Supporting Information

Enzyme-Guided Selection and Cascaded Emergence of Nanostructured Constitutional Dynamic Networks

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Materials and Instrumentation

*Eco*RI-High Fidelity restriction enzyme (20,000 units mL^{-1}), *Hin*dIII-High Fidelity restriction enzyme (100,000 units mL^{-1}) and 10× CutSmart® Buffer (1× Buffer Components: 50 mM Potassium Acetate, 20 mM Tris-acetate, 10 mM Magnesium Acetate, 100 μg mL^{-1} BSA, pH 7.9@25°C) were purchased from New England BioLabs Inc.. Acrylamide/bis-acrylamide, 19:1, 40% solution, was purchased from Sigma-Aldrich. "GelRed nucleic acid gel stain" was purchased from Invitrogen. DNA oligonucleotides were purchased from Integrated DNA Technologies Inc. (Coralville, IA). Ultrapure water from NANOpure Diamond (Barnstead) source was used in all of the experiments. Fluorescence spectra were recorded with a Cary Eclipse Fluorometer (Varian Inc.). Gel electrophoresis was performed on a Hoefer SE 600 electrophoresis unit.

The oligonucleic acid sequences used in the study are the following:

(**1**) A: 5'-CTGTTCAGCGATAAAA***GAATTC***AAAAGTCCACCCATGTTCGTCA-3'

(**2**) A': 5'-CTGCTCAGCGATGACTTTTGTTTTATCAGAACAG-3'

(**3**) $H_{BB'}$: 5'-GTCCTCAGCGATAAAAGAATAAAACAAAAGTCCACCCATGTTACTCTTGACGAT**r**

AGGAGCAGTTTTGTTT***GAATTC***TT TTCACCCATGTTTCAGT-3'

(**4**) Am': 5'-CTGCTCAGCGATGACTTTTGTTTTATCAGAACAGTTATTATTATTATTATTATT-3'

(**5**) $H_{CC'}$: 5'-CCATTCAGCGATAAACAATAAATCAATATCCGAGGACTAGTATGAAGAGTAT**rA**G

GAGCAGTTGTAAGTCCTCAGCGA TATT***AAGCTT***ATTGTTTCACCCATGTTTCAGT-3'

(**6**) $H_{DD'}$: 5'-CCATTCAGCGATATTAAGCCATTGATTTATTGTTTCACCCATGTTTAGCTAGAAGA

GTAT**rA**GGAGCAGATAAATAAATCAAT***AAGCTT***AATCACCCATGTTCCTGA-3'

(**7**) E: 5'-CTGCTCAGCGATCCATTCAGCGATAAA***GAATTC***AAAGTACACACACAATAGAAAAA

TCAATATCAGAGGAC-3'

(**8**) F: 5'-CTGTTCAGCGATAAAGAATAAACAAAGAATAAAAAAATAGAAAAATCAATATCAGA GGACCACCCATGTTTAGCT-3'

(**9**) G: 5'-GTCCTCAGCGATAAAGAATAAACAAAGAAAAAAATAAATAAAATCAA***AAGCTT***AA TCACCCATGTTTCAGT-3'

(**10**) E': 5'-CTGTTCAGCGATGTCCTCAGCGATATT***AAGCTT***TTCTAATCATATCTACACTTTGTT TATCAGAATGGCACCCATGTTACTCT-3'

(**11**) F': 5'-ATTAAGCATTGATTTTTCACTAACTATAATTTGTT***GAATTC***TTTCACCCATGTTCCT GA-3'

(**12**) G': 5'-CCATTCAGCGATGTCCTCTGTTCAGCGATATTAAGCATTGATTTTTACACTACACA CTTTGTTTATCAGAATGGCACCCATGTTCGTCA-3'

(**13**) sub1 (AA' and EG'): 5'-ROX-TGACGAT**rA**GGAGCAG-BHQ2-3';

(**14**) sub2 (AB' and GG'): 5'-FAM-ACTGAAT**rA**GGAACAG-BHQ1-3';

(**15**) sub3 (BA' and EE'): 5'-Cy5-AGAGTAT**rA**GGAGCAG-BHQ2-3';

(**16**) sub4 (BB' and GE'): 5'-Cy5-ACTGAAT**rA**GGAGGAC-BHQ2-3';

(**17**) sub5 (CC'): 5'-ROX-ACTGAAT**rA**GGAATGG-BHQ2-3';

