

Inter-Communication of DNA-Based Constitutional Dynamic Networks

Liang Yue,^{§,†} Shan Wang,^{§,†} Sivan Lilienthal,[†] Verena Wulf,[†] Françoise Remacle,[‡] R. D. Levine[†] and Itamar Willner^{*,†}

[†]Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.

[‡]Department of Chemistry, University of Liege, B6c, 4000 Liege, Belgium.

KEYWORDS: DNAzyme; Triplex; Feedback; Switch; Catalytic cascade; Adaptive.

ABSTRACT: Inter-communication between dynamic chemical networks plays a major role in cellular transformations. Bioinspired by nature, we introduce the inter-communication between two constitutional dynamic networks, CDNs, “S” and “T” composed, each, of four equilibrated supramolecular constituents AA', AB', BA' and BB', and of CC', CD', DC' and DD', respectively. Each of the constituents is conjugated Mg²⁺-ion-dependent DNAzyme unit that acts as a reporter element for the concentration of the respective constituent *via* the catalyzed cleavage of the fluorophore-quencher-functionalized substrate associated with the respective DNAzyme reporter. Also, constituents BB' (in CDN “S”) and CC' (in CDN “T”) include Mg²⁺-ion-dependent DNAzymes acting as activator units for generating triggering signals between the networks.

Subjecting CDNs “S” and “T” to the catalytically cleavable hairpin triggers H_{dd'} or H_{aa'} respectively, yields input strands that inter-communicate the CDNs by affecting the time-dependent re-equilibration of the constituents of the counter CDN without effecting the dynamic equilibrium of the constituents of the CDN that generates the triggering strands. Treatment of CDNs “S” and “T” with hairpins H_{dd'} and H_{aa'} (or H_{ba'}), respectively, stimulate autonomous positive/positive or positive/negative feedback to the programmed time-dependent up-regulation or down-regulation of the equilibrated constituents in the two CDNs.

INTRODUCTION

The up-regulation and down-regulation of dynamic chemical networks by environmental physical or chemical triggers play major roles in cellular transformations.¹ In addition, the inter-communication between dynamic networks presents, in nature, complex biotransformations that allow signal propagation,² branching and switching of reactions,³ feedback or oscillatory mechanisms,⁴ biocatalytic cascades⁵ and more.⁶ Inspired by nature, the area of systems chemistry that addresses the assembly of constitutional dynamic networks (CDNs) that mimic natural processes is rapidly advancing.⁷ Ingenious dynamically controlled self-organized supramolecular assemblies that adapt chemically-guided architectures were reported.⁸ For example, the metal-ion induced formation of metal-complex helicates⁹ or the self-organization of ligand binding receptors¹⁰ represent switchable, dynamically-controlled, structures dictated by external chemical triggers. A simple CDN consists of four equilibrated dynamically interconvertible constituents AA', AB', BA' and BB'. The stimuli-triggered stabilization of one of constituents, e.g., AA', shifts the system to a new dynamically equilibrated system where AA' is up-regulated on the expense of the constituents AB' and BA', and the concomitant up-regulation of constituent BB' that results from the stimuli-enforced separation of AB' and BA'. Subjecting the latter equilibrated constitutional network to a counter trigger that destabilizes the constituent AA' reconfigures the system to the originally equilibrated mixture of constituents. Different CDNs consisting of molecular or macromolar networks were reported.¹¹ A variety of external physical triggers, such as light,¹² temperature¹³ or electric field,¹⁴ and chemical triggers, such as pH,¹³ solvents,¹⁵ and supramolecular H-bonds¹⁶ were used to shift and adapt CDNs equilibria. For example, the equilibrated mixture of hydrazones or acylhydrazones were controlled by orthogonal

triggers, such as metal ions¹⁷ or light.¹⁸ The formation of programmed structural grids by the polymerization of equilibrated mixtures of hydrazone/hydrazine constituents, and using Lewis acids or metal ions as orthogonal triggers, that control the dynamically equilibrated mixtures of constituents,¹⁹ was reported. In addition to dynamically equilibrated mixtures consisting of four constituents, CDNs with enhanced complexities, such as triggered dynamically equilibrated mixtures composed of six-component constituents A, A', B, B', C and C' were reported.²⁰ Different applications of CDNs were suggested and these include the screening of drugs,²¹ design of self-sensing devices,¹⁴ and the dictated formation of switchable sol/gel materials.²² Nonetheless, the different CDNs, suffered till recently from several drawbacks: (i) There is no universal chemical scaffold for building the CDN systems. (ii) The diversity of chemical constituents to assemble the CDNs is limited, and the pre-design of programmable CDNs is difficult. (iii) The design of bio-inspired inter-communicating CDNs is unprecedented.

Recently, we suggested supramolecular structures of nucleic acids as versatile constituents for the assembly and triggered operation of CDNs.^{23,24} The advantages of using nucleic acids as functional components to construct CDNs include: (i) The nature and number of base-pairs comprising the constituents provide a means to delicately balance and control the composition of the constitutional constructs of the CDNs.²⁵ (ii) Different physical or chemical triggers control the stability of nucleic acid constructs. Particularly, reversible triggers/counter triggers can stabilize or destabilize the supramolecular nucleic acid constituents, thereby allowing the reversible reconfiguration of equilibrated CDNs, and the reversible switching and shifting of equilibrated CDNs.²⁶ Such triggers include, for example, the K⁺-ions stabilization of G-quadruplexes and their separation by 18-crown-6-ether,²⁷ the sta-

bilization of duplexes by T-Hg²⁺-T or C-Ag⁺-C bridges and their separation by thiol ligands,²⁸ the pH induced formation or separation of triplex nucleic acids²⁹ or i-motif structures.³⁰ Also, physical stimuli, such as light, were used to control the stabilities of duplex nucleic acids, e.g., the stabilization of duplex nucleic acids by *trans*-azobenzene intercalation units and their destabilization by photoisomerization of the units to *cis*-azobenzene.³¹ (iii) In addition to the diverse stimuli-responsive structures of nucleic acids, the biopolymer reveals unique recognition and catalytic functions. These include the sequence-specific binding of low-molecular-weight substrates or macromolecules (aptamers),³² and the catalytic properties of sequence-specific nucleic acids (DNAzymes),³³ e.g., metal-ion dependent hydrolytic DNAzymes that cleave phosphodiester bonds of nucleic acid substrates. The incorporation of these functional nucleic acid structures of CDN constituents, provides important “tools” to activate and operate reversible transitions of CDNs. Indeed, we have demonstrated that the rich “toolbox” provided by the base-sequences encoded in the nucleic acids biopolymer allows the control of the equilibrated mixtures of CDNs and the effective readout of the reconfigurable CDNs by DNAzymes conjugated to the biopolymer.^{23,24} For example, using nucleic acid inputs for the strand displacement of nucleic acids associated with the CDN constituents, the dictated self-organization of stabilized nucleic acid triplex structures guided shifts in the equilibrated mixture of constituents in the CDNs. The readout of the equilibrated mixture was then achieved by following the catalytic functions of DNAzymes integrated into the different constituents.

In the present study, we enhance the complexity of nucleic acid-based CDNs by demonstrating unprecedented inter-communication between CDNs. We reveal that the chemical functions of one CDN can trigger the reconfiguration of a second CDN system (up-regulation or down-regulation of the equilibrated second CDN). In addition, we demonstrate the inter-communication between two CDNs whereby one CDN controls the operation of the second CDN, and the controlled chemical functionalities of the second CDN dictate the functions and properties of the first CDN. Positive feedback and negative feedback mechanisms proceed upon the inter-communications of the CDNs. A major accomplishment of the study involves the engineering of the appropriate structures of the constituents comprising of inter-connected CDNs. A detailed description of the strands composing the constituents of the CDNs is provided in the supporting information.

