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Highly efficient catalysis of the Kemp elimination in the cavity of a cubic coordination cage

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Abstract

The hollow cavities of coordination cages can provide an environment for enzyme-like catalytic reactions of small-molecule guests . We report here a new example (catalysis of the Kemp elimination – reaction of benzisoxazole with hydroxide to form 2-cyanophenolate) in the cavity of a water-soluble M_8L_{12} coordination cage, with two features of particular interest. Firstly, the rate enhancement is amongst the largest so far observed: at pD 8.3, k_{cat}/k_{uncat} is 2 x 10^5, due to the accumulation of a high concentration of partially desolvated hydroxide ions around the bound guest arising from ion-pairing with the 16+ cage. Secondly, the catalysis is based on two orthogonal interactions: (i) hydrophobic binding of benzisoxazole in the cavity, and (ii) polar binding of hydroxide ions to sites on the cage surface, both of which were established by competition experiments. Hundreds of turnovers occur with no loss of activity due to expulsion of the hydrophilic, anionic product.

Since the realisation that hollow molecular container molecules can accommodate guest molecules in their central cavities,[1-3] their ability to modify the reactivity of their bound guests has been of great interest.[4-8] Well known examples include Cram's stabilisation of highly-reactive cyclobutadiene;[4] Fujita's 'ship-in-a-bottle' synthesis of cyclic silanol oligomers;[5] Nitschke's stabilisation of P_4 in a tetrahedral cage;[6] and the demonstration of unusual regioselectivity in a Diels-Alder reaction when the two reacting molecules are co-confined in a host cavity.[7]

The ultimate expression of this behaviour is efficient catalysis of a reaction occurring in the cavity of a container molecule.[8] These synthetic systems have the potential to achieve the selectivity and catalytic rate enhancements displayed by biological systems. For example, artificial container molecules provide relatively rigid and hydrophobic central cavities that may mimic binding pockets in enzymes. These containers may be purely organic hydrogen-bonded assemblies (such as Rebek's 'softball' dimer)[9] or may be metal-ligand polyhedral coordination cages [such as Fujita's Pd_6/tris(pyridyl)triazine cage].[10] Coordination cages offer particular promise in this field because of the ease with which they can be formed by a self-assembly process from very simple component parts, using the predictable coordination geometries of metal ions to provide the three-dimensional ordering of the components which generates the necessary cavity.[1-3,8,11,12]

In order for a container molecule to act as an efficient catalyst it needs to (i) recognise and bind the guest(s); (ii) accelerate the reaction by increasing the local concentration of reactants and/or stabilising the transition state; and (iii) expel the product to allow catalytic turnover.[8] Guest binding in cage cavities has been very well studied and is becoming a mature field,[1] to the extent that a modeling tool for quantitative prediction of guest binding has recently been reported by us.[13] For a reaction of the bound guest to be accelerated by the host, the transition state must be bound more tightly than the ground state. However, the factors that lead to efficient recognition of the transition state compared to the substrate are often linked to tight binding of the product. When the product binds much more tightly than the substrate, product inhibition restricts turnover and leads to inefficient catalysis.[8,14] Hence, the biggest challenge in establishing a catalytic cycle is often that step (ii) (the rate acceleration) occurs without step (iii) (release of the product to allow turnover).

