**Abstract:** Dynamic regulation of chemical reactivity is important in many complex chemical reaction networks, such as cascade reactions and signal transduction processes. Signal responsive catalysts could play a crucial role in regulating these reaction pathways. Recently, supramolecular encapsulation was reported to regulate the activities of artificial catalysts. We present a host-guest chemistry strategy to modulate the activity of commercially available synthetic organocatalysts. The molecular container cucurbit[7]uril was successfully applied to change the activity of four different organocatalysts and one initiator, enabling up- or down-regulation of the reaction rates of four different classes of chemical reactions. In most cases CB[7] encapsulation results in catalyst inhibition, however in one case catalyst activation by binding to CB[7] was observed. The mechanism behind this unexpected behavior was explored by NMR binding studies and pKa measurements. The catalytic activity can be instantaneously switched during operation, by addition of either supramolecular host or competitive binding molecules, and the reaction rate can be predicted with a kinetic model. Overall, this signal responsive system proves a promising tool to control catalytic activity.

**Introduction**

Dynamic regulation of chemical reactivity is important in many complex chemical reaction networks such as cascade reactions and signal transduction processes. In nature, these processes are heavily regulated by enzymatic catalysis, where the activity of these catalysts themselves are modulated to render such reaction networks responsive to external signals, changes in substrate levels or changes in the environment. Responsive artificial catalysts could play similar roles in chemical reaction networks, where regulation of catalytic activity is crucial to achieve efficient temporal and spatial control over chemical transformations without unnecessary waste or off-cycle reaction pathways. Furthermore, the reversible de-activation/re-activation of catalysts by external signals can make such artificial systems highly responsive to environmental stimuli, analogous to signal-responsive enzyme catalysis in nature. Still, to this date such responsive catalysts remain very rare, have a narrow application scope or rely on extensive synthetic efforts. Recently, there have been reports of regulation of the activity of synthetic catalysts by supramolecular encapsulation including rotaxanes, resorcin[4]arene, cyclodextrin and cucurbit[7]uril which is of high interest because it enables precise, reversible and responsive control over reaction rates by adjusting the amount of available catalyst in situ. Among them, cucurbit[7]uril (CB[7]) is a widely applied molecular container, a cyclic glycoluril heptamer that binds strongly to small neutral and cationic compounds, is commercially available, non-toxic and relatively soluble in water, which makes it possible to be used in aqueous environments or even biological systems. Examples of CB[7] catalytic activity regulation include the regulation of transition metal catalysts embedded in gold nanoparticles in cells, the enhancement of photocatalytic H2 evolution, promotion of the Fenton oxidation through supramolecularly modulated ferrocene catalysts, and control over copper catalyzed alkyne azide click chemistry. Most of these examples focus on transition metal catalysis. To date, only Leigh and coworkers reported a switchable secondary amine catalyst based on a rotaxane, but that system has a highly specialized design to enable complex formation between host and catalyst. As of now no generic method is available for tunable catalytic activity regulation of common simple, commercially available organocatalysts. Since organocatalysis is emerging as one of the main branches of synthetic science, we hypothesize that the exploration of CB to control readily accessible and widely used organocatalysts, would highly broaden the application scope of this method.

Herein we report a strategy to change and tune the catalytic activity in situ of diverse, widely applied organocatalysts by host-guest encapsulation in aqueous environment. Specifically, with supramolecular encapsulation we can control the catalytic activity of four different organocatalysts in various bond forming reactions: primary amine (1) aniline
and C5: benzimidazole-amine) catalyzed hydrazone formation, tertiary amine (C2: DABCO) catalyzed allylic substitution, secondary amine (C3: prolinol) catalyzed aldol formation, as well as the oligomerization of maleimide initiated by an amine initiator (C4: nornicotine) (Scheme 1c). These reactions all proceed in aqueous media under biologically relevant conditions. \[23\] In most of the cases, reaction rates can be down- and upregulated by binding the catalyst to CB[7] and subsequently releasing it by adding a competitive strong binder for CB as a chemical signal.

Scheme 1. The concept of using host-guest chemistry to control the activity of organocatalysts. a) Schematic representation of CB[7] binding to the organocatalyst (CAT), hindering its catalytic activity. Addition of the stronger binding signal leads to the release of the catalyst and restores its catalytic activity; b) Structure of CB[7]; c) Organocatalysts C1–C5 and their associated reactions.