RESULTS AND DISCUSSION

The first inter-linked two CDNs system is depicted in Figure 1. The system consists of two CDNs, “S” and “T”. The equilibrated CDN “S” includes four constituents, AA', AB', BA' and BB'. The equilibrated CDN “T” includes four other constituents, CC', CD', DC' and DD'. The two networks do not share any components and lack inter-communication. Each of the constituents in CDNs “S” or “T” is modified by a Mg²⁺-ion-dependent DNAzyme unit that acts as a reporter for the quantitative evaluation of the concentration of the respective constituent in CDNs “S” or “T”. Namely, the rates of hydrolysis of the fluorophore/quencher-functionalized substrates of each of the DNAzyme reporters, and the use of appropriate calibration curves, relating the rates of hydrolysis of the substrates to the variable concentrations of the eight different DNAzyme reporter units, provide a versatile means to quantitatively evaluate the concentrations of the equilibrated constituents in the two CDNs. In addition, two of the constituents, BB' and CC', in CDN “S” and CDN “T” include, each, besides the Mg²⁺-ion-dependent DNAzyme reporter unit, an additional Mg²⁺-ion-dependent DNAzyme unit that acts as functional catalytic

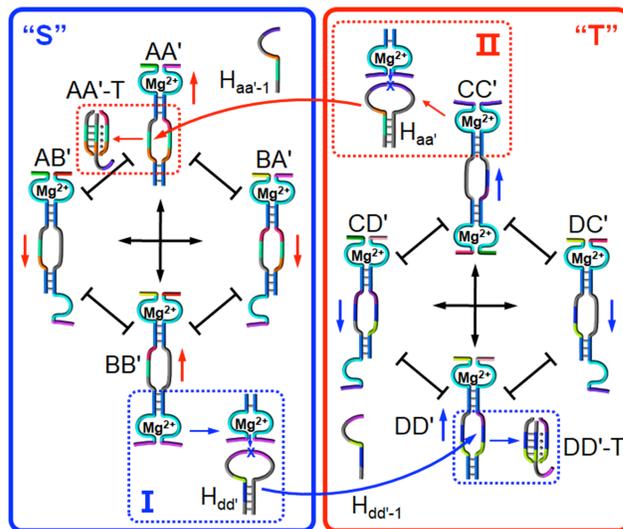


Figure 1. Schematic inter-communication between two CDNs (“S” and “T”) using two different hairpins H_{dd'} and H_{aa'} as triggers. Trigger H_{dd'} acts as a substrate for the activating DNAzyme unit associated with the constituent BB' of CDN “S” and trigger H_{aa'} acts as a substrate for the activating DNAzyme unit associated with the constituent CC' of CDN “T”.

activator unit to inter-connect the two networks (*vide infra*). The constituents, AA' and BA' in CDN “S”, and DD' in CDN “T”, include a two-loop thymine-rich domain that separates two duplex domains. Incorporation of a sequence-specific strand in the thymine-modified domains generates a T-A-T triplex. The stabilization of any of the constituents through the formation of the triplex structure leads to the stabilization of the respective constituent and to the reconfiguration and shift in the respective equilibrated CDN. For example, subjecting CDNs “S” and “T” to the hairpin substrate (input) H_{dd'} results in the cleavage of the substrate H_{dd'} by the constituent BB' in CDN “S”. The resulting cleave-off strand H_{dd'-1} is engineered to bind in the double-loop domain of DD' to yield the respective triplex functionalized DD' constituent, DD'-T. The stabilization of the constituent DD' via the formation of DD'-T shifts the equilibrium of CDN “T” by enriching the constituent DD'-T through the dissociation of the constituents CD' and DC'. The partial separation of the constituents CD' and DC' releases the components C and C' that recombine to enrich the constituent CC' of CDN “T”. That is, the cleavage of H_{dd'} by the constituent BB' induces a dynamic transition and reconfiguration of the equilibrated constituents of CDN “T” that results in the over-expression of constituents DD'-T and CC' and the down-regulation of CD' and DC'. The contents of the constituents associated with CDNs “S” and “T” before and after interaction of the two CDNs with H_{dd'} are read out by the DNAzyme reporter units associated with all eight constituents, Figure 2. Panel I shows the rates of the hydrolysis of the respective substrates by the constituents AA', AB', BA' and BB' before (i) and after (ii) subjecting the system to the trigger H_{dd'}. Evidently, no changes in the catalytic rates of the DNAzymes are observed, implying that, no changes in the concentrations of the constituents comprising CDN “S” occur upon treatment of the two CDNs with H_{dd'}. Conversely, panel II shows the DNAzyme-transduced catalytic rates corresponding to the constituents CC', CD', DC' and DD' before (i) and after (ii) subjecting the two CDNs system to the H_{dd'}. Evidently, the catalytic rate of the DNAzyme associated with DD' increases upon the addition of H_{dd'}, consistent with the stabilization of this constituent by the triplex-generating strand H_{dd'-1} (formation of DD'-T). This results in the increase in the concentration of DD'-T that leads to the enhanced catalytic DNAzyme functions. The triggered over-expression of

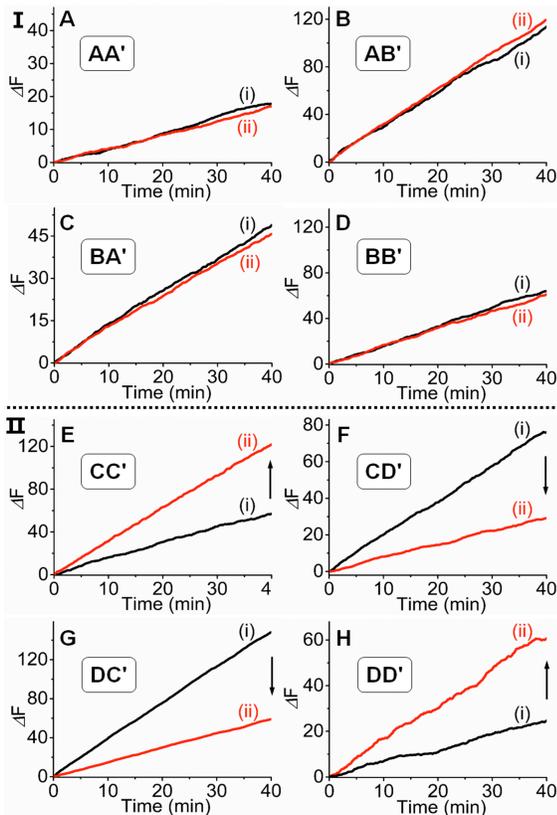


Figure 2. Time-dependent fluorescence changes generated from the cleavage of the respective fluorophore/quencher-modified substrates by the DNAzyme reporter units associated with the different constituents included in the CDNs “S” and “T” before (i) and after (ii) subjecting the coupled networks to the hairpin-trigger H_{dfr} for 24 hours: (I) The constituents associated with CDN “S”. (II) The constituents associated with CDN “T”.

DD'-T is accompanied by the down-regulation of CD' and DC', as evident by the lower catalytic rates of the DNAzyme reporter units associated with these constituents. The over-expression of DD'-T is accompanied by the concomitant increase in the concentration of CC', reflected by the higher catalytic activity of the DNAzyme reporter linked to this constituent. By applying the respective calibration curves (Figures S1 and S2), we evaluated quantitatively the concentration changes of the constituents of CDN “T” upon treatment of the two CDNs with H_{dfr} ; DD' and CC' increase by 125% and 91%, respectively, whereas CD' and DC' decrease by 57% and 53%, respectively. The dynamic equilibration of the CDN “T” by the triggered cleavage of H_{dfr} in CDN “S” is a slow process as compared to the catalytic cleavage of the substrates associated with the different DNAzyme reporter units. This allows us to probe the time-dependent changes of the concentrations of the constituents in CDN “T” during the equilibration of the CDN. Figures S3 and S4 show the rates of cleavage of the respective substrates by the DNAzyme reporter units associated with the four constituents CC', CD', DC' and DD' at different time-intervals of the dynamic equilibration of the CDN “T”. Evidently, a time-independent increase in the cleavage rates of the substrates corresponding to the DNAzyme reporter units associated with the constituents CC' and DD' is observed, whereas a time-dependent decrease in the cleavage rates of the substrates corresponding to the constituents CD' and DC' is detected, as expected. Using the respective calibration curves, Figures S1 and S2, we quantitatively evaluated the time-dependent concentration changes of the constituents in CDN “T” upon triggering the CDN by the strand $H_{\text{dfr-1}}$ generated by CDN “S”, Figure 3. One may realize a non-linear time-dependent increase in the concentrations of the

constituents CC' and DD' and analogous time-dependent decrease in the concentrations of CD' and DC'. Clearly, the system dynamically equilibrates after a time-interval of ca. 16 hours where the concentrations of all constituents reached a saturation value. Knowing the initial and final concentrations and the dissociation constants of the constituents, and the approximated rate-constants for cleavage of hairpins by the respective DNAzymes, the time-dependent profiles corresponding to the concentrations of the different constituents were computationally simulated, see Figure S5 (A) and accompanying discussion. A very good fit between the experimental results and the computationally simulated kinetic evaluation is obtained.