Ensuring turnover – and thereby obtaining genuine catalysis – can be achieved in a variety of ways. Rebek and co-workers have demonstrated how turnover occurs when the Diels-Alder reaction product from catalysis in a cavity has a substantially reduced affinity for the capsule due to unfavourable (or less favourable) steric or electronic interactions with the host compared to the starting materials(s).[7,15,16] An irreversible reaction of an initially-generated product to cause its release was reported by Raymond and Bergman: after an Aza-Cope cyclisation reaction in an anionic cage cavity,[17] the strongly-binding product (an iminium cation) is rapidly hydrolysed to give a neutral aldehyde, which binds weakly ensuring that the cavity is vacated and providing catalytic turnover. A change in charge between starting material and product can also be used as the basis of turnover. Raymond and Bergman demonstrated catalytic hydrolysis of a neutral orthoformate guest to an anionic carboxylate, which is expelled from the cage.[18] Rebek and co-workers have exploited the same principle, *viz.* conversion of a neutral, hydrophobic substrate that binds strongly in a water-soluble cavitand to an anionic, hydrophilic product which is accordingly released, facilitating turnover and catalysis.[19] Examples of catalysis in cage cavities now vary from the Knoevenagel reaction[20] to the enantioselective hydrolysis of an organophosphate dichloride,[21] with the best example reported so far being an acid-catalysed Nazarov cyclisation of pentamethyl-cyclopentadienol in the cavity of an anionic cage, which displays a rate enhancement of 2.1 million compared to the uncatalysed reaction with hundreds of turnovers.[22]

We report here a new example of catalysis in a coordination cage cavity, which occurs with very high rate enhancements and multiple turnovers making this system comparable to the best that are currently known in supramolecular catalysis. The system is unusual in that it uses two orthogonal supramolecular interactions to bring together the two components of a bimolecular reaction.

Results and Discussion

The cage catalyst, guest substrate, and structure of the host-guest complex

The catalyst is the octanuclear $[Co_8L_{12}]^{16+}$ coordination cage shown in Fig. 1, with a Co(II) ion at each vertex of an approximate cube and a bridging ligand spanning every edge.[23-25] The presence of hydroxyl groups on the external surface renders the cage water-soluble, whilst the hydrophobic interior cavity (volume ≈ 400 Å^3) allows binding

of hydrophobic organic guests that are a good shape / size match for the cavity with binding constants of up to 10^8 M^{-1} in water.[13,23,26] Windows in the centre of each face allow ingress / egress of guests. Importantly, only neutral guests bind strongly: cationic (protonated amines) or anionic (carboxylate) guests bind much more weakly as they are hydrophilic and preferentially solvated by bulk water, which means that pH can be used to control uptake and release of ionisable guests.[27,28] In the catalytic system we report here, conversion of a neutral starting material to an anionic product is the basis of product release and catalytic turnover.

The reaction that is catalysed is the Kemp elimination (Fig. 2) in which benzisoxazole undergoes a ring-opening reaction with hydroxide to afford 2-cyano-phenolate.[29,30] The Kemp elimination has been studied in particular detail as an exemplar E2 elimination that can be adapted to a wide range of reaction rates, and as a sensitive probe for catalytic systems, both biological and artificial.[31-36] The reaction is first order in OH^- under basic conditions and reaches a minimum rate at around pH 6 when water, rather than hydroxide, acts as the base. The observed rate constants for the base catalysed (dashed black line) and spontaneous (dashed blue line) reactions are shown in Fig. 3; our measurements (black circles) of the uncatalysed Kemp elimination reaction agree very well with the published values.[29,30]

Benzisoxazole was identified by a recent computational screening experiment as a possible guest for the cavity of the cage, and it does indeed bind in water with an association constant of $K_{ass} = 4 \times 10^3$ M^{-1}.[13] Treatment of single crystals of the cage with neat liquid benzisoxazole resulted in the guest being taken up into the empty cage cavity,[26,27] allowing a crystal structure of the cage/benzisoxazole complex to be determined (Fig. 4a; see Supplementary Information). The cage has twofold crystallographic symmetry, and the structure shows that two equivalent sites in the cavity are both partially occupied by a guest molecule: the structure refines best with all guest atoms having a site occupancy of 0.5 such that there is (on average) one guest molecule present which is disordered over the two possible sites. This agrees with solution measurements of guest binding which confirm a 1:1 host:guest ratio in solution.[13] The CH hydrogen atom of the guest that is removed in the elimination reaction is directed towards one of the windows in the cage. The guest is oriented inside the cavity, as we have seen in other structures of cage/guest complexes,[26,27] such that the electron-rich N and O atoms are directed towards the polar pockets. These pockets

lie at the two diagonally opposed *fac* tris-chelate metal sites where there is a convergent array of inwardly-directed ligand C–H atoms in regions of relatively high electrostatic potential.[37] From the structure, we can see that there are CH•••N and CH•••O hydrogen bonds between the guest and the internal surface of the cage (Fig. 4b). Fig. 3c shows how the windows in the centre of each face (*cf.* Fig. 1) are all occupied by tetrafluoroborate anions in the solid state, which form multiple CH•••F hydrogen-bonding interactions with the cage.