Results and Discussion

System design considerations and selection of organocatalysts

The applied catalysts (Scheme 1c) were first selected based on the binding affinity with CB[7]. CB[7] binds strongly to somewhat hydrophobic, positively charged molecules with an appropriate size for the CB[7] cavity. \[12\] To be able to use CB[7] to modify catalyst activity by encapsulation, it is essential that the catalyzed reaction works in aqueous environments, and that the affinity of the catalysts with CB[7] is high enough to ensure that the majority of catalyst is encapsulated at the operational concentrations. Meanwhile, the substrates and products should not bind to CB[7]. On the other hand, the signal molecules should have a much larger affinity for CB[7] than the catalyst, to allow efficient liberation of the catalyst through competitive binding, analogous to the indicator displacement assay (IDA). \[24\] From this principle, four organocatalysts and one organic initiator: aniline C1, DABCO C2, L-prolinol C3, nornicotine C4 (an initiator for maleimide oligomerization), 1H-benzimidazole-2-methanamine C5, and three signal molecules (SG1–3) were selected and used separately in a range of reactions. NMR binding studies of these catalysts and initiator also indicated their affinity to CB[7] (Figures S5–S10). Table 1 summarizes binding studies of these catalysts and initiator also indicated selected and used separately in a range of reactions. NMR binding studies of these catalysts and initiator also indicated their affinity to CB[7].

Control over aniline (C1) catalysis in hydrazone formation

We first focused on the hydrazone formation reaction, a widely applied condensation reaction between an aldehyde and a hydrazide that takes place in aqueous buffer and is accelerated by a variety of organocatalysts. \[27,28\] Aniline C1 is often used as a catalyst in this reaction, although in (super)-stochiometric amounts because of its low efficiency. \[29,30\] The reaction between aldehyde SM1 (0.4 mM) and hydrazide SM2 (0.04 mM) in aqueous buffer (10 mM sodium phosphate buffer pH 6.0) leads to the formation of hydrazone product P1 (Figure 1), where both catalyzed and uncatalyzed reactions follow second-order reaction kinetics. However, under the operational conditions (with \[ [\text{SM1}] > [\text{SM2}] \]) we calculate the reaction rate constant based on the pseudo-first order assumption (Equation S1). As is apparent from Figure 1a,c and Table S1, catalyst C1 (0.4 mM) increases the reaction rate 13-fold with respect to the uncatalyzed reaction. A blank reaction with CB[7] (0.42 mM) alone increases the reaction rate 1.9-fold with respect to the uncatalyzed reaction, indicating that the macrocycle shows a small catalytic activity towards the hydrazone formation reaction. \[29\] Addition of

Table 1: Binding constants of organocatalysts and signal molecules with CB[7].

| Compound | Structure | \( K_a [M^{-1}] \) |
|----------|-----------|------------------|
| C1       | ![Image](image1.png) | \((1.3 \pm 0.038) \times 10^{11}\) |
| C2       | ![Image](image2.png) | \((3.6 \pm 0.032) \times 10^{11}\) |
| C3       | ![Image](image3.png) | \((5.75 \pm 0.16) \times 10^{11}\) |
| C4       | ![Image](image4.png) | \((4.6 \pm 0.035) \times 10^{11}\) |
| C5       | ![Image](image5.png) | \((2.8 \pm 0.20) \times 10^{10}\) |
| SG1      | ![Image](image6.png) | \((4.2 \pm 1.0) \times 10^{12}\) |
| SG2      | ![Image](image7.png) | \((2.5 \pm 0.6) \times 10^{12}\) |
| SG3      | ![Image](image8.png) | \((6.1 \pm 0.5) \times 10^{12}\) |

[a] Measured by ITC in 10 mM sodium phosphate buffer pH 6.0, 25°C; [b] Measured by ITC in 100 mM sodium phosphate buffer pH 7.4, 25°C; [c] Values from Ref. [19], measured by NMR in NaOCCD3 (50 mM) buffer, pH 4.74; [d] Values from Ref. [25], measured by NMR in D2O, 25°C; [e] Values from Ref. [26], measured by ITC in H2O, 25°C.

