Chemical signal cascading in a supramolecular network

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Abstract: A chemically-triggered signalling cascade between cucurbituril host-guest complexes by means of multi-step competitive displacement was demonstrated. The inter-complex communication of chemical information yields the release of bio-relevant cargo, reminiscent of cellular signalling pathways.

Signal transduction is the process by which chemical information is communicated along a pathway in a cell. This is achieved by concatenating a series of molecular processes, being the output of an upstream event the input of a downstream event. Nature uses mainly kinase-catalyzed phosphorylation or secondary messengers such as calcium ions or inositol triphosphate to effectuate directed signal propagation. The mimicking of such archetypal processes with stimuli-responsive chemical structures is an important goal in systems chemistry where regulatory functions and chemical communication are key features.[1] Recent works with supramolecular assemblies had a strong focus on the networking between (catalytical or non-catalytical) reaction events[2-6] and information processing.[7, 8] Networks of supramolecular assemblies, relying on inherently reversible interactions, can adapt their equilibrium composition in function of the stimulation by external triggers (e.g., chemical species or light)[9-17] and are therefore ideal models for the demonstration of chemical communication features.[18] Cucurbituril macrocycles were identified as prime hosts in supramolecular self-sorting systems,[19, 20] owing to their differential binding properties towards structurally variable guests.[21, 22] The biomimetic features have favored, for example, the use of CBs in pharmacological[23-25] and analytical applications[26-30] or the control and monitoring of enzymatic catalysis.[31-33]

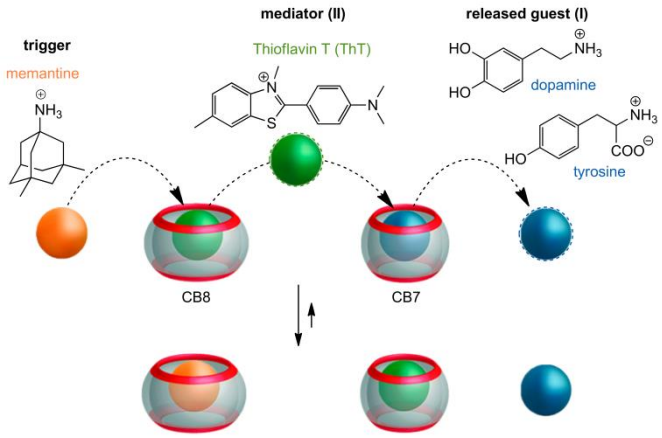

Figure 1. Chemically-triggered supramolecular communication cascade with memantine as trigger, Thioflavin T (ThT) as mediator, and tyrosine or dopamine as finally released guest.

The design of stimuli-responsive host-guest complexes in release applications builds commonly on the direct competition between potential guests.[34-41] However, the demonstration of multi-step guest displacement in small networks of host-guest complexes, leading to chemical communication, adds another layer of complexity to those designs.[42] Herein we pursue the use of a four-component system (see Figure 1), consisting of two guests (**I** and **II**) and two cucurbituril homologues (i.e., CB7 and CB8) to demonstrate externally triggered chemical communication that yields the release of bio-relevant cargo **I** (i.e., the neurotransmitter dopamine or the essential amino acid tyrosine), reminiscent of a cascade reaction; see Figure 1. The role of the mediator, which is dislocated from CB8 by action of the external stimulus and then interacts with the **I**•CB7 complex via competitive displacement, is taken over by Thioflavin T (ThT, **II**). The latter is well-known to form complexes with CB7 and CB8 that are characterized by differential binding strength.[43-45] The binding constants of the guests with CB7 and CB8 are compiled in Table 1.

Table 1. Binding constants of memantine, dopamine, tyrosine, and Thioflavin T (ThT) with CB7 and CB8.

Complex	K/ M^{-1}
dopamine•CB7[a]	$(1.1 \pm 0.3) \times 10^6$
dopamine•CB8[a]	$(4.2 \pm 0.9) \times 10^4$
tyrosine•CB7[a]	$(6.3 \pm 1.1) \times 10^4$
tyrosine•CB8[b]	$\leq 10^4$
ThT•CB7[a]	$(1.0 \pm 0.5) \times 10^6$
ThT•CB8[c]	$(2.0 \pm 0.5) \times 10^7$
memantine•CB7[d]	$(5.9 \pm 0.2) \times 10^4$
memantine•CB8[e]	$(1.1 \pm 0.2) \times 10^{12}$