Measurements of catalysis and its pD dependence

It was immediately apparent from ^{1}H NMR experiments that conversion of benzisoxazole to 2-cyanophenolate in aqueous base was accelerated by the presence of cage. To measure the rate constant for the reaction in the cage (k_{cat}) with little interference from the background reaction (k_{uncat}), we first monitored the reaction in aqueous solution at pD 10.2 under conditions where there was excess catalyst (1 mM cage, 0.85 mM benzisoxazole). Under these conditions around 65% of the benzisoxazole is bound at the start of the experiment. There is a clear difference in rate due to the presence of the cage (Supporting Information, Fig. S1 and Table S1), and at this pD, k_{cat}/k_{uncat} is 4500. An important point is that at the pD of the reaction, the product 2-cyanophenolate (pK_a = 6.9) is deprotonated and therefore hydrophilic and shows no sign of binding to the cage by NMR spectroscopy.[27,28] Thus, the anionic product of the reaction is expelled from the cavity, facilitating catalytic turnover and we did not observe the reaction rate decreasing with time: the reaction was not being inhibited by accumulation of product.

To confirm that the reaction is indeed associated with the benzisoxazole being bound in the cage cavity, we added an excess of a strongly-binding competing guest (20 mM cycloundecanone, $K = 1.2 \times 10^6$ M^{-1}).[26] With this inhibitor present, the reaction rate dropped to that of the uncatalysed reaction (Supplementary Information, Fig. S9 and Table S2), because the competing guest prevents substrate binding in the cage cavity. This demonstrates that the rate acceleration does not occur due to some interaction between the cage exterior and the substrate. We also showed that in the absence of cage but in the presence of 1mM of Co^{2+} ions there was no rate acceleration: the metal ions have no catalytic effect on their own (Supplementary information, Fig. S9 and Table S2).

We measured the catalysed reaction rate at a range of pD values by ^{1}H NMR spectroscopy in D_2O, and the results are shown in Fig. 3 (see Supporting Information, Figs. S3 – S8 and Table S1). At pD ≈ 12 the cage starts to decompose which provides an upper limit to the pD range. In the pD window 8.5 – 11.4, the rate of the catalysed reaction does not change: it is completely independent of the concentration of DO^- ions (Fig. 3, red line). Over this pD range, the rate of the uncatalysed reaction drops by an order of magnitude for every decrease in pD by 1 unit (Fig. 3, black dotted line), so it follows that the k_{cat}/k_{uncat} ratio increases by a factor of 10 for every decrease in pD by 1 unit until at pD 8.5, the directly observed k_{cat}/k_{uncat} ratio is 2×10^5. If we compare the rates of the catalysed and uncatalysed reactions when they are both pH independent, we find that k_{cat}/k_{uncat} is 6×10^6.

Two questions arise from these observations. Firstly, how does the cage act as a catalyst for this reaction? As the reaction proceeds the transition state involves a buildup of negative charge on the O atom, which could be stabilised by adjacent H-bond donors.[31,37] However, the H-bond donor pocket on the interior surface of the cage cavity is less effective at stabilising H-bond acceptor sites than is water: a carbonyl-containing guest in this pocket is actually destabilised relative to solvation by water by about 7 kJ mol^{-1},[26] and the penalty must be even larger for a negatively charged transition state. The interior of the cage therefore provides a *poorer* medium than water for the reaction because of preferential solvation of the developing negative charge by water, and the weak binding of the product anion underlines this.[27,28] In these terms, binding the substrate is anti-catalytic.

