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Non-covalent allosteric regulation of capsule catalysis†

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Allosteric regulation is an essential biological process that allows enzymes to modulate their active site properties by binding a control molecule at the protein exterior. Here we show the first example of capsule catalysis in which activity is changed by exotopic binding. This study utilizes a simple Pd₂L₄ capsule that can partition substrates and external effectors with high fidelity. We also present a detailed, quantitative understanding of how effector interactions alter both substrate and transition state binding. Unlike other allosteric host systems, perturbations are not a consequence of large mechanical changes, rather subtle electronic effects resulting from weak, non-covalent binding to the exterior surface. This investigation paves the way to more sophisticated allosteric systems.

Introduction

Compartmentalization is a common theme that underpins various areas from logic gates,¹ trans-membrane transport² through to molecular machines³ and allosteric regulation of catalysis.⁴ Recently, multicavity metallosupramolecular architectures⁵ able to allocate different guest molecules in localized cavities have been reported (Fig. 1, left).⁶ However, there are both challenges and unexplored opportunities with such systems. The

challenges are most obviously complex ligand synthesis and difficult self-assembly reactions. While selective binding in different sites has been demonstrated,^{6a,b} as has allosteric influence of one cavity by another,^{6a,c} quantifying how binding at one site affects another is limited.⁷ Such allosteric regulation usually take place through steric factors,^{6a,c} wherein the binding at one site produces a large mechanical re-arrangement of the global structure. To date, the influence that allosteric binding has on capsule catalysis has not been described.

The multi-binding site approach we envisaged negates the need for complex architectures (Fig. 1, left), rather it uses a minimalist, mono-cavity system at which external effectors could interact (Fig. 1, right).⁷ The exterior of most coordination capsules are swamped by counter-ion interactions, leaving little scope for binding more specific or weaker effectors. Also, counter-ion binding is often poorly defined, a consequence of dominant non-directional coulombic effects. In contrast, cage compounds C-1 and C-2 (Fig. 1) only interact weakly with the associated BARF counteranions (BARF = B(3,5-(CF₃)₂C₆H₃)₄⁻),^{8a} moreover, they possess both inner and outer H-bond donor pockets (shown in blue and red, respectively) that can interact with complementary neutral molecules. Furthermore, the functional properties of these cages make them prime candidates to explore allosterically regulated binding and catalysis.⁸

Results and discussion

Experiments with C-1 and C-2 have shown that no observable exotopic binding occurs with quinone compounds.⁹ This emphasises that the synergistic interaction of both internal H-bond pockets is crucial for quinone encapsulation.⁸ It was therefore clear that any outside effector would need to be a very strong hydrogen bond acceptor *and* be size and/or shape mismatched for the cavity. We were thus pleased to see that the

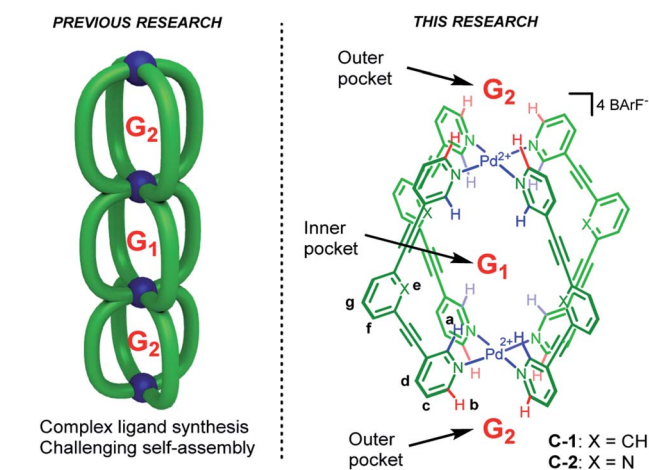


Fig. 1 Compartmentalized guest binding using metallosupramolecular hosts.