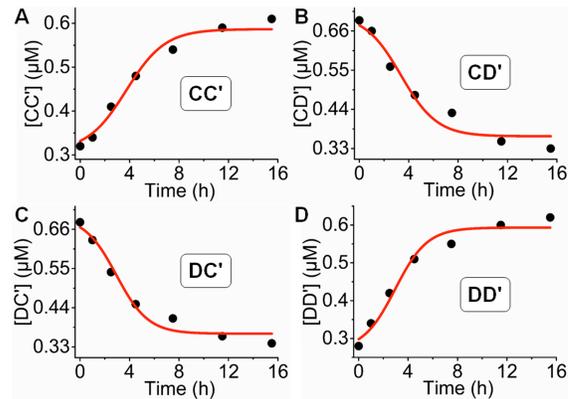


Figure 3. Time-dependent dynamically-equilibrated concentration changes of the constituents of CDN “T” upon subjecting the CDNs “S” and “T” to the trigger H_{dfr} .

In analogy, the initially equilibrated system CDNs “S” and “T” were treated with the hairpin H_{aa} that acts as substrate for the activator of the Mg^{2+} -ion-dependent DNAzyme associated with the constituent CC' of CDN “T”, Figure 1, inset, panel II. The constituent CC' cleaves H_{aa} , and the released strand $H_{\text{aa-1}}$ yields the T-A-T triplex structure in the double-loop domain of AA'. The stabilization of AA' results in its up-regulation (increased content), and the down-regulation of AB' and BA' through their dissociation. The partial release of B and B' leads to the concomitant increase in the concentration of BB'. Thus, the generation of $H_{\text{aa-1}}$ by CDN “T” leads to a shift in the dynamically equilibrated network “S”, where the constituents AA'-T and BB' increase in their contents, and the constituents BA' and AB' are expected to decrease in their contents, as compared to the initial mixture of constituents in CDN “S”. The quantitative assessment of the concentrations of the different constituents is, then, recorded by the activities of the different DNAzyme reporters associated with all constituents of the two CDNs. Note that cleavage of H_{aa} is not expected to affect the equilibrium of CDN “T”, but only to dynamically shift the equilibrium of the constituents in the adjacent CDN “S”. Figure 4 shows the activities of the Mg^{2+} -ion-dependent DNAzymes reporter units associated with the different constituents before (i) and after (ii) treatment of the two interconnected CDNs with the hairpin H_{aa} . Evidently, the activities of the constituents AA' and BB' are enhanced, implying the up-regulation of these constituents, and a decrease in the activities of AB' and BA' constituents is observed, as a result of their down-regulation. Using the appropriate calibration curves (Figures S1 and S2), the contents of AA' and BB' increase by 116% and 88%, respectively, and the concentrations of AB' and BA' decrease by 55% and 63%, respectively. Conversely, the activities (or concentrations) of the constituents comprising CDN “T” are unchanged upon addition of H_{aa} to the two CDNs system. Note, that the catalytic operation of a constituent in one of the CDNs does not perturb the equilibrium

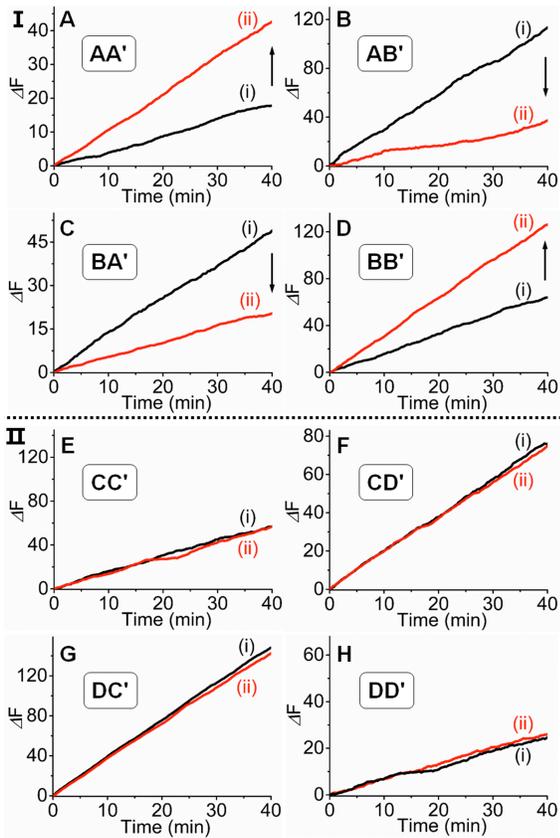


Figure 4. Rates of the cleavage of the respective fluorophore/quencher-modified substrates by the DNAzyme reporter units coupled to the respective constituents before (i) and after (ii) subjecting the two CDNs to the trigger $H_{aa'}$ for 24 hours. Panel I: The constituents associated with CDN “S”. Panel II: The constituents associated with CDN “T”.

of its own CDN, but it reveals a drastic equilibrium transition on the counter CDN. The cleavage of the substrate $H_{aa'}$ by the constituent CC' associated with CDN “T”, triggers the time-dependent increases in the contents of constituents AA' and BB' , and the concomitant time-dependent decreases of the constituents AB' and BA' . Figures S6 and S7 show the rates of cleavage of the substrates by the DNAzymes coupled to the constituents of CDN “S” at different time-intervals. Using the appropriate calibration curves (Figures S1 and S2), we were able to formulate the concentrations of the constituents at time-intervals of the dynamic equilibration of the system, Figure S8. (Similarly, these results were computationally simulated, see Figure S5 (B).)

The possibility to inter-connect, and selectively control, the operation of two CDNs suggested that the treatment of the two CDNs with two input triggers $H_{aa'}$ and $H_{dd'}$ could yield a feedback-driven inter-network operation, as outlined in Figure 1. That is, subjecting CDN “T” to $H_{aa'}$ results in the CC' -stimulated cleavage of $H_{aa'}$ to form $H_{aa'-1}$ that up-regulates the constituent AA' . Similarly, the BB' -induced cleavage of $H_{dd'}$ yields the strand $H_{dd'-1}$ that up-regulates the constituent DD' of CDN “T”. The up-regulation of constituent AA' in CDN “S” is, however, accompanied by the concomitant up-regulation of BB' in CDN “S”, while the up-regulation of DD' in CDN “T” (in the form of DD' -T) is accompanied by the concomitant up-regulation of CC' in CDN “T”. That is, subjecting the two networks to the two inputs $H_{aa'}$ and $H_{dd'}$ leads to the inter-connection of the two networks by an autocatalytic feedback cycle where dynamic time-dependent equilibria changes in the equilibrated mixtures of constituents in the two networks proceed. These changes are anticipated to proceed as long as $H_{aa'}$ and $H_{dd'}$ are present in the system and reflected by a

time-dependent increase in the contents of AA' and BB' (in CDN “S”) and of DD' and CC' (in CDN “T”) and the concomitant time-dependent decrease in the concentrations of BA' and AB' (in CDN “S”) and of DC' and CD' in CDN “T”. Note, that the changes in the equilibrated contents of constituents in any one of the CDNs does not affect the equilibrium of its own constituents but it affects the dynamic equilibrium of the connected CDN. Figure 5 presents the changes in the equilibrated contents of the constituents associated with the CDNs “S” and “T” before (i) and after (ii) subjecting the systems to the inputs $H_{dd'}$ and $H_{aa'}$ (equilibration time-interval 24 hours). The contents of the different constituents are reflected by the activities of the DNAzymes associated with the respective constituents and the quantitative assessment of the concentrations of the constituents by the calibration curves (Figures S1 and S2). As expected, in CDN “S” the constituents AA' and BB' increase in their contents by 116% and 76%, respectively, while the constituents BA' and AB' are down-regulated by 65% and 53%, respectively. Similarly, in CDN “T”, the constituents CC' and DD' are up-regulated by 97% and 132%, whereas the constituents DC' and CD' are down-regulated by 51% and 54%, respectively.