Secondly, what is the reason for the pD-independence of k_{cat}? As there are no basic sites associated with the cage, the rate invariance in the pD 8.5 – 11.4 range could be explained by the use of water as the base for the reaction rather than hydroxide. However if the cage interior does not stabilise the developing negative charge on the product, it is unlikely that a weak base (water) could replace the hydroxide involved in the solution reaction and give the high rates observed for the catalysed reaction. Thus neither the origin of the catalysis, nor the pD independence of its rate, can be explained just by consideration of the environment inside the cage cavity.

Proposed catalytic mechanism based on orthogonal interactions for substrate and DO^- binding

The high catalytic reactivity and region of pD independence are both consistent with a model that has been developed for catalysis by micelles and vesicles.[32-34] It is proposed in this model that ion-pairing effects result in accumulation of hydroxide ions around the positively charged surfaces of the micelle or vesicle, resulting in both a high local concentration of hydroxide ions and partial desolvation of the hydroxide ions which increases their reactivity. For cationic vesicles, these effects lead to a maximum observed rate acceleration for the reaction of benzisoxazoles of about 800 fold.[34] We propose that the surface of the highly positively charged cage catalyst (16+) acts in a similar way, concentrating partially desolvated hydroxide ions around its surface (Fig. 5). We know from numerous structural studies that the windows in each face of the cage are invariably occupied by anions in the crystal structures (*cf.* Fig. 4c),[23,24,26,27,37] which would position hydroxide close to the CH of the substrate constrained in the cavity. If these sites around the cage are saturated with hydroxide ions at pD 8.5 due to the high positive charge, increasing the pD to 11.4 will not result in an increase in the *local* hydroxide concentration and the rate of the reaction should therefore be independent of pD in this range. To test this, we added a large excess of chloride ions (47 mM) to the solution to compete for the sites on the cage surface: this reduced the observed rate of reaction to that of the background rate (Fig. 3, green point; Supplementary Information, Fig. S9 and Table S2). This cannot just be an effect of changing the medium as the Kemp elimination is known to be insensitive to ionic strength[29,30] and the addition of chloride has no effect on the rate of the background reaction.[32] [We note that, as well as being present in ~180 fold excess relative to hydroxide in solution at the pD of the experiment, chloride ions are preferentially bound to the interface region of cationic micelles (typically 10 fold[32,38,39]) as they are less strongly solvated by water than hydroxide. This explains why the reduction in concentration of the catalytically active cage in the presence of chloride is greater than the ratio of the anion concentrations].

Overall, the catalytic activity of the cage can be accounted for by a combination of concentration of the base around the bound guest (which itself may be positioned by the internal binding sites such that the reactive CH is directed towards the base in one of the cage windows), and desolvation of the bound base to make it more potent for the elimination reaction. The intersection of the two lines in Fig. 3 (rate constants of catalysed and uncatalysed reactions as a function of pD) occurs at pD 13.8, which means

that the accumulation of DO^- ions around the cage by ion-pairing, even when the bulk pD is as low as 8.5, affords an environment around the substrate that is equivalent to an aqueous solution of pD 13.8 (100 mM [DO^-]). Formally, the cage provides 440 fold enhancement ((k_{cat}•K_{ass})/k_{DO-}) of the reaction of the substrate with DO^- compared to bulk water, which represents 15 kJ mol^{-1} stabilisation of the transition state. We note that there is an interesting parallel with the Raymond / Bergman cage based on anionic metal tris-catecholate vertices, in which the high negative charge encourages protonation of bound guests, and thereby facilitates acid-catalysed reactions even in basic conditions.[18,40] These observations imply that our cage may be able to catalyse reactions of a wide range of bound guests that require an anionic base or nucleophile and so could offer a versatile framework for catalysing bimolecular reactions.