Generally, the binding constants of the catalysts are in the range of \(10^3–10^5\) M\(^{-1}\) and the signal molecules are \(10^5–10^7\) M\(^{-1}\), while the reaction substrates and products are chosen such that they bind with \( K_a < 10\) M\(^{-1}\) (Supporting information Table S5).
CB[7] (0.42 mM) to catalyst C1 (0.4 mM) should lead to an estimated >89% of the catalyst bound in CB[7] [Eq. (S10)]. This mixture gives a reaction rate constant of 0.28 M⁻¹ s⁻¹, which is 3.5-fold lower than the catalyzed reaction, showing a substantial reduction of the catalytic activity of C1. On top of that, hydrazone formation in the presence of CB[7] (0.42 mM), catalyst C1 (0.4 mM) and signal molecule SG1 (0.8 mM) gives a reaction rate constant of 1.2 M⁻¹ s⁻¹, showing that the signal molecule effectively replaces the catalyst by competitive binding with CB[7], restoring the catalytic activity of catalyst C1. Noteworthy, the reaction rate in the presence of CB[7], catalyst C1 and signal molecule SG1 is slightly higher than the reaction rate with only catalyst C1. A reason might be that the catalytic activity of CB[7] adds up to the catalytic activity of catalyst C1, leading to a higher reaction rate. Signal guest molecule SG1 (0.8 mM) alone does not show any catalytic activity, while the reaction in the presence of CB[7] (0.42 mM) and signal guest SG1 (0.8 mM) is 1.3-fold faster than the blank reaction, showing that a guest inside the cavity of CB[7] does not have a significant effect on the CB[7] catalytic background activity. In essence, CB[7] encapsulation thus reduces the catalytic activity of organo-catalyst C1, which can be restored by competitive binding with a signal molecule.

**Control over DABCO (C2) catalysis in allylic substitution**

The successful control of the hydrazone formation reaction rate via CB[7] catalyst encapsulation encouraged us to extend the application of this strategy to other organocatalysts. 1,4-Diazabicyclo[2.2.2]octane (DABCO, C2) is a widely used catalyst in many organic reactions. From ITC, we learned that the binding constant of DABCO (C2) with CB[7] is 3.6 × 10⁶ M⁻¹ (Table 1), which is in a similar range as C1 and a suitable value for reaction rate control. Moreover, DABCO was reported to accelerate the allylic substitution reaction between diethyl(o-acetoxymethyl) vinylphosphonate SM3 and nitrogen-based nucleophiles in aqueous solvents.[33,34] Hence, we used glycine (SM4; 100 mM) as nucleophile in phosphate buffer (100 mM, pH 7.4) to react with SM3 (10 mM), giving the double substituted compound as the major product (Figure 2). Similar to the hydrazone reaction, with SM4 = [SM3] in this substitution reaction, we measured a pseudo-first order reaction rate by ¹H NMR following the consumption of SM3. With 20 mol% of DABCO (2 mM), the SM3 consumption is 13-fold faster than the uncatalyzed reactions conditions (15.86 M⁻¹ h⁻¹ vs. 1.19 M⁻¹ h⁻¹; Figure 2a,b). Addition of 3.5 mM CB[7], encapsulating about 99.8% of the present DABCO, decreased the reaction constant to 1.23 M⁻¹ h⁻¹, giving a similar rate constant as the blank reaction. To avoid any side-reactions of the substrate with SG1 (a primary amine) signal molecule, SG2 was used in this particular system to release the catalyst. In the presence of CB[7] (3.5 mM), catalyst C2 (2 mM, 20%) and signal molecule SG2 (6 mM), the reaction rate is accelerated again, about 11.4-times faster than the blank reaction, although slightly lower than the catalytic reaction which may be cause by the slight inhibitory effect of CB[7] itself on this reaction (Figure 2b). Neither the signal molecule SG2 (6 mM), or CB[7] (3.5 mM) separately or together show any catalytic activity. This demonstrates a successful re-activation of the substitution reaction through the release of catalyst C2 from the CB cavity by competitive binding of the signal molecule. From these results, we prove that the catalytic activity of DABCO can be tuned by CB[7] encapsulation and competitive binding of a signal molecule.

**Control over prolinol catalysis in aldol reaction**

Host-guest regulation of catalytic activity is also applicable to the aldol reaction, one of the most popular synthetic and biochemical means to construct carbon-carbon bonds. The aldol reaction can be catalyzed by a variety of organo-
signal molecules are charged (SG2) or neutral (SG3). The origin of this unexpected result remains unclear, as $^1$H-NMR did not show unforeseen binding of reaction products or intermediates to CB[7] or the catalyst, which might interfere with catalyst reactivation.