[a]Measured in this work by ITC (298 K) in water; compare with K(CB7) = 1.0×10^5 M^{-1} (ref. 46) and 4.7×10^5 M^{-1} (ref. 47) for dopamine in water; K(CB7) = 2.2×10^4 M^{-1} (ref. 48) for tyrosine in 10 mM NH_4OAc buffer (pH 6); K(CB7) = 1.2×10^5 M^{-1} (ref. 43) for ThT in water. [b]Conservative estimate by NMR titration; limited by the scarce solubility of CB8. [c]Apparent binding constant; estimated from a competition experiment with CB7 for a limited quantity of ThT. [d]Taken from ref. 27. [e]Taken from ref. 41.

On a first qualitative inspection of the binding constants it can be said that dopamine and tyrosine (guest **I**, both have comparable binding constants) show preference for CB7, while ThT (guest **II**) binds stronger to CB8. Noteworthy, as reported previously, ThT does form not only a 1:1 complex with CB8 but also higher-order complexes of 2:1 and 2:2 stoichiometry.[43, 44] The herein used binding constant is an apparent value, which was estimated by the competition between CB8 and CB7 for a limiting quantity of the dye. Based on the global binding preferences of guest **I** and **II** it is expected that in the four-component system (mixture of **I**, **II**, CB7, and CB8) thermodynamic self-sorting will take place. The simulation of the distribution of the four possible complexes supports this notion: *ca*. 70% of guest **I** (tyrosine or dopamine) are bound by CB7 and *ca*. 93% of guest **II** (ThT) is encapsulated by CB8 (see Figure 2 for tyrosine and ESI for dopamine).

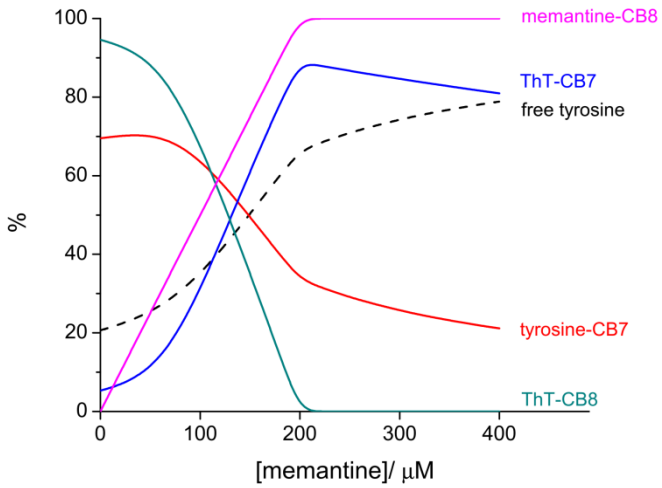

Figure 2. Distribution of the main species upon titration of the four-component mixture of ThT (140 μM), tyrosine (200 μM), CB7 (200 μM), and CB8 (200 μM) with memantine. The percentage of each species is expressed relative to the maximum possible concentration. Sub-stoichiometric amounts of ThT were chosen to maximize the complexation degree of this dye with CB8. ThT-CB8 stands for the mixture of the different possible complexes with varying stoichiometric compositions.

These predictions were confirmed in ^{1}H-NMR spectroscopic experiments in D_2O; see Figure 3 for tyrosine and the ESI for dopamine as guest **I**. On the one hand, in the NMR spectrum of the four-component mixture, containing tyrosine as guest **I**, the resonance signals of ThT•CB8 are unambiguously identified (Figure 3a and c). No significant amount of free ThT or ThT•CB7 was detected by ^{1}H-NMR spectroscopy of the four-component mixture, in agreement with a strong and preferential binding of this guest to CB8. On the other hand, tyrosine is mainly bound by CB7 and only a minor quantity of free guest is detected (Figure 3b and c).

Having the self-sorting of the four-component adequately documented we sought to demonstrate the chemically-triggered cascade reaction. As trigger the adamantane derivative memantine was chosen, because of its strong and selective binding to CB8 (see Table 1).[20, 27] Based on the involved equilibria it can be predicted that the titration of the four-component mixture with memantine leads to a competitive re-distribution of the guests: memantine displaces ThT from CB8, which in consequence competes with tyrosine for CB7 and leads to the release of the amino acid (Figure 2). The corresponding simulation for the equivalence point indicates that CB8 would be quantitatively occupied by memantine and 89% of the ThT is bound by CB7, resulting in the effective release of tyrosine.

