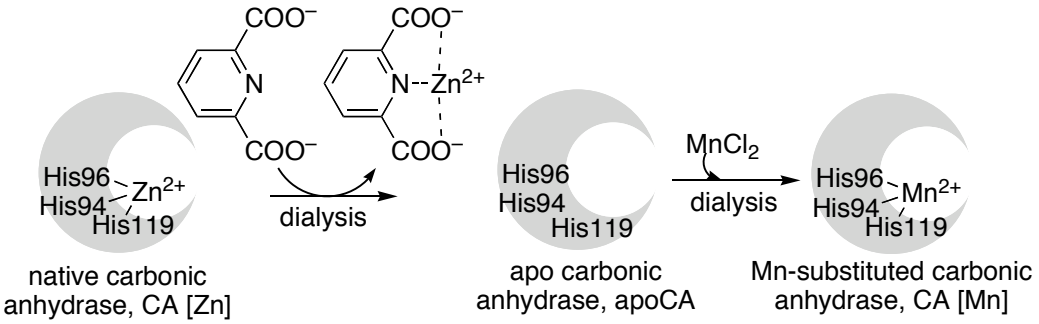
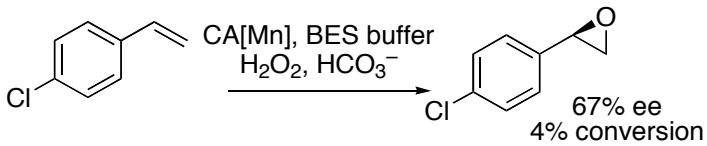
Manganese-Substituted Carbonic Anhydrase as a New Peroxidase

(*Chem. Eur. J.* **2006**, *12*, 1587 – 1596.)

Carbonic anhydrase is a zinc metalloenzyme that catalyzes the hydration of carbon dioxide to bicarbonate. Replacing the active-site zinc with manganese yielded manganese-substituted carbonic anhydrase (CA[Mn]), which shows peroxidase activity with a bicarbonate-dependent mechanism. In the presence of bicarbonate and hydrogen peroxide, (CA[Mn]) catalyzed the efficient oxidation of *o*-dianisidine with $k_{cat}/K_M = 1.4\times10^6\ M^{-1}s^{-1}$, which is comparable to that for horseradish peroxidase, $k_{cat}/K_M = 57\times10^6\ M^{-1}s^{-1}$. CA[Mn] also catalyzed the moderately enantioselective epoxidation of olefins to epoxides ($E = 5$ for *p*-chlorostyrene) in the presence of an amino-alcohol buffer, such as *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES). This enantioselectivity is similar to that for natural heme-based peroxidases, but has the advantage that CA[Mn] avoids the formation of aldehyde side products.



Scheme 1. Dialysis of carbonic anhydrase against a zinc chelator, 2,6-pyridinedicarboxylate, removed 90–95 % of the active-site zinc. Subsequent dialysis against manganese(II) yielded manganese-substituted carbonic anhydrase (CA[Mn]).



Scheme 2. Epoxidation of *p*-chlorostyrene catalyzed by CA[Mn] . Reaction conditions: 30 °C, 16 h, BES buffer (0.1 m), pH 7.2, CA[Mn] (41 mm), sodium bicarbonate (147 mm), *p*-chlorostyrene (7.4 mm), H_2O_2 (7.4 mm).

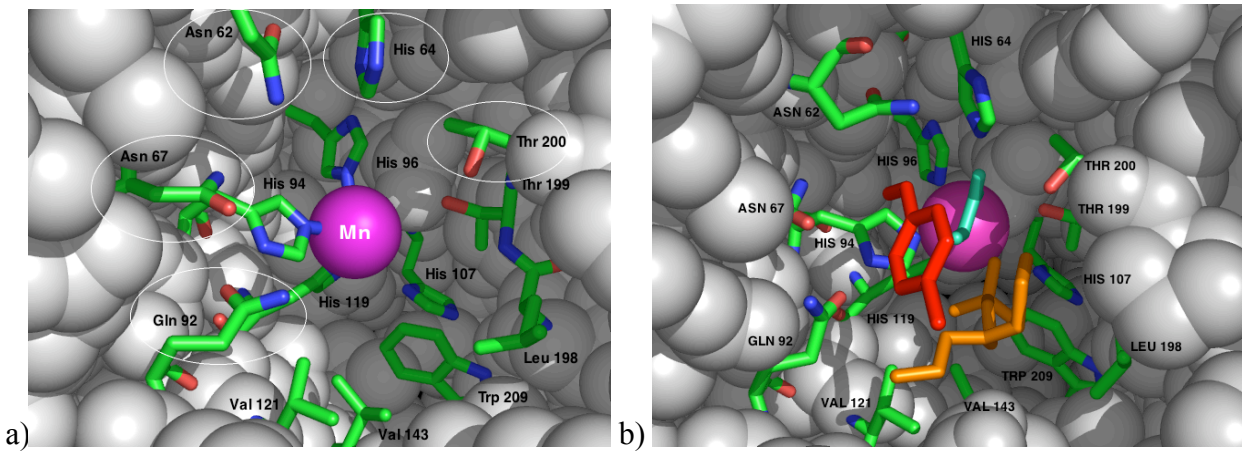


Figure 1. Structures of the active site of manganese-substituted human carbonic anhydrase II. a) X-ray crystal structure. b) Computer model created by adding peroxycarbonate (pastel blue sticks), BES (orange sticks) and *p*-chlorostyrene (red sticks) to an X-ray crystal structure of manganese-substituted human carbonic anhydrase. The peroxybicarbonate lies closest to the favored face of *p*-chlorostyrene for epoxidation.