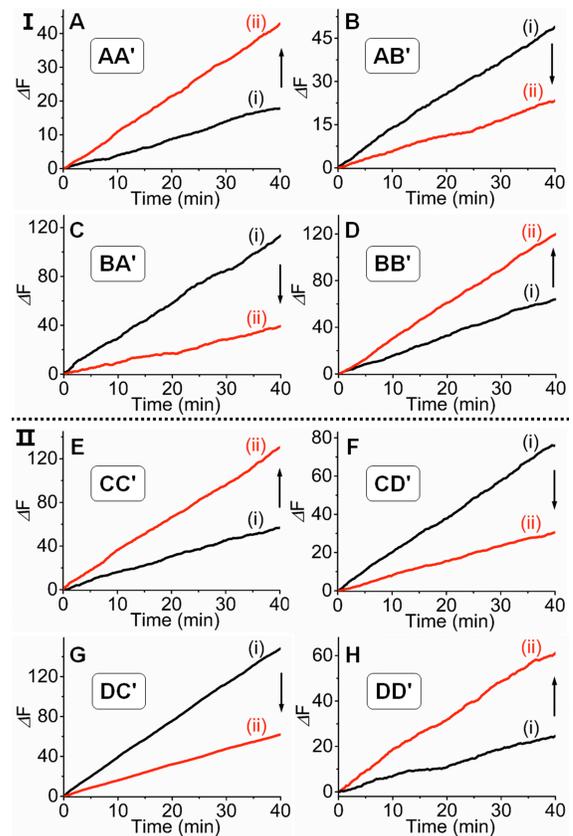


Figure 5. Rates of the cleavage of the respective fluorophore/quencher-modified substrates by the DNAzyme reporter units coupled to the respective constituents before (i) and after (ii) subjecting the two CDNs to the triggers $H_{dd'}$ and $H_{aa'}$ for 24 hours. Panel I: The constituents associated with CDN “S”. Panel II: The constituents associated with CDN “T”.

The inter-communication between the CDNs “S” and “T” represents a time-dependent change in the composition of the systems. The results shown in Figure 5 show the equilibrated contents of the constituents, reported by the activities of the conjugated DNAzymes, after a time interval of 24 hours. Thus, by monitoring the output of the different reporter units within a relatively short time-interval of 40 minutes, and using the appropriate calibration curves, the time-dependent profiles of the changes in the contents of the different constituents, as a result of the feedback-

driven inter-communication between the CDNs, can be evaluated. Figures S9 and S10 show the time-dependent activity changes of the DNazyme reporter units as a result of the inter-network communication. The activities of the DNazymes associated with AA', BB', CC' and DD' increase with time and reach a saturation value after ca. 16 hours, whereas the activities of the DNazyme reporters associated with AB', BA', CD' and DC' decrease with time and reach a saturation value after this time-interval. Using the fluorescence changes corresponding to the time-dependent activities of the different reporter DNazymes, and the respective calibration curves (Figures S1 and S2), the time-dependent changes in the concentrations of the constituents during the dynamic inter-communication between the networks is shown in Figure 6.

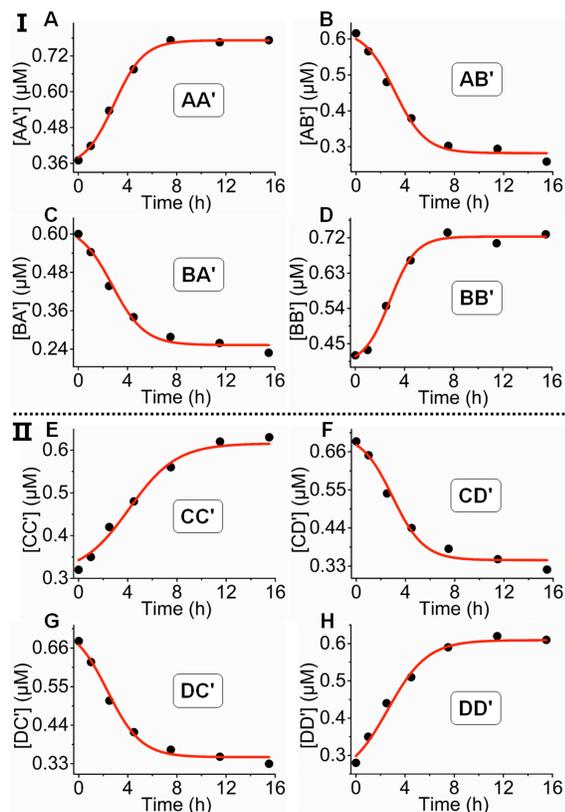


Figure 6. Time-dependent concentration changes of the constituents associated with CDNs “S” and “T” upon the dynamic feedback-driven equilibration of the CDNs in the presence of the triggers $H_{dd'}$ and $H_{aa'}$. Panel I: The constituents of CDN “S”. Panel II: The constituents of CDN “T”.

Table 1 summarizes the concentrations of the different constituents in CDNs “S” and “T” before and after subjecting the CDNs to the input triggers $H_{dd'}$ and $H_{aa'}$ (for a time-interval of 24 hours). Further support that the concentration changes of the constituents occurring in the CDNs upon subjecting the system to the $H_{dd'}$ and $H_{aa'}$, using the activities of the reporter DNazyme units, are correct, was obtained by complementary quantitative electrophoretic experiments. Figure 7 shows the electrophoretic separation of the different constituents before (lane 11) and after (lane 12) subjecting the CDNs “S” and “T” to the triggers $H_{dd'}$ and $H_{aa'}$. Note that in order to reveal this separation, some of the constituents were modified with conjugated tethers, that do not participate in the stabilization/destabilization of the constituents, in order to enhance the separation (for further discussion see supporting information). In lane 1 to lane 8 are the reference bands corresponding to the constituents AA', AB', BA', BB', CC', CD', DC' and DD', respectively. In lane 9 is shown the band of the AA'-T whereas in lane 10 is shown the band corresponding to DD'-T. The comparison of the separated bands of the different constituents of CDNs

“S” and “T” before subjecting the CDNs to $H_{dd'}$ and $H_{aa'}$, lane 11, and after treatment of the CDNs with $H_{dd'}$ and $H_{aa'}$, lane 12, allows to identify the following conclusions. (Note that not all of the constituents could be fully separated and these non-separated bands were not used to quantify the respective constituents, *vide infra*). (i) The stained bands associated with the constituents of the CDNs before subjecting the system to $H_{dd'}$ and $H_{aa'}$ are fully separable. (ii) The following separated stained bands are intensified upon treatment of the CDNs with $H_{dd'}$ and $H_{aa'}$: BB' and CC' (belonging to the different CDNs). Conversely, the band intensities associated with the constituents BA' and CD' are depleted. Using the ImageJ software, we quantified the concentrations of the separated bands before and after subjecting the system to the triggers. Table 1 summarizes the concentrations of the constituents derived by the DNazyme reporters and the concentration of the respective constituents derived from the stained electrophoretic bands (data provided in brackets). Evidently, excellent agreement in the evaluated concentrations of the constituents using the two methods is demonstrated. (iii) We note that the bands associated with AA'-T and AB' and of DD'-T and DC' are non-separable and thus the quantitative evaluation of concentrations of these constituents, after addition of $H_{dd'}$ and $H_{aa'}$ is prohibited.

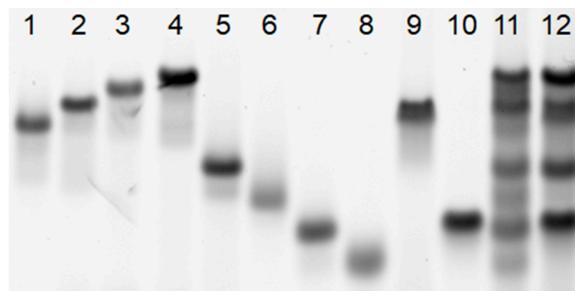


Figure 7. Native page gel electrophoresis separation of the constituents of CDNs “S” and “T” before and after subjecting the inter-connected CDNs to the triggers $H_{dd'}$ and $H_{aa'}$ (the two triggers yield the inter-connected feedback-driven equilibrated mixtures). Lanes 1 to 10 control bands corresponding to the individual constituents: lane 1-AA', lane 2-AB', lane 3-BA', lane 4-BB', lane 5-CC', lane 6-CD', lane 7-DC', lane 8-DD', lane 9-AA'-T and lane 10-DD'-T. Lane 11-The separated bands corresponding to the mixture of the constituents of CDNs “S” and “T” before applying the triggers $H_{dd'}$ and $H_{aa'}$. Lane 12-Separated bands corresponding to the mixture of the constituents of CDNs “S” and “T” after subjecting the CDNs to the triggers $H_{dd'}$ and $H_{aa'}$ for a time-interval of 24 hours.