Catalytic turnover

Finally we demonstrate that the catalytic reaction occurs with a large number of turnovers. To a 1 mM solution of the cage in water at pD = 10.2 we added several successive portions of benzisoxazole (0.85 equivalents each), waiting until each aliquot had completely reacted before adding the next. Under these conditions the uncatalysed reaction does not contribute significantly, partly as it is slow at this pH and partly because under these conditions almost all of the added guest is bound to the cage. We can see from Fig. 6 (see also Supplementary Information, Table S3) that after multiple additions of guest the reaction profile is completely unchanged, and so there is no detectable change in activity after 5 turnovers. In a separate experiment we added 100 equivalents of benzisoxazole to a 0.1 mM solution of cage, at pD 9.9 where the k_{cat}/k_{uncat} ratio is *ca.* 8800. After conversion of all the benzisoxazole to 2-cyanophenolate (100 turnovers), the ^{1}H NMR spectrum of the cage was unchanged (Supporting Information, Fig. S10).

Conclusions

In conclusion we have demonstrated that the $[Co_8L_{12}]^{16+}$ coordination cage is an effective catalyst for the Kemp elimination using benzisoxazole as substrate due to a combination of (i) a high local concentration of partially-desolvated hydroxide ions around the cavity arising from ion-pairing with the cationic cage, and (ii) localisation of the hydrophobic substrate in this cavity. Thus, the catalyst uses two different types of

supramolecular interaction, associated with different recognition sites on the cage, to bring the two reacting components into close proximity. The significance of both recognition sites was confirmed by competitive inhibition experiments: addition of a competitive guest, cycloundecanone, prevents binding of the substrate inside the cage cavity; and addition of a competitive anion, chloride, prevents binding of hydroxide to the cage windows. k_{cat} is independent of pD in the range 8.5 – 11.4 leading to a maximum observed rate acceleration of 2 x 10^5 fold. This is much greater than previously observed for catalysis by vesicles and micelles, and these cages accordingly present more specific binding cavities and robust structures than these weakly bound supramolecular aggregates.

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Author contributions

W. C. performed the synthesis, crystallography and most of the experimental measurements; M. C. M. helped to design the experiment and performed some of the initial experimental measurements; C. A. H., N. H. W. and M. D. W. jointly conceived and designed the experiments, analysed the results and wrote the manuscript. Correspondence and requests for material should be sent to M. D. W.

The authors declare no competing financial interests.

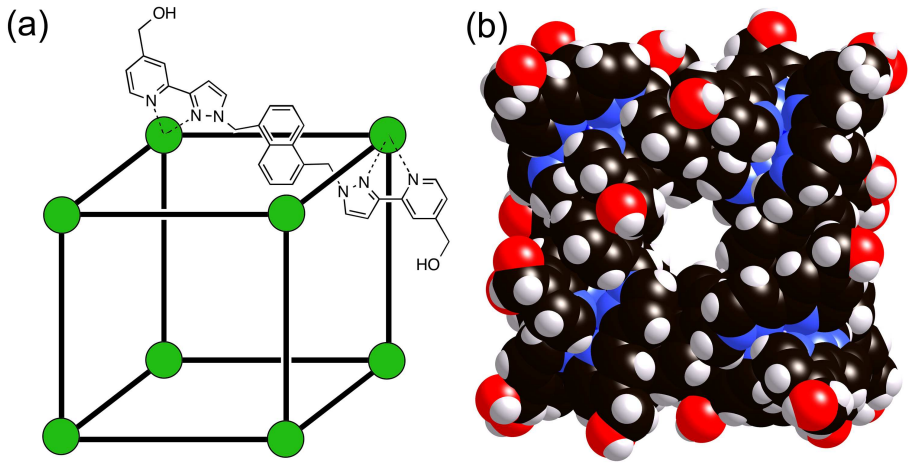

Fig. 1 Structure of the host cage $[Co_8L_{12}](BF_4)_{16}$. (a) A sketch showing the arrangement of metal ions and bridging ligands. Green circles indicate the metal ions that define the vertices of an approximate cube; the bridging ligands lie along the edges of the cube, with each ligand spanning two metal ions. (b) Structure of the empty cage (from ref. 23); note the apertures in the faces of the cube that allow ingress and egress of guests.

