechanics/molecular mechanics (QM/MM) calculations of the proposed reaction pathway. QM/MM is an established computational method for studying the mechanisms of enzymatic reactions^{32–35}. We first investigated the docking of phenylsilane to hCAII. The zinc hydroxide, three histidine side chains and phenylsilane were chosen as the core structure for quantum calculations using density functional theory with the B3LYP functional, while the structure of the remaining protein was calculated using molecular mechanics with the OPLS force field. Geometry optimization, which was initiated with the most populated structures from molecular mechanics, converged to a structure in which the phenylsilane docks into the substrate binding site. Driven by the oxophilicity of the silane, the silicon atom associates with the zinc hydroxide in the computed structure with an O–Si bond distance of 1.77 Å. The silicon hydride is also close to the zinc centre, with a H–Zn distance of 3.58 Å. This structure is well poised for the metathesis reaction between the zinc hydroxide and the silicon hydride (Fig. 4a).

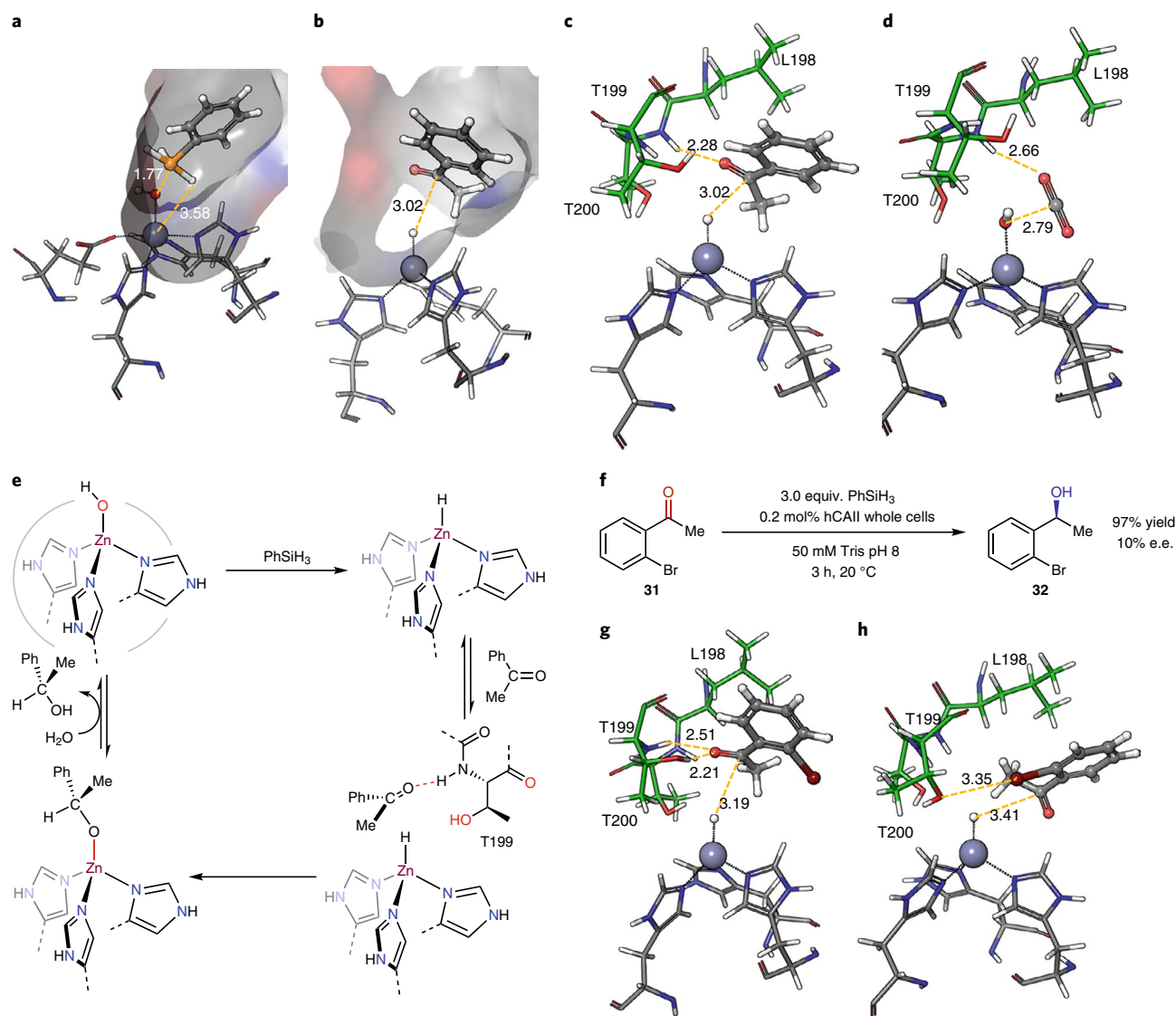


Fig. 4 | Computational study of hydride generation and transfer. **a**, QM/MM optimized structure of phenylsilane docked into zinc hydroxide in hCAII. **b**, QM/MM optimized structure of acetophenone docked into zinc hydride in hCAII. **c**, Hydrogen-bonding interaction of Thr199 and Thr200 with the carbonyl group of acetophenone. **d**, Hydrogen-bonding interaction of Thr199 with the carbonyl group of CO₂. **e**, Proposed overall mechanism for hCAII-catalysed reduction of ketone with phenylsilane. **f**, hCAII-catalysed reduction of 2-bromoacetophenone with phenylsilane. **g**, The docking modes of 2-bromoacetophenone in hCAII through carbonyl hydrogen bonding. **h**, The docking modes of 2-bromoacetophenone in hCAII through halogen bonding with the bromine. All distances between atoms are shown in Å.

We also calculated the docking of acetophenone to hCAII containing a zinc hydride by applying the QM/MM method to the most populated structures from molecular mechanics (Fig. 4b). In the resulting structure, acetophenone docks in the substrate binding site and the carbonyl group of acetophenone associates with the amide N–H of Thr199 by a hydrogen-bonding interaction in which the CO–HN distance is 2.28 Å (Fig. 4c). Such docking of acetophenone positions the carbonyl carbon close to the zinc hydride with a H–C distance of 3.02 Å. This proximity facilitates the subsequent hydride transfer. This docking mode resembles the docking of the natural substrate, CO₂, in the same active site of hCAII (Fig. 4d, PDB ID 3D92). Moreover, the computational model predicts that the hydride transfers to the prochiral *re* face of the ketone, matching the experimental formation of the *S* configuration of the alcohol. Thus, both experimental and computational studies are consistent with a catalytic cycle that involves the formation of a zinc hydride intermediate and reaction of the hydride with the ketone that inter-