Table 1. Quantitative assessment of the concentrations of the constituents associated with CDNs “S” and “T” before subjecting the inter-connected CDNs to triggers $H_{dd'}$ and $H_{aa'}$ (i) and after subjecting the CDNs to the triggers for a time-interval of 24 hours (ii).

system	concentration (μM)							
	[AA']	[AB']	[BA']	[BB']	[CC']	[CD']	[DC']	[DD']
(i) ^(a) _(b)	0.37	0.62	0.60	0.42	0.32	0.69	0.68	0.28
	(0.35)	(0.59)	(0.53)	(0.45)	(0.28)	(0.63)	(0.67)	(0.29)
(ii) ^(a) _(b)	0.80	0.29	0.21	0.74	0.63	0.32	0.33	0.65
	(-) ^(c)	(-) ^(c)	(0.18)	(0.72)	(0.58)	(0.25)	(-) ^(c)	(-) ^(c)

^(a)Concentration data provided by the DNazyme reporter units.

^(b)Concentration data extracted by quantitative analysis of the electrophoretically-separated stained bands. ^(c)Concentrations cannot be evaluated due to the overlap of the bands.

The base sequence encoded in the substrates $H_{dd'}$ and $H_{aa'}$ and the fragmented products, $H_{dd'-1}$ and $H_{aa'-1}$ generated by the BB' and CC' DNAzyme activators dictated the up-regulation and down-regulation of the dynamically inter-connected constituents. One may, however, control an orthogonal regulation of the constituents by the appropriate programming of the hairpin substrate conjugated to the constituent CC' as outlined in Figure 8. In this system we use the hairpin $H_{ba'}$ as the substrate for the DNAzyme activator associated with constituent CC', that is a part of CDN "T". The cleavage of $H_{ba'}$ yields the strand $H_{ba'-1}$ that forms a triplex with the double-loop region of BA', that is a part of CDN "S", resulting in the stabilized constituent BA'-T. The stabilization of BA'-T shifts the equilibrium of the constituents in CDN "S" leading to an increase in its concentration, the up-regulation of the constituent AB', and the concomitant down-regulation of AA' and of BB'. That is, treatment of the CDNs "S" and "T" with the two inputs $H_{dd'}$ and $H_{ba'}$ is anticipated to shift the equilibrated mixtures of the two inter-communicating CDNs, by the up-regulation of the constituents DD'-T and CC' and the down-regulation of DC' and CD' in CDN "T", and to stimulate the up-regulation of BA' and AB' and the concomitant down-regulation of AA' and BB' constituents associated with CDN "S". Figure S11 shows the effect of the added hairpin $H_{ba'}$ on the equilibrated inter-connected CDNs "S" and "T" as reflected by the catalytic activities of the DNAzymes conjugated to the different constituents. Cleavage of the hairpin $H_{ba'}$ has no effect on the catalytic activities of DNAzyme reporters associated with the constituents CC', DD', DC' and CD' of CDN "T" but induces a reconfiguration in the equilibrated CDN "S". Figure S11, panel I, depicts the catalytic activities of the DNAzyme reporters that are conjugated to the constituents of CDN "S", before (i) and after (ii) subjecting the system to $H_{ba'}$. The increase/decrease in the concentration of the constituents were evaluated by using the respective calibration curves (Figures S1 and S2). The constituents AB' and BA' are up-regulated by 31% and 40%, respectively, while the constituents AA' and BB' are down-regulated by 49% and 50%, respectively. As before, the catalytic rates of the DNAzyme reporter units, coupled to the constituents of CDN "S", at time-intervals of interaction with the cleaved product of CDN "T", $H_{ba'-1}$, are shown in Figures S12 and S13. The concentration changes of the constituents AA', AB', BA' and BB' upon the triggered, time-dependent, dynamic equilibration of the system are presented in Figure S14.

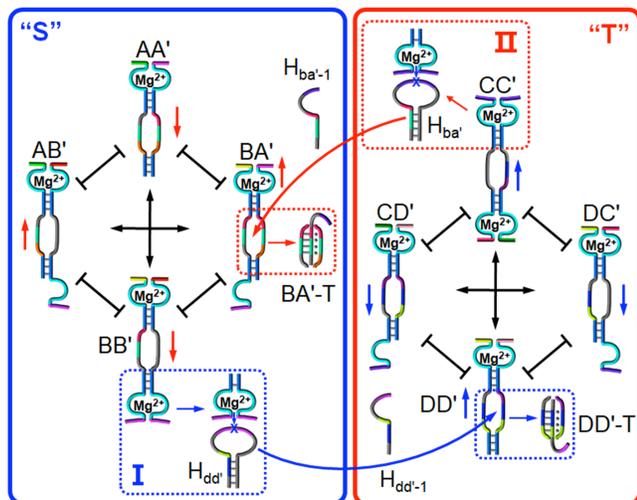


Figure 8. Schematic inter-communication between two CDNs ("S" and "T") using two different hairpins $H_{dd'}$ and $H_{ba'}$ as triggers. Trigger $H_{dd'}$ acts as a substrate for the activating DNAzyme unit associated with the constituent BB' of CDN "S" and trigger $H_{ba'}$ acts as a substrate for the activating DNAzyme unit associated with the constituent CC' of CDN "T".

The inter-communication between the CDNs "S" and "T", upon subjecting the two CDNs to the inputs $H_{dd'}$ and $H_{ba'}$, on the equilibration of the two CDNs is presented in Figure 9. The cleavage of $H_{ba'}$ yields the strand $H_{ba'-1}$ that affects the CDN "S" by enriching the concentrations of AB' and BA' by 32% and 38%, respectively, while the constituents AA' and BB' are down-regulated by 46% and 45%, respectively. The opposite cleavage of $H_{dd'}$ by the DNAzyme activator conjugated to BB' in CDN "S" yields $H_{dd'-1}$ that guides and controls the equilibrium of CDN "T". This is reflected by the up-regulation of the constituents CC' and DD' by 97% and 125%, respectively, and the down-regulation of CD' and DC' by 51% and 51%, respectively. The equilibrated CDNs shown in Figure 9 represent the result of a dynamic transition of the contents of the constituents upon the continuous time-dependent cleavage of the hairpins $H_{dd'}$ and $H_{ba'}$ (24 hours). During this time-interval of the dynamic equilibration of the contents of the constituents, the contents of the constituents are continuously changed, and their time-dependent concentration changes can be probed by following the activities of the DNAzyme reporter units at different time-intervals (the dynamic changes in the concentrations of the constituents are slow as compared to the kinetic assay that probes the concentrations of the constituents). Figures S15 and S16 show the activities of the catalytic reporter units associated with the different constituents of the CDNs "S" and "T" upon the dynamic equilibration of the CDNs. These rates are then translated, using the appropriate calibration curves (Figures S1 and S2), into the time-dependent concentrations of the different constituents at time-intervals of equilibration of the systems, as shown in Figure 10.