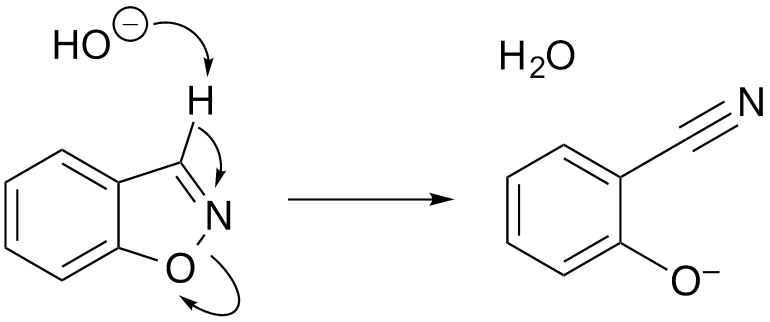

Fig. 2 The Kemp elimination reaction. Deprotonation of benzisoxazole at the 3-position leads to ring-opening and formation of 2-cyanophenolate.

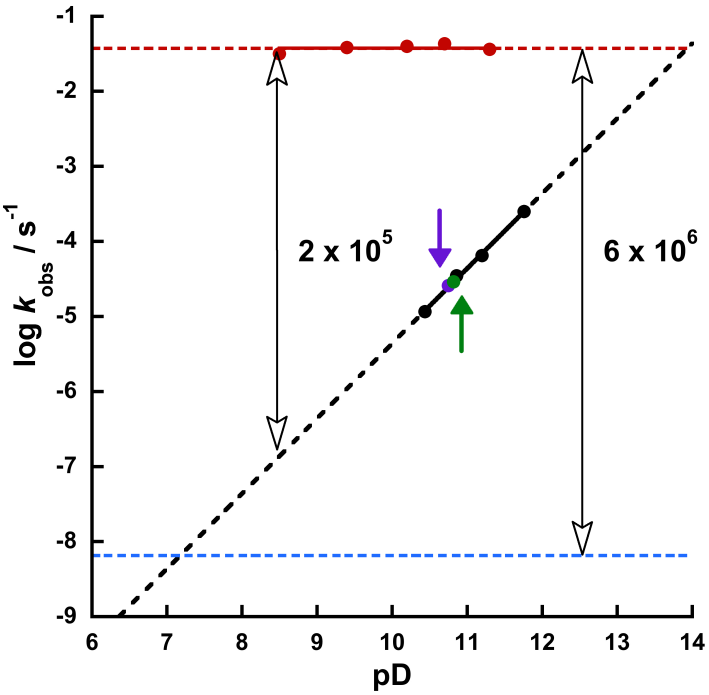

Fig. 3 pD-dependence of the reaction rate constants. Black circles show the pD-dependence of the uncatalysed reaction, re-measured in this work. The black and blue dashed lines show the rate dependence of the base-catalysed and the spontaneous (water rather than hydroxide as nucleophile) reactions, respectively, calculated from data in ref. 29 and 30. The red circles show the pD-dependence of the cage-catalysed reaction (D_2O, 298 K). For the catalysed reactions the concentration of cage was 1 mM; the concentration of benzisoxazole was 0.85 mM. The purple circle (arrowed) shows the rate of the reaction in the presence of catalyst but with a competing guest (20 mM cycloundecanone) present as an inhibitor. The green circle (arrowed) shows the rate of the reaction in the presence of catalyst but with 47 mM LiCl as a competitor for the DO^- binding sites. The vertical black arrows indicate the acceleration of the reaction due to catalysis by the coordination cage. Error bars are not shown as the uncertainty in each individual measurement is ≤ the diameter of the dot; the standard deviations are tabulated in Table S1.
In summary the data show that (i) catalysis of the reaction is very effective; (ii) it requires binding of the substrate in the cavity and binding of DO^- anions around the cage surface; and (iii) it is pD independent over a wide range because of saturation of the cage surface with DO^- ions even at pD 8.5.