acts with the amide N–H bond of Thr199, leading to the highly enantioselective reductions (Fig. 4e).

Among the 26 ketones we investigated for reduction with PhSiH₃, only 2-bromoacetophenone reacted with a low e.e. of 10% (Fig. 4f). To determine if the low selectivity for reaction of this ketone could be explained by our proposed mechanism, we computed the docking of this substrate to hCAII. These calculations showed two major docking modes of 2-bromoacetophenone, and hydride transfer would occur to opposite faces of the ketone in these two modes (Fig. 4g). In the first mode, 2-bromoacetophenone docks by hydrogen bonding of the carbonyl with the amide hydrogen of Thr199 in an analogous fashion to the docking of acetophenone and leading to the *S* enantiomer. However, in the second mode, the 2-bromo group participates in a halogen-bonding interaction with the hydroxy group of Thr200, with a linear arrangement of the C–O–Br atoms and an O–Br distance of 3.35 Å to form a structure in which the hydride would transfer to the *si* face of the ketone to

form the *R* enantiomer (Fig. 4h). Such halogen-bonding interactions are frequently observed in the structures of enzyme–inhibitor complexes³⁶. While this halogen-bonding interaction is not captured by the molecular dynamics calculations, which do not include such orbital interactions, competitive binding of the ketone in these two orientations and addition of the hydride to them can tentatively explain the distinct enantioselectivity of this ketone containing a bromine atom in an appropriate position for a halogen bond to override the hydrogen bond.

In summary, we show that carbonic anhydrases catalyse the enantioselective reduction of ketones with silanes at an active site in which the metal is directly bound to the protein without an exogenous cofactor by the intermediacy of a monomeric hydride of an electropositive metal, which is a class of intermediate unknown in nature. Our data show that enzymatic reactions with silanes as purely abiological reducing agents are feasible. Moreover, the sequence of hydride transfer from silanes to zinc and subsequent transfer of the hydride to the ketone is a mechanism that is distinct from those of biological reductions. Yet, this biocompatible approach to form metal hydrides can be envisioned to enable the generation of additional types of metal hydrides as reactive intermediates inside enzymes to create new pathways for additional abiotic enzymatic reactions. Future studies will seek to generate analogous metal hydrides that are more stable in the protein than the zinc hydride and that could be characterized directly.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41557-020-00633-7>.