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addition of excess Ph_3PO (10 eq.) to a sample of **C-1** induced significant change in the exotopic ^1H NMR signals: a significant downfield shift (*ca.* 1 ppm) of the exterior H-bond donor atom (H_b , Fig. 2a and b) and smaller yet significant changes to neighbouring protons ($\text{H}_{c/d}$). Also, only small changes in the internal resonances (H_a and H_e) were observed. When an excess (10 eq.) of benzoquinone (Bq) was added to the Ph_3PO containing sample (Fig. 2c), changes characteristic of guest encapsulation were observed: H_a moved downfield by 0.4 ppm and the inner equatorial protons (H_e) shift upfield due to shielding by the guest. At the same time, the peaks associated with the outside resonances change little, showing high fidelity binding of both molecules. Similar spectroscopic changes were observed for **C-2** (see Fig. S1†).

Initially, the exotopic association constants (K_{11} , K_{12} ; Table 1) were determined using ^1H NMR titration. The data from these experiments fits the binding isotherm for a statistical 1 : 2 model (see Fig. S6 and S11†) and also a 1 : 1 model based on twice the concentration of host (Fig. S7 and S12†). The statistical model yielded association constants of $K_{11} = 8200 \text{ M}^{-1}$ and $K_{12} = 2100 \text{ M}^{-1}$ for both **C-1** and **C-2** with Ph_3PO (Table 1, entries 1 and 6), using the assumptions that $K_{11} = 4 \times K_{12}$ and $\Delta\delta_{\text{HG}2} = 2 \times \Delta\delta_{\text{HG}1}$.¹⁰ The lack of cooperativity between exotopic sites likely stems from the remoteness of the exterior recognition sites, and that binding requires little reorganization. These results also show that unlike guest encapsulation, the central phenyl (**C-1**) and pyridyl (**C-2**) rings have little effect on exterior binding.

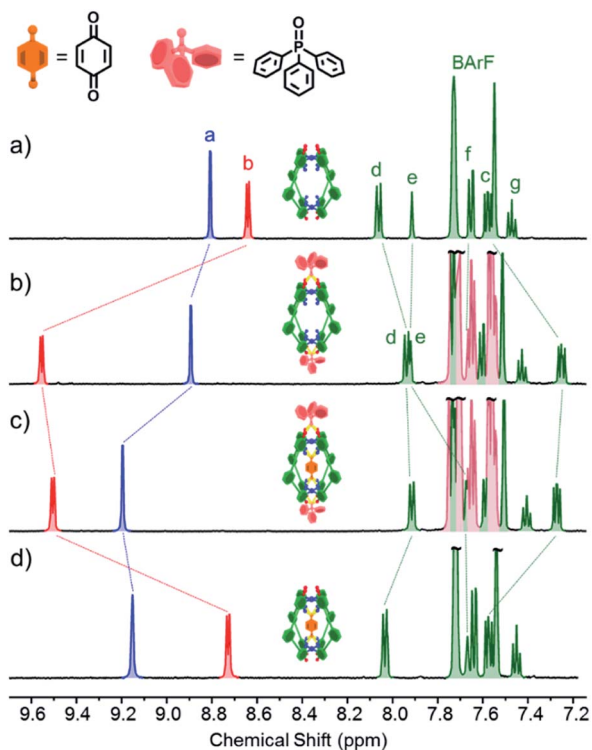


Fig. 2 ^1H NMR (500 MHz, CD_2Cl_2 , 300 K) of (a) cage **C-1** (0.5 mM); (b) **C-1** (0.5 mM) + Ph_3PO (5 mM); (c) **C-1** (0.5 mM) + Ph_3PO (5 mM) + benzoquinone (5 mM); (d) cage **C-1** (0.5 mM) + benzoquinone (5 mM). Exo and endotopic CH acidic protons coloured red and blue respectively. See Fig. 1 for assignments.

Table 1 Microscopic equilibrium association constants for the cage–quinone– Ph_3PO compartmentalized binding system. All association constants measured in dichloromethane. Errors estimated to be 10%

Entry	Equilibrium	Cage	Guest	$K_A \text{ (M}^{-1}\text{)}$
1	K_{11}	C-1	—	8200
	K_{12}			2100
2 ^a	K_Q	C-1	Benzoquinone	7900
3	K_{Q2}	C-1	Benzoquinone	2800
4 ^a	K_Q	C-1	Pentacenedione	8×10^8
5	K_{Q11}	C-1	Pentacenedione	2900
	K_{Q12}			725
6	K_{11}	C-2	—	8200
	K_{12}			2100
7 ^b	K_Q	C-2	Benzoquinone	1100
8	K_{Q2}	C-2	Benzoquinone	250
9 ^c	K_Q	C-2	Pentacenedione	8.9×10^5
10	K_{Q2}	C-2	Pentacenedione	2.5×10^5

^a Ref. 8a. ^b Ref. 8b. ^c Ref. 8c.