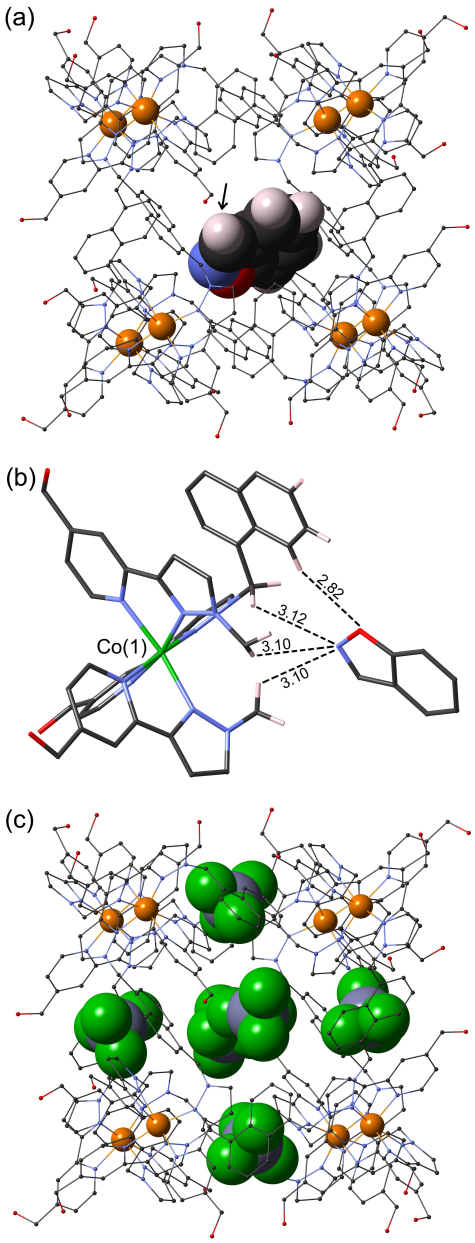

Fig. 4. **Crystal structure of the cage / benzisoxazole complex**. (a) A view of the cage complex cation (wireframe) showing a bound guest (space-filling); guests have a site occupancy of 0.5 in each of two equivalent sites in the cavity such that there is one guest per cavity. The arrow denotes the CH proton that is removed in the Kemp elimination; it is clearly visible through the 'window'. (b) A view showing the H-bonding environment around the guest (O•••H and N•••H separations in Å). (c) A view from the same viewpoint as that in (a), but showing the array of six tetrafluoroborate anions which occupy the windows in the face-centres surrounding the central cavity.

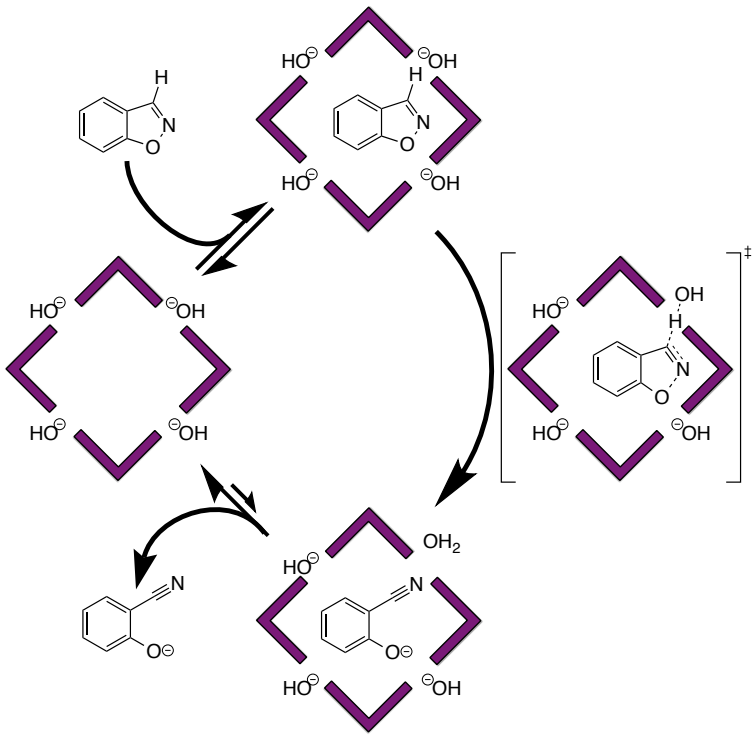

Fig. 5 **Cartoon of the catalytic reaction cycle**. Starting center left, hydrophobic benzisoxazole binds in the cavity with the proton at the 3-position 'visible' to a hydroxide ion that is bound in the adjacent cage window (top). The elimination reaction occurs to produce a 2-cyanophenolate ion as the product which binds less strongly due to the negative charge which renders it hydrophilic; it is therefore ejected to give catalytic turnover.