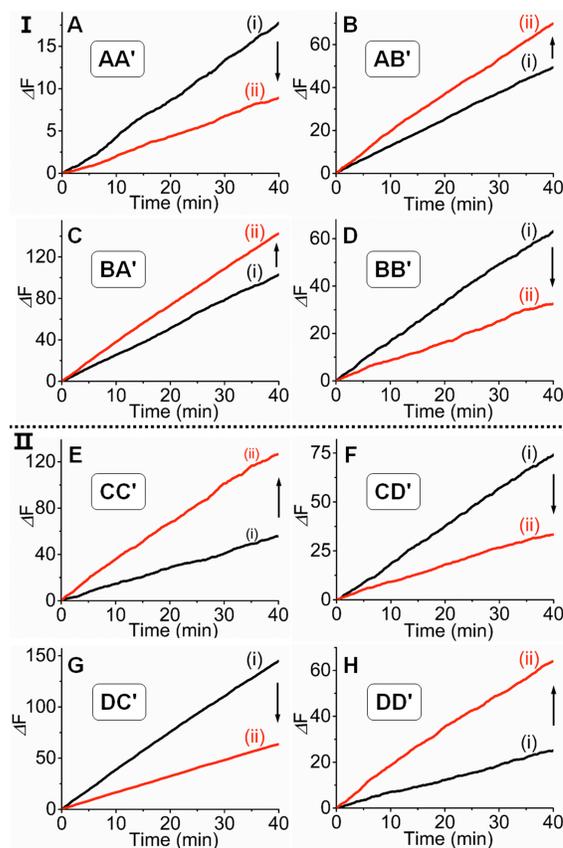


Figure 9. Rates of the cleavage of the respective fluorophore/quencher-modified substrates by the DNAzyme reporter units coupled to the respective constituents before (i) and after (ii) subjecting the two CDNs to the triggers $H_{dd'}$ and $H_{ba'}$ for 24 hours. Panel I: The constituents associated with CDN "S". Panel II: The constituents associated with CDN "T".

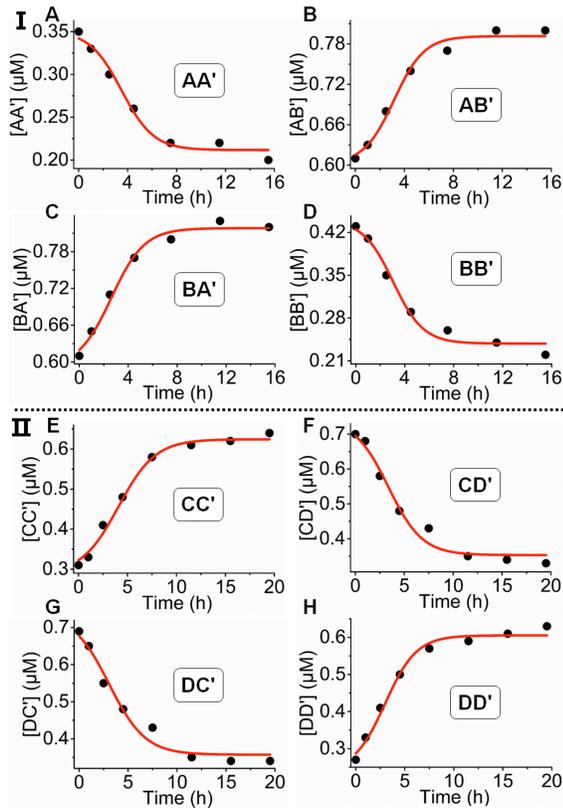


Figure 10. Time-dependent concentration changes of the constituents associated with CDNs “S” and “T” upon the dynamic feedback-driven equilibration of the CDNs in the presence of the triggers $H_{dd'}$ and $H_{ba'}$. Panel I: The constituents of CDN “S”. Panel II: The constituents of CDN “T”.

Table 2 summarizes the concentrations of the different constituents in CDNs “S” and “T” before and after subjecting the CDNs to the input triggers $H_{dd'}$ and $H_{ba'}$ (for a time-interval of 24 hours). The evaluation of the concentrations of the constituents before and after subjecting the CDNs “S” and “T” to $H_{dd'}$ and $H_{ba'}$ were further supported by quantitative electrophoretic experiment. Figure S17 and accompanying discussion present the detailed analysis of the electrophoretic experiment. The derived values of the concentrations of the constituents derived from the electrophoretic experiment are provided in Table 2 (in brackets). Excellent agreement in the evaluated concentrations of the constituents using the two methods is demonstrated.

Table 2. Quantitative assessment of the concentrations of the constituents associated with CDNs “S” and “T” before subjecting the inter-connected CDNs to triggers $H_{dd'}$ and $H_{ba'}$ (i) and after subjecting the CDNs to the triggers for a time-interval of 24 hours (ii).

system	concentration (μM)							
	[AA']	[AB']	[BA']	[BB']	[CC']	[CD']	[DC']	[DD']
(i) ^(a)	0.37	0.62	0.60	0.42	0.32	0.69	0.68	0.28
(i) ^(b)	(0.35)	(0.59)	(0.53)	(0.45)	(0.28)	(0.63)	(0.67)	(0.29)
(ii) ^(a)	0.20	0.82	0.83	0.23	0.63	0.34	0.33	0.63
(ii) ^(b)	(0.16)	(0.83)	(-) ^(c)	(-) ^(c)	(0.55)	(0.24)	(-) ^(c)	(-) ^(c)

^(a)Concentration data provided by the DNAzyme reporter units.

^(b)Concentration data extracted by quantitative analysis of the electrophoretically-separated stained bands. ^(c)Concentrations cannot be evaluated due to the overlap of the bands.

CONCLUSIONS

The study has introduced means to inter-communicate two CDNs consisting of nucleic acid constituents. Besides enhancing the complexity of nucleic acid based CDNs systems by communicating two CDNs, the study has demonstrated the transfer of signals from one network to another, and the signal-guided operation of inter-CDNs feedback mechanisms. These unique features of the artificial CDNs represent bioinspired functionalities of natural networks. These achievements made use of the diverse properties and functions introduced by the base-sequence of nucleic acids that include: (i) The design of nucleic acid constituents that allowed dynamic reconfiguration across different equilibrated networks. (ii) The feasibility to stabilize programmed constituents by auxiliary strands, e.g., stabilization of T-A-T triplex structures. (iii) The conjugation of catalytic nucleic acid units (DNAzymes) to the constituents that allowed the quantitative assessment of the equilibrated concentrations of the constituents before, and after, subjecting the CDNs to the re-equilibration triggers, using the DNAzyme as “reporter” units. (iv) Furthermore, additional DNAzyme units provided activator sites for the functional inter-connection of the CDNs. Specifically, the DNAzyme catalyzed cleavage of hairpin substrates acting as inputs (e.g., $H_{dd'}$ and $H_{aa'}$ or $H_{ba'}$) generated the strands that guided the re-equilibration of the different CDNs. Interestingly, the engineered structures of the two CDNs revealed that the chemical transformation driven by one CDN does not affect the equilibrium of the constituents of its own CDN, but yields a guiding strand that controls the equilibration of the coupled CDN. This property allowed the application of two different hairpins, acting as inputs to drive the continuous dynamic reconfiguration of the two interacting CDNs. We note, however, that we did not reach the maximum complexity from these CDNs. At present, the cleavage of the two hairpins, e.g., $H_{dd'}$ and $H_{aa'}$ or $H_{ba'}$ led to two strands ($H_{dd'-1}$ and $H_{aa'-1}$ or $H_{ba'-1}$) that activated the re-equilibration of the CDNs. The cleavage of the hairpins yields concomitantly two “waste” strands. These strands could trigger two additional independent CDNs, thus further enhancing the diversity and complexity of coupled inter-communicating CDNs, by branching the inter-communication across additional CDNs. Specifically, we may envisage the subsequent use of the “waste” strands to operate autocatalytic cascades of networks. In addition, we demonstrated the possibility to construct kinetic models that simulate computationally the experimental results. These simulations were presented for the individual operations of CDN “S” and CDN “T”. We note, however, that the simulations of the different inter-connected CDNs presented in the paper are beyond the scope of the present report. A comprehensive description of the computational simulations corresponding to the different inter-communicating CDNs will be presented in a forthcoming scientific report. Specifically, we will address the predictive power of such simulations to control the composition of the different CDNs (e.g. by altering the concentration of the constituents) and the experimental verification of the simulated predictions.