Stereoselective Hydrogenation of Olefins Using Rhodium-Substituted Carbonic Anhydrase
(*Chem. Eur. J*. **2009**, *15*, 1370 – 1376.)

One notable reaction missing from nature's toolbox is the direct hydrogenation of substrates using hydrogen gas. Instead nature uses cofactors like NADH to reduce organic substrates, which adds complexity and cost to these reductions.

Carbonic anhydrase (CA), a zinc-contained protein, catalyzes the hydration of carbon dioxide with very high activity but it does not catalyze any hydrogenation. Interestingly, the zinc atom at active site is able to be removed by some chemical regents and replaced by other transition metals such as rhodium to produce hydrogenation catalyst. The initial results disclosed that hydrogenation occured with significant isomerization and the latter probably catalyzed by the unspecific rhodium – metals binded at the protein surface. The further research suggested chemical modification and/or mutagenesis were very effective strategies to improve activity and stereoselectivity of the metalloenzyme. The hydrogenation of stilbene, a model substrate, catalyzed by CA-Rh provided up to 20/1, ratio of hydrogenation/isomerization. This enzyme is the first cofactor-independent reductase that reduces organic molecules using hydrogen as well as a good starting point to create variants with tailored reactivity and selectivity.

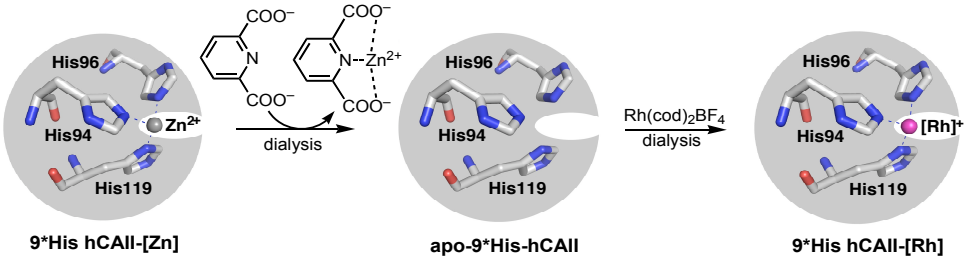
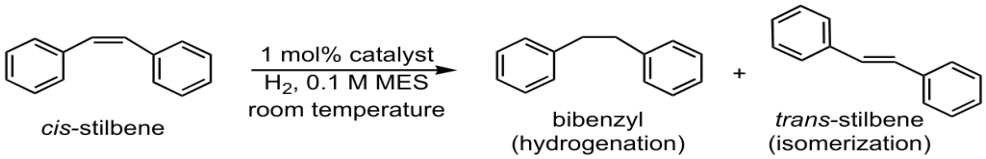


Figure 1. Dialysis of carbonic anhydrase against a zinc chelator - 2,6-pyridinedicarboxylate - removed 90–95 % of the active-site zinc. Subsequent dialysis against a solution of $Rh(cod)_2BF_4$ yielded rhodium(I)-substituted carbonic anhydrase.



Scheme 1. Hydrogenation of *cis*-stilbene with up to 20/1, ratio of hydrogenation/isomerization.

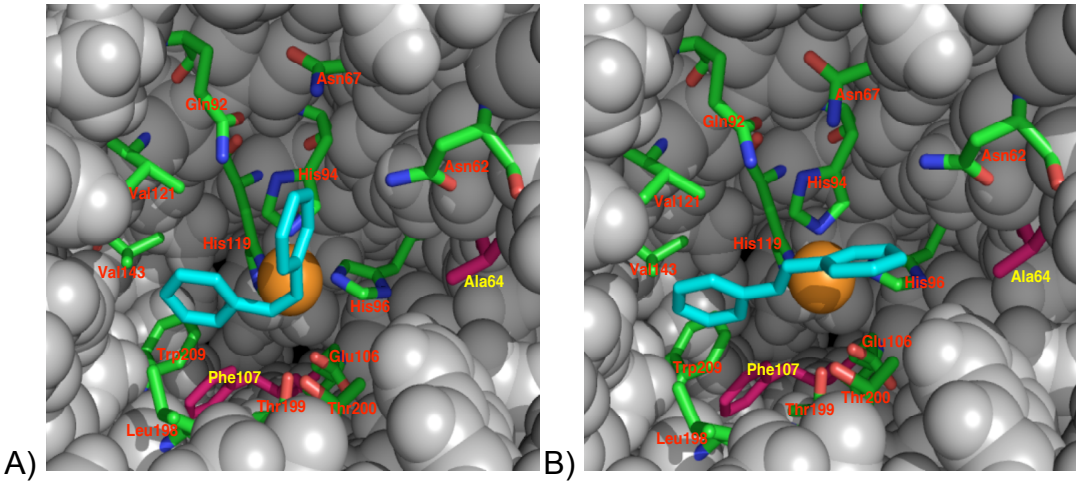


Figure 2. Modeling fits the double bond of *cis*-stilbene facing the metal ion in human carbonic anhydrase II (Fig. A), but the double bond of *trans*-stilbene does not face the metal ion (Fig. B). And *cis*-stilbene stands closer to metal center than *trans* isomer.