To determine how the binding of the guest is affected by the exotopic interactions, benzoquinone was titrated into cage solutions containing 10 equivalents of Ph_3PO . Under these conditions the major species in solution are the 1 : 2 cage–(Ph_3PO)₂ complexes (89% of 1 : 2 complex and 11% of 1 : 1 complex and less than 1% free cage). These titration sets were analysed using a 1 : 1 binding isotherm (Fig. S17 and S19†), producing excellent fits that gave a K_{Q2} value of 2800 M^{-1} for **C-1**(Ph_3PO)₂ (Table 1, entry 3) and 250 M^{-1} for **C-2**(Ph_3PO)₂ (Table 1, entry 8). These values represent reductions of 65% and 77% with respect to the association constants of 7900 M^{-1} and 1100 M^{-1} in the absence of Ph_3PO (Table 1, entries 2 and 7).⁸

In order to determine how the binding of the allosteric sites is affected by the internal guest, we reasoned that utilising a sub-stoichiometric ratio of strong binding, slow exchange



quinone would expedite this process as it would facilitate simultaneous monitoring of both free and filled cages. As anticipated, titrating Ph_3PO into C-1 in the presence of half an equivalent of pentacenedione ($K_A = 8 \times 10^8 \text{ M}^{-1}$; Table 1, entry 4) showed the characteristic downfield shift in the exotopic protons of both the empty and filled cages (Fig. 3a). It was also apparent that the changes in the filled cage were more gradual. Fitting this data (Fig. 3b and S9†) confirmed this empirical observation, with $K_{Q11} = 2900 \text{ M}^{-1}$ and $K_{Q12} = 725 \text{ M}^{-1}$ for pentacenedione \subset C-1 (Table 1, entry 5), a 65% reduction in exotopic Ph_3PO binding compared to empty C-1. The low solubility of pentacenedione \subset C-2 supramolecular complex hampered a similar ^1H NMR titration with Ph_3PO .¹¹ However, we were able to use a UV/Vis titration at much lower concentration to measure how Ph_3PO exterior binding affects the association of the same strong guest pentacenedione with C-2 (Fig. 3c and d). This approach yielded a K_{Q2} value for C-2 with pentacenedione of $2.5 \times 10^5 \text{ M}^{-1}$ compared to K_Q of $8.9 \times 10^5 \text{ M}^{-1}$ in the absence of Ph_3PO (Table 1, entries 9 and 10) corresponding to a 72% reduction of the association constant. This indicates that exterior binding affects both weak and strong binding quinone guests similarly.

The destabilization of quinone binding by Ph_3PO and *vice versa* is interesting, especially considering the direction of the

^1H NMR chemical shifts. These spectra show that (a) Ph_3PO binding to the exterior site causes a slight deshielding of the interior H-bond protons (H_a , Fig. 2a and b) and (b) quinone binding also causes slight deshielding of the exterior protons (H_b , Fig. 2a and d). The reduction in electron density of these binding site H-bond atoms should correspond to an increased H-bond donor capacity, which should translate to mutually stronger binding. We have also analysed the known X-ray data of C-1 and C-2 to gain insight into the structural changes that occur upon guest encapsulation (Fig. S42–S47†). These structures show little conformational change between “empty”, quinone and simple anion containing cages, suggesting there is no obvious mechanical effect that could explain the reduction in binding. Considering these contradictory observations, we suggest that field effects¹² contribute to binding, wherein the positively charged Pd^{2+} ions are attracted to the electron rich oxygen atoms of the guest and effector. Binding one species therefore partially “neutralizes” the charge on Pd^{2+} , leading to the observed mutual destabilization.