EXPERIMENTAL SECTION

Materials. The oligonucleic acid sequences used in the study include:

(1) A: 5'-GATATCAGCGATCAGTAAACACTTTATTTAATTTCTCCTTTCACAAATGA C-3'; (2) B: 5'-CTGCTCAGCGATCAGTAAACACTTCTCTTTTATTTATTTAACAATGACCACCCATGTTCTGTC-3'; (3) A': 5'-GTCATTGTGAATTCCTCTTCTTCTTAATGTTACTGCACCCATGTTACTCT-3'; (4) B': 5'-CAACTCAGCGATGTCATTTGTATTTATTTATTTATTTATTTAATGTTACTGCACCCATGTTCTGTC-3'; (5) C: 5'-GTCCTCAGCGATCTCAAATTGACTTATTATCTTTCCTTTCGTTAAACACCAACCCATGTTGAGTG-3'; (6) D: 5'-CTGTTACGGATCTCAAATTGACTTCTCTTCTTTATTTATTAGTTA

AACAC-3'; (7) C': 5'-GTAGTCAGCGATGTGTTAAACCTTTA TTTATTTATTTATTTCCAATTTGAGCACCCATGTTTCAGT -3'; (8) D': 5'-GIGTTTAAACATTTTCCCTTCTCTCTTACCA ATTGAGCACCATGTTCTCTGA-3'; (9) H_{ddr}: 5'-ATGCAAAG AAGAGACATGACAGATrAGGAGTTGAAACAATCTTCTT TGCATA-3'; (10) H_{aa}: 5'-AGCAAAGGAGAAACATACACTCA TrAGGACTACAAACAATCTCCTTTGCTAT-3'; (11) H_{ba}: 5'-T GCAAGGAAGAAACATACACTCATrAGGAC-TACAAACAAT CTTCTTGG CATA-3'; (12) sub1 (AA'): 5'-FAM-AGAGTATrA GGATATC-BHQ1-3'; (13) sub2 (BB'): 5'-ROX-TGACGATrAG GAGCAG-BHQ2-3'; (14) sub3 (BA'): 5'-CY5-AGAGTATrAGG AGCAG-BHQ2-3'; (15) sub4 (AB'): 5'-CY5.5-TGACGATrAGG ATATC-IBRQ-3'; (16) sub5 (DC'): 5'-FAM-ACTGAATrAGGA ACAG-BHQ1-3'; (17) sub6 (CD'): 5'-ROX-TCAGGATrAGGAG GAC-BHQ2-3'; (18) sub7 (CC'): 5'-CY5-ACTGAATrAGGAGG AC-BHQ2-3'; (19) sub8 (DD'): 5'-CY5.5-TCAGGATrAGGAAC AG-IBRQ-3'; (20) sub1-noFQ (AA'): 5'-AGAGTATrAGGATAT C-3'; (21) sub2-noFQ (BB'): 5'-TGACGATrAGGAGCAG-3'; (22) sub3-noFQ (BA'): 5'-AGAGTATrAGGAGCAG-3'; (23) sub4-noFQ (AB'): 5'-TGACGATrAGGATATC-3'; (24) sub5-noFQ (DC'): 5'-ACTGAATrAGGAACAG-3'; (25) sub6-noFQ (CD'): 5'-TCAGGATrAGGAGGAC-3'; (26) sub7-noFQ (CC'): 5'-ACTGA ATrAGGAGGAC-3'; (27) sub8-noFQ (DD'): 5'-TCAGGATrAG GAACAG-3'; (28) A_m: 5'-GATATCAGCGATCAGTAAACAC TTTATTTAATTTCTCCTTTCCACAAATGACCCACCACCAC ACCACCACCACA-3'; (29) B_m: 5'-CTGCTCAGCGCATCAG TAAACACCTTCTTCTTTTATTTATTTAAACAAATGACCA CCCATGTTCTGTCCCACCACCACCACCACCACCACCACC ACCA-3'; (30) A'_m: 5'-ACCACCACCACCACCACCACCACC CGTCATTTGTAATTTCTTCTTTCTTCTTAATGTTTACTGC ACCCATGTTACTCT-3'; (31) B'_m: 5'-ACCACCACCACCACC ACCACCACCAACTCAGCGATGTCATTTTGTATTTATTTA TTTATTTATTTAATGTTTACTGCACCACCATGTTCTGTC-3'; (32) C_m: 5'-GTCCTCAGCGACTCTCAAATGACTTATTATCT TTCCTTTCCGTTAAACACCACCCATGTTGAGTGCCACC ACCA-3'; (33) H_{ddr-1}: 5'-ATGCAAAGAAGAGACATAGACAG AT-3'; (34) H_{aa-1}: 5'-AGCAAAGGAGAAACATACACTCAT-3'; (35) H_{ba-1}: 5'-TGCAAGGAAGAAACATACACTCAT-3'.

The ribonucleobase cleavage site, rA, in the substrates of the different Mg²⁺-ion-dependent DNazymes is indicated in bold, the respective Mg²⁺-ion-dependent DNazyme sequences are underlined, and the triplex domains associated with the different structures are presented in italic.

Details on the preparation of the CDNs, the quantitative evaluation of the constituents of the CDNs, and details on the quantitative separation of the constituents associated with the different CDNs are provided in the supporting information.

An example for the probing the inter-communications between the CDNs "S" and "T" by catalytic functions of the CDNs. The equilibrated mixture of AA', AB', BA', BB' (CDN "S"), and CC', CD', DC', DD' (CDN "T"), 1000 μL, was subjected to the two hairpins H_{aa}, 100 μL 50 μM, and H_{ba} or H_{bb}, 66.7 μL 50 μM, and 833.3 μL HEPES buffer 10 mM, pH = 7.2, that included MgCl₂, 20 mM, and allowed to equilibrate at 28 °C. At different time, aliquots of 60 μL were withdrawn from the respective mixture, and were treated with the sub1 (12), sub2 (13), sub3 (14), sub4 (15), sub5-noFQ (24), sub6-noFQ (25), sub7-noFQ (26) and sub8-noFQ (27), 3 μL 100 μM each (for CDN "S"), or with the sub5 (16), sub6 (17), sub7 (18), sub8 (19), sub1-noFQ (20), sub2-noFQ (21), sub3-noFQ (22) and sub4-noFQ (23), 3 μL 100 μM each (for CDN "T"). Subsequently, the time-dependent fluorescence changes driven by the cleavage of the different catalytic constituents were followed. Using the appropriate calibration curves corresponding to the rates of cleavage of the different substrates by different concentrations of the intact constituents (see detailed description in Figures S1 and S2), the contents of the constituents in the different CDNs were evaluated.

ASSOCIATED CONTENT

Supporting Information.

Materials and instrumentation, methods, composition of systems, the quantitative evaluation of the contents of the constituents of the CDNs described in Figure 8 by gel electrophoresis, calibration curves, and additional results are provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*willnea@vms.huji.ac.il

Author Contributions

§Both authors contributed equally to this study.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This research is supported by The Minerva Center for Biohybrid Complex Systems.