**Control over nornicotine in maleimide oligomerization**

After demonstrating the capability of CB[7] to control the activity of organocatalysts, we wondered whether the same strategy can be used for the regulation of other organic molecules, such as an organic initiator for polymerization, in order to extend the scope of our strategy. In that context, we used CB[7] to control the oligomerization of a maleimide derivative. Maleimide is a widely used functional building block in polymer materials. The homo-polymerization of maleimide can be initiated through an anionic mechanism by an aliphatic amine sticks out beyond the CB[7] carbonyl rim. Moreover, to avoid maleimide N-additions as side reactions, N-acetic acid maleimide (SM6) was synthesized, which also increased substrate solubility and removed any affinity for CB[7]. In the presence of C4, the substrate consumption was accelerated ($>90\%$ conversion in 50 h) compared to the initiator-free blank reaction, resulting in a 5.9-fold faster reaction ($5.29 \text{ h}^{-1}$ vs. $0.89 \text{ h}^{-1}$, Figure 4b). Addition of 7 mM CB[7] into the reaction mixture slowed down the rate to $1.27 \text{ h}^{-1}$. Analogous to the organocatalyzed reactions above, addition of signal molecule SG2 (12 mM) leads to recovery of the reaction rate back to the same level as with only nornicotine present, while the signal molecule and CB[7] alone did not show any activity.

**Catalysis enhancement (C5)**

So far, we have demonstrated the inhibiting effect of CB[7] on the activity of organocatalysts C1, C2, C3 and initiator C4. Yet, CB[7] can also be used to increase organo-catalytic activity. 1H-benzimidazole-2-methanamine C5 (0.4 mM) is a catalyst for the same hydrazone formation reaction as shown in Figure 1b,c. Addition of CB[7] (0.42 mM) to that reaction leads to a 3-fold higher reaction rate than the reaction rate with only C5 (0.57 vs. 0.19 $M^{-1} \text{s}^{-1}$). CB[7] encapsulation in this case increased the catalytic activity of C5, which is an opposite effect compared to what we observe for catalyst C1 in the same reaction. Next, addition of signal molecule SG1 (0.8 mM) to the reaction with catalyst C5 (0.4 mM) and CB[7] (0.42 mM) gives a reaction rate of $0.17 M^{-1} \text{s}^{-1}$, thus restoring the catalytic activity of catalyst C5 to its original value. As such, in this opposite activation model the catalyst release from CB[7] with a signal molecule also works effectively. We were interested in exploring the mechanism behind the unexpected inverse effect of CB[7] encapsulation on the two catalysts. $^1$H-NMR binding studies (Figure S5) indicate that catalyst C1 is fully sequestered inside the CB[7] host. For catalyst C5, $^1$H-NMR shows that only the aromatic part is inside the host but the aliphatic amine sticks out beyond the CB[7] carbonyl rim.
In-situ control over catalytic activity

Using this supramolecular encapsulation strategy, we hypothesized that we should be able to change the reaction rate at any given moment of time during the reaction, by adding CB[7] to encapsulate the catalyst or by releasing the catalyst with addition of a signal molecule. We performed these in situ control experiments with CB[7] for the organocatalysts in the allylic substitution reaction and the hydrazone formation reaction (Figure 5). In the allylic substitution reaction with catalyst C2 (2 mM), adding CB[7] (3.5 mM) after 5 h caused an immediate flattening of the conversion curve (Figure 5a), demonstrating that the host molecule can very rapidly change the activity of the catalyst by encapsulating it. Subsequent addition of signal molecule SG2 after 10 h shifted the curve back to a higher rate. The decrease of the reaction rate constant after CB[7] addition at 5 h and re-initialization with SG2 at 10 h confirms the effective regulation of the catalytic activity of DABCO (Figure 5b). Similarly, for catalyst C1 in the hydrazone formation reaction, we also performed an in situ (de-)activation experiment. When monitoring the reaction using catalyst C1 (0.4 mM), upon adding CB[7] (0.42 mM) after 10 min we immediately observed a decrease in reaction rate (Figure 5c). Subsequent addition of signal molecule SG1 (0.8 mM) after 20 min resulted in an increased reaction rate, back to the original value. For the activated catalyst C5, in situ activity control also works. As shown in Figure 5d, adding CB[7] after 10 min to the reaction mixture with catalyst C5 (0.4 mM) increases the reaction rate immediately. Addition of signal molecule SG1 (0.8 mM) 10 min later liberated the catalyst again from the CB[7] cavity restoring the reaction rate to the original level. These results of two reaction examples with three different organocatalysts confirm successful in situ control of the catalytic activity where CB[7] can thus be used to switch off the catalyst, and a signal molecule can switch the system back on again.