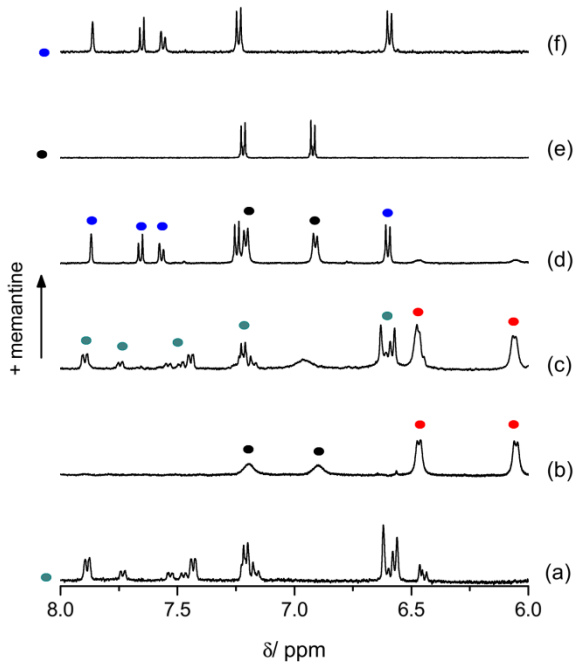

Figure 3. Partial ^{1}H-NMR spectra (D_2O) of a) ThT•CB8 (green dots); b) tyrosine•CB7 (red dots – complex; black dots – free tyrosine); c) the four-component mixture of ThT, tyrosine, CB7, and CB8; d) the four-component mixture after addition of memantine; e) free tyrosine (black dots); f) ThT•CB7 (blue dots). [ThT] = 140 µM, [tyrosine] = [memantine] = [CB7] = [CB8] = 200 µM.

At a first point the individual displacement processes were experimentally monitored by ^{1}H-NMR spectroscopy (see ESI). The addition of memantine to ThT•CB8 resulted in the complete displacement of ThT from the CB8 macrocycle and the clear-cut observation of the memantine•CB8 complex. Likewise, the addition of ThT to a solution of tyrosine and CB7 (76% complexation degree) yielded the ThT•CB7 complex and unbound tyrosine. For dopamine the same observation of efficient displacement by ThT was made (see ESI). As a side note and having a closer look at the binding constants in Table 1, it turns out that memantine could displace tyrosine or dopamine directly to some extent. However, in presence of CB8 the six orders of magnitude higher binding constant with CB8 prevents memantine from competing with guest **I** for CB7, except when a larger excess of memantine is introduced. Hence, based on the combined observations the projected cascade reaction seemed feasible.

^{1}H-NMR spectroscopic experiments for the addition of memantine to the four-component mixture provided clear evidence for chemical communication. As can be observed in Figure 3d, the resulting NMR spectrum clearly shows the signals corresponding to ThT•CB7 (*cf*. Figure 3f), while the resonances assigned to the ThT•CB8 complex have completely vanished. In addition, the signals of the unbound tyrosine (*cf*. Figure 3e) and the memantine•CB8 complex are seen (see ESI). Hence, memantine took the place of ThT in CB8 and in turn ThT displaced

tyrosine from CB7. Likewise, dopamine is released in the same manner by the memantine-triggered cascade (see ESI). Noteworthily, ThT offers unique optical spectroscopic fingerprints that can be used as well for following the cascade reaction (see ESI).

Based on obtained results the following general design rules for supramolecular cascades can be formulated: (a) guests and hosts should be chosen so that a clear self-sorting is achieved for the initial network state, (b) the addition of the trigger should give rise to a different self-sorting situation, displacing only the guest from one of the hosts which itself is a competitor for the guest of the other host, (c) the selective binding of the trigger should prevent the direct displacement of the guest at the end of the cascade.

In conclusion, by drawing on the potential of cucurbituril macrocycles as components of orthogonally assembled host-guest complexes, the externally triggered chemical communication between them was demonstrated. This culminated in the release of biologically relevant guests, i.e., tyrosine or dopamine. The cascade is strictly defined by the thermodynamic characteristics of the involved host-guest complexes. The reported case corresponds to a one-input (trigger)/one-output (finally released guest) situation. However, variations of this should be possible when employing triggers that are activated by a combination of chemical/physical inputs, e.g., light and pH.[36, 49]

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CONFLICTS OF INTEREST

There are no conflicts to declare.

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