REFERENCES

- (1). (a) Jaenisch, R.; Bird, A. *Nat. Genet.* **2003**, *33*, 245–254. (b) Vogel, C.; Marcotte, E. M. *Nat. Rev. Genet.* **2012**, *13*, 227–232. (c) Barabási, A. L.; Oltvai, Z. N. *Nat. Rev. Genet.* **2004**, *5*, 101–113.
- (2). (a) Ma'ayan, A.; Jenkins, S. L.; Neves, S.; Hasseldine, A.; Grace, E.; Dubin-Thaler, B.; Eungdamrong, N. J.; Weng, G.; Ram, P. T.; Rice, J. J.; Kershenbaum, A.; Stolovitzky, G. A.; Blitzer, R. D.; Iyengar, R. *Science* **2005**, *309*, 1078–1083. (b) Zhang, Q.; Bhattacharya, S.; Andersen, M. E. *Open Biol.* **2013**, *3*, 130031.
- (3). Bhalla, U. S.; Ram, P. T.; Iyengar, R. *Science* **2002**, *297*, 1018–1023.
- (4). (a) Angeli, D.; Ferrell Jr., J. E.; Sontag, E. D. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 1822–1827. (b) Mangan, S.; Zaslaver, A.; Alon, U. *J. Mol. Biol.* **2003**, *334*, 197–204. (c) Atkinson, M. R.; Savageau, M. A.; Myers, J. T.; Ninfa, A. J. *Cell* **2003**, *113*, 597–607. (d) Yeager-Lotem, E.; Sattath, S.; Kashtan, N.; Itzkovitz, S.; Milo, R.; Pinter, R. Y.; Alon, U.; Margalit, H. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 5934–5939.
- (5). Weber, W.; Fussenegger, M. *Chem Biol.* **2009**, *16*, 287–297.
- (6). Rao, C. V.; Arkin, A. P. *Annu. Rev. Biomed. Eng.* **2001**, *3*, 391–419.
- (7). (a) Lehn, J.-M. *Chem. Soc. Rev.* **2007**, *36*, 151–160. (b) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898–952. (c) Corbett, P. T.; Leclair, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. *Chem. Rev.* **2006**, *106*, 3652–3711. (d) Herrmann, A. *Chem. Soc. Rev.* **2014**, *43*, 1899–1933. (e) Li, J.; Nowak, P.; Otto, S. *J. Am. Chem. Soc.* **2013**, *135*, 9222–9239.
- (8). (a) Belowich, M. E.; Stoddart, J. F. *Chem. Soc. Rev.* **2012**, *41*, 2003–2024. (b) Hunt, R. A. R.; Otto, S. *Chem. Commun.* **2011**, *47*, 847–855.
- (9). (a) Hasenknopf, B.; Lehn, J.-M.; Kneisel, B. O.; Baum, G.; Fensha, D. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1838–1840. (b) Hasenknopf, B.; Lehn, J.-M.; Boumediene, N.; Dupont-Gervais, A.; Van Dorsselaer, A.; Kneisel, B.; Fenske, D. *J. Am. Chem. Soc.* **1997**, *119*, 10956–10962.
- (10). (a) Northrop, B. H.; Aricó, F.; Tangchiavang, N.; Badjić, J. D.; Stoddart, J. F. *Org. Lett.* **2006**, *8*, 3899–3902. (b) Valade, A.; Urban, D.; Beau, J.-M. *ChemBioChem* **2006**, *7*, 1023–1027.
- (11). Lehn, J.-M. *Angew. Chem., Int. Ed.* **2015**, *54*, 3276–3289.
- (12). (a) Ingerman, L. A.; Waters, M. L. *J. Org. Chem.* **2009**, *74*, 111–117. (b) Chaur, M. N.; Collado, D.; Lehn, J.-M. *Chem.—Eur. J.* **2010**, *17*, 248–258.

- (13). Giuseppone, N.; Lehn, J.-M. *Chem.—Eur. J.* **2006**, *12*, 1715–1722.
- (14). Giuseppone, N.; Lehn, J.-M. *Angew. Chem., Int. Ed.* **2006**, *45*, 4619–4624.
- (15). Ramirez, J.; Stadler, A.-M.; Kyritsakas, N.; Lehn, J.-M. *Chem. Commun.* **2007**, 237–239.
- (16). (a) Berl, V.; Huc, I.; Lehn, J.-M.; DeCian, A.; Fischer, J. *Eur. J. Org. Chem.* **1999**, 3089–3094. (b) Au-Yeung, H. Y.; Cougnon, F. B. L.; Otto, S.; Pantoş , G. D.; Sanders, J. K. M. *Chem. Sci.* **2010**, *1*, 567–574.
- (17). Holub, J.; Vantomme, G.; Lehn, J.-M. *J. Am. Chem. Soc.* **2016**, *138*, 11783–11791.
- (18). Vantomme, G.; Jiang, S.; Lehn, J.-M. *J. Am. Chem. Soc.* **2014**, *136*, 9509–9518.
- (19). (a) Giuseppone, N.; Schmitt, J.-L.; Lehn, J.-M. *Angew. Chem., Int. Ed.* **2004**, *43*, 4902–4906. (b) Kovaříček, P.; Lehn, J.-M. *J. Am. Chem. Soc.* **2012**, *134*, 9446–9455.
- (20). (a) Men, G.; Lehn, J.-M. *J. Am. Chem. Soc.* **2017**, *139*, 2474–2483. (b) Hafezi, N.; Lehn, J.-M. *J. Am. Chem. Soc.* **2012**, *134*, 12861–12868.
- (21). Ramström, O.; Lehn, J.-M. *Nat. Rev. Drug. Discovery* **2002**, *1*, 26–36.
- (22). (a) Roche, C.; Percec, V. *Isr. J. Chem.* **2013**, *53*, 30–44. (b) Sreenivasachary, N.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 5938–5943.
- (23). Wang, S.; Yue, L.; Shpilt, Z.; Ceconello, A.; Kahn, J. S.; Lehn, J.-M.; Willner, I. *J. Am. Chem. Soc.* **2017**, *139*, 9662–9671.
- (24). Yue, L.; Wang, S.; Ceconello, A.; Lehn, J.-M.; Willner, I. *ACS Nano* **2017**, *11*, 12027–12036.
- (25). (a) Soloveichik, D.; Seelig, G.; Winfree, E. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 5393–5398. (b) Zhang, D. Y.; Turberfield, A. J.; Yurke, B.; Winfree, E. *Science* **2007**, *318*, 1121–1125. (c) Zhang, D. Y.; Seelig, G. *Nat. Chem.* **2011**, *3*, 103–113.
- (26). Wang, F.; Liu, X.; Willner, I. *Angew. Chem., Int. Ed.* **2015**, *54*, 1098–1129.
- (27). (a) Lu, C.-H.; Qi, X.-J.; Orbach, R.; Yang, H. H.; Mironi-Harpaz, I.; Seliktar, D.; Willner, I. *Nano Lett.* **2013**, *13*, 1298–1302. (b) Aleman-Garcia, M. A.; Orbach, R.; Willner, I. *Chem.—Eur. J.* **2014**, *20*, 5619–5624.
- (28). (a) Miyake, Y.; Togashi, H.; Tashiro, M.; Yamaguchi, H.; Oda, S.; Kudo, M.; Tanaka, Y.; Kondo, Y.; Sawa, R.; Fujimoto, T.; Machinami, T.; Ono, A. *J. Am. Chem. Soc.* **2006**, *128*, 2172–2173. (b) Ono, A.; Cao, S.; Togashi, H.; Tashiro, M.; Fujimoto, T.; Machinami, T.; Oda, S.; Miyake, Y.; Okamoto, I.; Tanaka, Y. *Chem. Commun.* **2008**, 4825–4827. (c) Freeman, R.; Finder, T.; Willner, I. *Angew. Chem., Int. Ed.* **2009**, *48*, 7818–7821. (d) Park, K. S.; Jung, C.; Park, H. G. *Angew. Chem., Int. Ed.* **2010**, *49*, 9757–9760.
- (29). (a) Leitner, D.; Schröder, W.; Weisz, K. *Biochemistry* **2000**, *39*, 5886–5892. (b) Chen, Y.; Lee, S.-H.; Mao, C. *Angew. Chem., Int. Ed.* **2004**, *43*, 5335–5338. (c) Hu, Y.; Ceconello, A.; Idili, A.; Ricci, F.; Willner, I. *Angew. Chem., Int. Ed.* **2017**, *56*, 15210–15233.
- (30). (a) Gehring, K.; Leroy, J.-L.; Guéron, M. *Nature* **1993**, *363*, 561–565. (b) Chen, L.; Cai, L.; Zhang, X.; Rich, A. *Biochemistry* **1994**, *33*, 13540–13546.
- (31). (a) Asanuma, H.; Ito, T.; Yoshida, T.; Liang, X.; Komiyama, M. *Angew. Chem., Int. Ed.* **1999**, *38*, 2393–2395. (b) Asanuma, H.; Liang, X.; Yoshida, T.; Komiyama, M. *ChemBioChem* **2001**, *2*, 39–44. (c) Kamiya, Y.; Asanuma, H. *Acc. Chem. Res.* **2014**, *47*, 1663–1672. (d) Meng, F.-N.; Li, Z.-Y.; Ying, Y.-L.; Liu, S.-C.; Zhang, J.; Long, Y.-T. *Chem. Commun.* **2017**, *53*, 9462–9465.
- (32). (a) Osborne, S. E.; Matsumura, I.; Ellington, A. D. *Curr. Opin. Chem. Biol.* **1997**, *1*, 5–9. (b) Famulok, M.; Mayer, G. *Acc. Chem. Res.* **2011**, *44*, 1349–1358.
33. (a) Joyce, G. F. *Annu. Rev. Biochem.* **2004**, *73*, 791–836. (b) Famulok, M.; Hartig, J. S.; Mayer, G. *Chem. Rev.* **2007**, *107*, 3715–3743. (c) Willner, I.; Shlyahovsky, B.; Zayats, M.; Willner, B. *Chem. Soc. Rev.* **2008**, *37*, 1153–1165.

TOC graphic