Received: 4 June 2020; Accepted: 16 December 2020;
Published online: 18 February 2021

References

- Schwizer, F. et al. Artificial metalloenzymes: Reaction scope and optimization strategies. *Chem. Rev.* **118**, 142–231 (2018).
- Rosati, F. & Roelfes, G. Artificial metalloenzymes. *ChemCatChem* **2**, 916–927 (2010).
- Coelho, P. S., Brustad, E. M., Kannan, A. & Arnold, F. H. Olefin cyclopropanation via carbene transfer catalyzed by engineered cytochrome P450 enzymes. *Science* **339**, 307–310 (2013).
- Key, H. M. et al. Beyond iron: Iridium-containing P450 enzymes for selective cyclopropanations of structurally diverse alkenes. *ACS Cent. Sci.* **3**, 302–308 (2017).
- Key, H. M., Dydio, P., Clark, D. S. & Hartwig, J. F. Abiological catalysis by artificial haem proteins containing noble metals in place of iron. *Nature* **534**, 534–537 (2016).
- Kan, S. B. J., Lewis, R. D., Chen, K. & Arnold, F. H. Directed evolution of cytochrome *c* for carbon–silicon bond formation: Bringing silicon to life. *Science* **354**, 1048–1051 (2016).
- Green, M. L. H. A new approach to the formal classification of covalent compounds of the elements. *J. Organomet. Chem.* **500**, 127–148 (1995).
- Jing, Q. & Kazlauskas, R. J. Regioselective hydroformylation of styrene using rhodium-substituted carbonic anhydrase. *ChemCatChem* **2**, 953–957 (2010).
- Horitani, M. et al. Radical SAM catalysis via an organometallic intermediate with an Fe–[5′-C]-deoxyadenosyl bond. *Science* **352**, 822–825 (2016).
- Gloaguen, F. & Rauchfuss, T. B. Small molecule mimics of hydrogenases: hydrides and redox. *Chem. Soc. Rev.* **38**, 100–108 (2009).
- Skander, M. et al. Artificial metalloenzymes: (strept)avidin as host for enantioselective hydrogenation by achiral biotinylated rhodium–diphosphine complexes. *J. Am. Chem. Soc.* **126**, 14411–14418 (2004).
- Lin, C.-C., Lin, C.-W. & Chan, A. S. C. Catalytic hydrogenation of itaconic acid in a biotinylated Porphyrin–rhodium(I) system in a protein cavity. *Tetrahedron Asymmetry* **10**, 1887–1893 (1999).
- Rebele, J. G., Cotel, Y., Garabedian, B. & Ward, T. R. Chemical optimization of whole-cell transfer hydrogenation using carbonic anhydrase as host protein. *ACS Catal.* **9**, 4173–4178 (2019).
- Chevalley, A., Cherrier, M. V., Fontecilla-Camps, J. C., Ghasemi, M. & Salmain, M. Artificial metalloenzymes derived from bovine β -lactoglobulin for the asymmetric transfer hydrogenation of an aryl ketone – synthesis, characterization and catalytic activity. *Dalton Trans.* **43**, 5482–5489 (2014).
- Merkler, D. J. & Schramm, V. L. Catalytic mechanism of yeast adenosine 5′-monophosphate deaminase. Zinc content, substrate specificity, pH studies, and solvent isotope effects. *Biochemistry* **32**, 5792–5799 (1993).
- Håkansson, K., Carlsson, M., Svensson, L. A. & Liljas, A. Structure of native and apo carbonic anhydrase II and structure of some of its anion–ligand complexes. *J. Mol. Biol.* **227**, 1192–1204 (1992).
- Friedfeld, M. R., Zhong, H., Ruck, R. T., Shevlin, M. & Chirik, P. J. Cobalt-catalyzed asymmetric hydrogenation of enamides enabled by single-electron reduction. *Science* **360**, 888–893 (2018).
- Knowles, W. S. Asymmetric hydrogenations (Nobel lecture). *Angew. Chem. Int. Ed.* **41**, 1998–2007 (2002).
- Mukherjee, D., Ellern, A. & Sadow, A. D. Conversion of a zinc disilazide to a zinc hydride mediated by LiCl. *J. Am. Chem. Soc.* **132**, 7582–7583 (2010).
- Kläui, W., Schilde, U. & Schmidt, M. Fluoro[η^3 -hydrotris(3-*R*-5-methylpyrazol-1-yl)borato]zinc(II): The first TpZnF complexes, convenient precursors to zinc hydride complexes. *Inorg. Chem.* **36**, 1598–1601 (1997).
- Sattler, W. & Parkin, G. Zinc catalysts for on-demand hydrogen generation and carbon dioxide functionalization. *J. Am. Chem. Soc.* **134**, 17462–17465 (2012).
- Ilic, S., Alherz, A., Musgrave, C. B. & Glusac, K. D. Thermodynamic and kinetic hydricities of metal-free hydrides. *Chem. Soc. Rev.* **47**, 2809–2836 (2018).
- Sun, Z. et al. Catalytic asymmetric reduction of difficult-to-reduce ketones: Triple-code saturation mutagenesis of an alcohol dehydrogenase. *ACS Catal.* **6**, 1598–1605 (2016).
- Paul, C. E., Lavandera, I., Gotor-Fernández, V., Kroutil, W. & Gotor, V. *Escherichia coli*/ADH-A: An all-inclusive catalyst for the selective biooxidation and deracemisation of secondary alcohols. *ChemCatChem* **5**, 3875–3881 (2013).
- Kitamura, M. et al. Homogeneous asymmetric hydrogenation of functionalized ketones. *J. Am. Chem. Soc.* **110**, 629–631 (1988).
- Li, W., Lu, B., Xie, X. & Zhang, Z. Ru-catalyzed chemo- and enantioselective hydrogenation of β -diketones assisted by the neighboring heteroatoms. *Org. Lett.* **21**, 5509–5513 (2019).
- Gajewy, J., Gawronski, J. & Kwit, M. Mechanism and enantioselectivity of [zinc(diamine)(diol)]-catalyzed asymmetric hydrosilylation of ketones: DFT, NMR and ECD studies. *Eur. J. Org. Chem.* **2013**, 307–318 (2013).
- Mukherjee, D., Thompson, R. R., Ellern, A. & Sadow, A. D. Coordinatively saturated tris(oxazolonyl)borato zinc hydride-catalyzed cross dehydrocoupling of silanes and alcohols. *ACS Catal.* **1**, 698–702 (2011).
- Rauch, M., Rong, Y., Sattler, W. & Parkin, G. Synthesis of a terminal zinc hydride compound, [Tp^{But,Me}]ZnH, from a hydroxide derivative, [Tp^{But,Me}]ZnOH: Interconversions with the fluoride complex, [Tp^{But,Me}]ZnF. *Polyhedron* **103**, 135–140 (2016).
- Bergquist, C., Koutcher, L., Vaught, A. L. & Parkin, G. Reactivity of the B–H bond in tris(pyrazolyl)hydroborato zinc complexes: Unexpected example of zinc hydride formation in a protic solvent and its relevance towards hydrogen transfer to NAD⁺ mimics by tris(pyrazolyl)hydroborato zinc complexes in alcoholic media. *Inorg. Chem.* **41**, 625–627 (2002).
- Bergquist, C. & Parkin, G. Modeling the catalytic cycle of liver alcohol dehydrogenase: Synthesis and structural characterization of a four-coordinate zinc ethoxide complex and determination of relative Zn–OR versus Zn–OH bond energies. *Inorg. Chem.* **38**, 422–423 (1999).
- Senn, H. M. & Thiel, W. QM/MM methods for biomolecular systems. *Angew. Chem. Int. Ed.* **48**, 1198–1229 (2009).
- Field, M. J., Bash, P. A. & Karplus, M. A combined quantum mechanical and molecular mechanical potential for molecular dynamics simulations. *J. Comput. Chem.* **11**, 700–733 (1990).
- Lewis, R. D. et al. Catalytic iron–carbene intermediate revealed in a cytochrome *c* carbene transferase. *Proc. Natl Acad. Sci. USA* **115**, 7308–7313 (2018).
- Baker Dockrey, S. A., Lukowski, A. L., Becker, M. R. & Narayan, A. R. H. Biocatalytic site- and enantioselective oxidative dearomatization of phenols. *Nat. Chem.* **10**, 119–125 (2018).
- Howard, E. I. et al. Ultrahigh resolution drug design I: Details of interactions in human aldose reductase–inhibitor complex at 0.66 Å. *Proteins* **55**, 792–804 (2004).