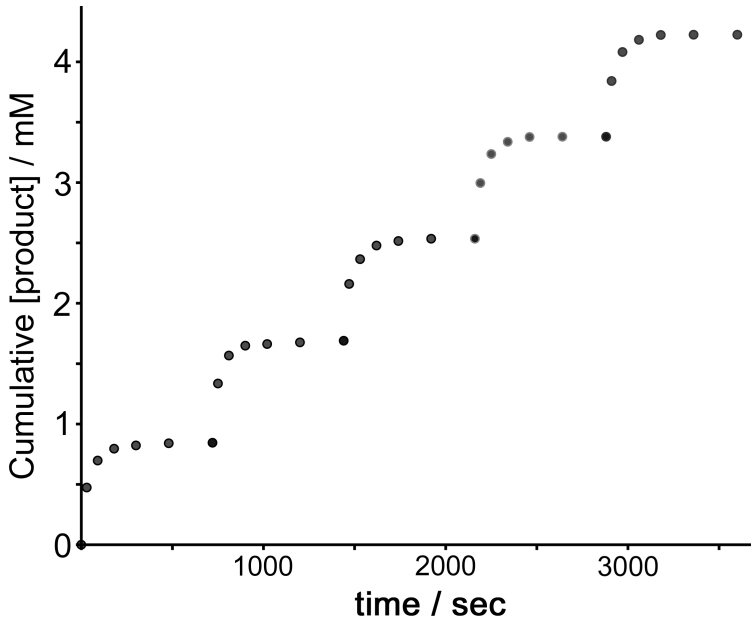

Fig. 6 Demonstration of catalytic turnover with the cage catalyst. The graph shows the accumulation of 2-cyanophenolate (based on integration of its ^{1}H NMR signal as it forms) following addition of a series of aliquots of benzisoxazole substrate (0.85 equivalents each) to a solution of cage (1 mM) in D_2O at pD 10.2. Aliquots of substrate were added at 720 second intervals; the five individual reaction profiles are superimposable, giving a total of 4.2 turnovers. Error bars are not shown as the uncertainty in each individual measurement is ≤ the diameter of the dot.

Highly efficient catalysis of the Kemp elimination in the cavity of a cubic coordination cage

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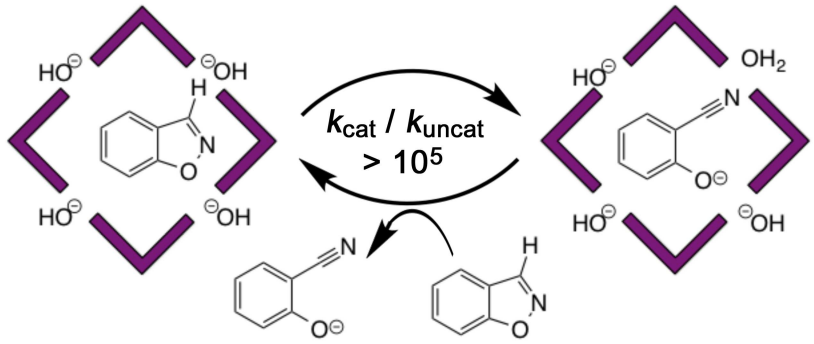
Graphical Abstract



The Kemp elimination is catalysed in the cavity of a coordination cage with a rate enhancement (k_{cat}/k_{uncat}) of 200,000 at pD 8.5. The catalysis requires two orthogonal interactions to bring together the components: hydrophobic binding of benzisoxazole, and accumulation of hydroxide ions at the cationic cage surface by ion-pairing.