Remotely regulating activity is a key aspect of biological catalysis. Considering the frequently-drawn parallels between enzyme and capsule catalysis,¹³ it is therefore surprising that external regulation of the latter has not been previously described, as far as we are aware. We were therefore interested to determine the consequence of effector binding on the previously described Diels–Alder activity of cages C-1 and C-2.^{8b} Considering that free C-1 does not accelerate the reaction of benzoquinone and isoprene (or other small dienes), it is perhaps unsurprising that adding excess Ph_3PO does not change this (Fig. 4). In contrast, when the effector was added to the C-2 catalyzed reaction, a marked reduction in the overall rate was observed (Fig. 4), from a k_{obs} of $3.4 \text{ M}^{-1} \text{ h}^{-1}$ with C-2 only, to $1.4 \text{ M}^{-1} \text{ h}^{-1}$ in the presence of Ph_3PO (Table 2). We note that Ph_3PO does not affect the uncatalyzed reaction. We have also examined the effect of adding a small phosphine oxide, which can bind to both the inside and outside pockets. As expected, adding Et_3PO has a much larger influence on catalysis, reducing the rate of product formation to not much above the background reaction (Fig. 4), indicating that this sterically unencumbered hydrogen bond acceptor also competes with benzoquinone for the capsule's cavity.

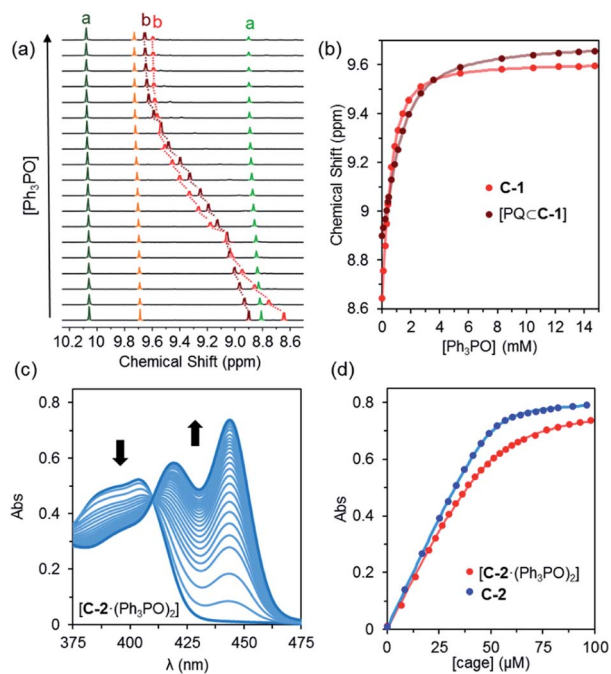


Fig. 3 (a) Partial ^1H NMR spectra (600 MHz, CD_2Cl_2 , 300 K) for the titration of C-1 (0.48 mM) and pentacenequinone (0.25 mM) with Ph_3PO . (b) Binding curves based on proton H_b for C-1 only (red) and pentacenequinone \subset C-1 (brown). The solid points are experimental data, the continuous brown line the fitted binding isotherm, and the continuous red line is the predicted binding isotherm using the previously determined association constants for cage only. (c) UV-Vis titration of pentacenedione (50 μM) with cage C-2 (0–2 equiv.) in the presence of Ph_3PO (5 mM). (d) A comparison of binding curves for the encapsulation of pentacenedione with free C-2 and C-2(Ph_3PO)₂. Absorbance measured at 444 nm C-2(Ph_3PO)₂ and 447 nm (C-2).

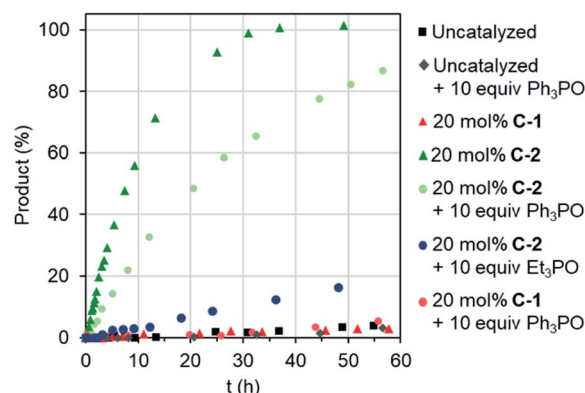


Fig. 4 Allosteric regulation of capsule catalysis. Evolution of Diels–Alder product for the reaction of benzoquinone and isoprene.