A kinetic model to predict reaction rates based on speciation

With this CB[7] responsive catalyst systems in hand, we wondered whether we could control the rate of hydrazone formation precisely by varying the ratio of [catalyst] versus [CB[7]] and predict the reaction rate with a kinetic model. We followed the reactions with different concentrations of catalyst C1 and CB[7] and determined the reaction rates constants experimentally (Figure 6; black dots). The developed kinetic model to predict the reaction rate constants is shown in Figure 6 (red lines). In the kinetic model we assumed that hydrazone formation occurred without catalyst (k1), via organocatalysis (k2), catalyzed by CB[7] (k3) and catalyzed by the catalyst-CB[7] complex (k4) (Equation 1). The partial reaction rate constants were determined by fitting the concentration profiles of the formation of hydrazone with the least square error method, giving: k1 = 0.0568 M\(^{-1}\)s\(^{-1}\), k2 = 2.46 \times 10^3 M\(^{-1}\)s\(^{-1}\), k3 = 150 M\(^{-1}\)s\(^{-1}\), k4 = 221 M\(^{-1}\)s\(^{-1}\) (see Supplementary information).

\[ k_{\text{total}} = k_1 + k_2 \cdot [\text{cat}] + k_3 \cdot [\text{CB7}] + k_4 \cdot [\text{cat} \subset \text{CB7}] \]  

(1)
We quantified how well the model (Figure 6, red line) fits the experimental values by determining the coefficients of determination $R^2$. In Figure 6a we kept the concentration of CB[7] (0.42 mM) constant and varied the concentration of catalyst C1. The reaction rate hardly increases due to the inhibiting effect of CB[7] encapsulation, until all CB cavities are occupied and free catalyst becomes available to the system. When $[C5]$, the concentration of catalyst C1 is varied between 0–1.8 mM, $R^2 = 0.990$. In Figure 6b, where the reaction rate is 25-fold higher than without catalyst (Figure 6c). Similarly, in Figure 6d, the reaction rate also shows a stark increase with increasing excess of CB[7] when the concentration of catalyst C5 (0.4 mM) is kept constant. These activities are among the highest recorded for hydrazone formation using small molecule catalysts. The linear kinetic model [Eq. (1)] used before does not describe the measurements ($R^2$ values of -0.372 and 0.210). When comparing the host-guest-complex speciation (free CB[7], free catalyst C5, the C5:CB[7] complex) at varying ratios of CB[7] and catalyst C5 to the observed rates, a correlation appears to exist between the rate and the product of the complex and excess species concentrations. Such a correlation suggests the existence of a synergistic effect between the excess species (either free CB[7] or free catalyst C5) and the product of the complex and excess species concentrations. Nevertheless, the mechanism behind this synergistic behavior remains unclear, as Equation (2) and (3) indicate that a large number of catalytic species is involved in the rate determining step, which has a reduced likelihood with increasing complexity.

$$k_{\text{total}} = k_1 + k_2 \cdot [\text{cat}] + k_3 \cdot [\text{CB7}] + k_4 \cdot [\text{cat} \cdot \text{CB7}] + k_5 \cdot [\text{cat} \cdot \text{CB7}]$$

$$k_{\text{total}} = k_1 + k_2 \cdot [\text{cat}] + k_3 \cdot [\text{CB7}] + k_4 \cdot [\text{cat} \cdot \text{CB7}] + k_5 \cdot [\text{cat} \cdot \text{CB7}].$$

**Conclusion**

In this work, we show that supramolecular encapsulation of organocatalysts with CB[7] is a powerful tool to control and tune catalytic activity. Addition of stoichiometric amounts of CB[7] to the catalysts or initiator leads to an immediate reaction rate decrease for catalysts C1 to C4, where CB[7] acts as an inhibitor, and an rate increase for C5, where CB[7] acts as a catalyst.
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[45] I. Ghosh, W. M. Nau, *Adv. Drug Delivery Rev.* **2012**, *64*, 764–783.

[46] L. Magee, *Am. Stat.* **1990**, *44*, 250–253.

[47] Y. Zhou, I. Piergentili, J. Hong, M. P. van der Helm, M. Macchione, Y. Li, R. Eelkema, S. Luo, *Org. Lett.* **2020**, *22*, 6035–6040.

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Tuneable Control of Organocatalytic Activity through Host–Guest Chemistry

Binding an organocatalyst inside a cucurbituril molecular host can reversibly up- or downregulate catalytic activity, which is demonstrated in temporal control over the rate of a range of organocatalyzed transformations.