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2021

Data availability

The data supporting the findings of this study are available within the article and its Supplementary Information.

Acknowledgements

The work on enantioselective reductions was supported by the NIH (grant no. R37 GM130387) and the studies pertaining to the intermediacy of the metal hydride were supported by the Director, Office of Science, of the US Department of Energy under Contract No. DEAC02-05CH11231. We thank the Berkeley DNA Sequencing Facility for plasmid sequencing. We thank the College of Chemistry's Molecular Graphics and Computing Facility for resources provided and K. Durkin for her assistance. The COC-MGCF is supported in part by the NIH (grant no. S10OD023532). P.J. acknowledges support from the Miller Institute for Basic Research in Science at the University of California Berkeley for a postdoctoral fellowship.

Author contributions

P.J. and J.F.H. conceived and designed the project. P.J. performed initial discovery and optimization of reaction conditions, mechanistic studies and all computational

experiments. P.J. and J.-Y.P. performed the study of substrate scope. Y.G. and P.J. screened the activity of different mutants. P.J., D.S.C. and J.F.H. analysed the data. P.J. and J.F.H. wrote the manuscript with input from all other authors.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41557-020-00633-7>.

Correspondence and requests for materials should be addressed to J.F.H.

Peer review information *Nature Chemistry* thanks the, anonymous, reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.