Table 2 The effects of Ph_3PO binding on the kinetic and thermodynamic parameters for the C-2 catalyzed reaction of benzoquinone with isoprene and 1,3-cyclohexadiene

Catalyst	Diene	k_{obs} ($\text{M}^{-1} \text{h}^{-1}$)	k_{cat} ($\text{M}^{-1} \text{h}^{-1}$)	$k_{\text{cat}}/k_{\text{unecat}}$	$K_{\text{Ass TS}}$ (M^{-1})
C-2	Isoprene	3.4	24	400	4.3×10^5
C-2(Ph_3PO) ₂	Isoprene	1.4	17	290	7.2×10^4
C-2	1,3-Cyclohexadiene	6	61	300	3.3×10^5
C-2(Ph_3PO) ₂	1,3-Cyclohexadiene	2.5	37	190	4.6×10^4

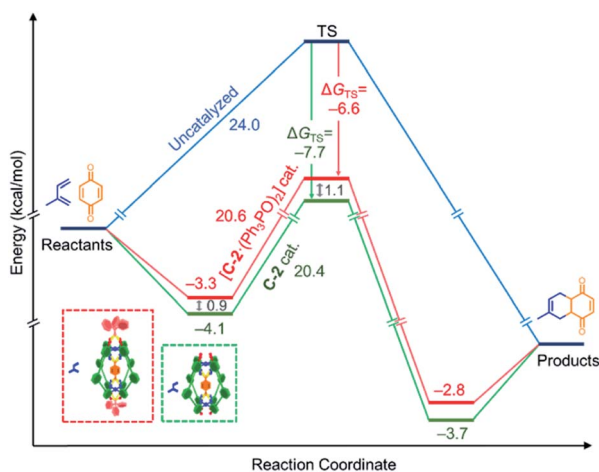


Fig. 5 "Diels-Alderase" catalyst activity rationalized using substrate and TS stabilization effects: whereas the association constants are reduced by binding of Ph_3PO to the allosteric pockets, the catalytic activity remains good. The uncatalyzed reaction is represented in blue, the C-2 catalyzed reaction in green, and the C-2(Ph_3PO)₂ in orange.

We have sought to understand and rationalize the multiple factors that contribute to this reduction in activity. The k_{cat} values, and by extension $k_{\text{cat}}/k_{\text{unecat}}$, are surprisingly close considering the nearly 3-fold reduction in k_{obs} (Table 2). This can be understood in terms of relative stabilization of the substrate and the transition state (TS) energies (Fig. 5). While the effector lowers the TS affinity ($K_{\text{Ass TS}}$) by nearly an order magnitude compared to C-2 only, corresponding to a $1.1 \text{ kcal mol}^{-1}$ energy difference, this is offset by a smaller lowering of the substrate energy. This combination leads to only $0.2 \text{ kcal mol}^{-1}$ difference in the catalytic energy barriers. However, the lower binding affinity for benzoquinone in the presence of Ph_3PO corresponds to a drop from 14% to 7% initially bound substrate. Considering all the factors, it is therefore apparent that the effector-induced decrease in catalytic activity stems principally from the lower catalyst-substrate concentration. The DA reaction between benzoquinone and 1,3-cyclohexadiene shows similar trends (Table 2).

Conclusions

We have shown how a simple, single cavity Pd_2L_4 capsules can recognize and partition multiple neutral molecules using endotopic and exotopic binding sites. Outer binding also modulates both guest binding and catalytic properties. The

reduction in activity we observe also sheds further light on the non-covalent interactions involved in both substrate and TS recognition. We envisage that this greater understanding will pave the way to more sophisticated bio-mimetic catalyst systems.

Experimental section

See ESI† for association constant determination by ^1H NMR, UV-Vis and kinetic experiments.

Conflicts of interest

There are no conflicts to declare.

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