(**18**) sub6 (CD' and GF'): 5'-ROX-TCAGGAT**rA**GGAGGAC-BHQ2-3';

(**19**) sub7 (DC' and FG'): 5'-FAM-AGCTAAT**rA**GGAATGG-BHQ1-3';

(**20**) sub8 (DD' and EF'): 5'-ROX-TCAGGAT**rA**GGAATGG-BHQ2-3';

(**21**) sub9 (FE'): 5'-Cy5-AGCTAAT**rA**GGAACAG-BHQ2-3';

(**22**) sub10 (FF'): 5'-Cy5.5-TCAGGAT**rA**GGAACAG-IBRQ-3';

(**23**) sub1-noFQ (AA' and EG'): 5'-TGACGAT**rA**GGAGCAG-3';

(**24**) sub2-noFQ (AB' and GG'): 5'-ACTGAAT**rA**GGAACAG-3';

(**25**) sub3-noFQ (BA' and EE'): 5'-AGAGTAT**rA**GGAGCAG-3';

(**26**) sub4-noFQ (BB' and GE'): 5'-ACTGAAT**rA**GGAGGAC-3';

(**27**) sub5-noFQ (CC'): 5'-ACTGAAT**rA**GGAATGG-3';

(**28**) sub6-noFQ (CD' and GF'): 5'-TCAGGAT**rA**GGAGGAC-3';

(**29**) sub7-noFQ (DC' and FG'): 5'-AGCTAAT**rA**GGAATGG-3';

(**30**) sub8-noFQ (DD' and EF'): 5'-TCAGGAT**rA**GGAATGG-3';

(**31**) sub9-noFQ (FE'): 5'-AGCTAAT**rA**GGAACAG-3';

(**32**) sub10-noFQ (FF'): 5'-TCAGGAT**rA**GGAACAG-3';

The ribonucleobase cleavage site, **rA**, in the substrates of the different Mg^{2+}-ion-dependent DNAzymes is indicated in bold, the respective Mg^{2+}-ion-dependent DNAzyme sequences are underlined, and the sequence domains cut by the restriction enzymes are italic and in bold.

Measurements

The excitations of FAM, ROX, Cy5 and Cy5.5 were performed at 496, 588, 648 and 685 nm, respectively. The emissions of FAM, ROX, Cy5 and Cy5.5 were recorded at 516, 608, 668 and 706 nm, respectively.

Methods

Preparation of hairpins $H_{BB'}$, $H_{CC'}$, $H_{DD'}$. Taking $H_{BB'}$ as an example: $H_{BB'}$, 50 μM, in the prepared 1× CutSmart® Buffer which does not contain BSA, was annealed at 75 °C for 10 min, cooled down quickly to 25 °C, and allowed to equilibrate at 25 °C for 2 h.

Probing the CDN systems using the DNAzyme reporter units. For the CDN "X" system, a mixture of A and A', 10 μM each, in 1× CutSmart® Buffer, was annealed at 40 °C for 10 min and then allowed to equilibrate at 25 °C for 2 h to yield constituent AA'. The prepared AA' (200 μL) was subjected to hairpin $H_{BB'}$, 40 μL of 50 μM, and 760 μL 1× CutSmart® Buffer, and allowed to equilibrate at 28 °C for 24 h to yield CDN "X". The resulting CDN "X" (300 μL) was treated with 10 μL *Eco*RI and 290 μL 1× CutSmart® Buffer, annealed for 20 cycles at 40 °C for 1 min, then at 25 °C for 1 min and finally at 37 °C for 10 min, and allowed to equilibrate at 25 °C for 2 h to select BA'. Aliquots of 60 μL were withdrawn from the respective solutions and treated with sub1, sub2-noFQ, sub3-noFQ and sub4-noFQ (for AA'), or with sub1-noFQ, sub2, sub3-noFQ and sub4-noFQ (for AB'), or with sub1-noFQ, sub2-noFQ, sub3 and sub4-noFQ (for BA'), or with sub1-noFQ, sub2-noFQ, sub3-noFQ and sub4 (for BB'), 3 μL of 100 μM each. Subsequently, the time-dependent fluorescence changes were followed (catalytic rate = d(*Δ*F)/dt). Using the appropriate calibration curves corresponding to the cleavage rates of the different substrates by the isolated constituents at various concentrations (Figures S3 and S4), we quantified the concentrations of the constituents in the different states.

For the CDN "Y" system, the selected constituent BA' generated upon the treatment of CDN "X" with *Eco*RI was prepared (3 μM of 700 μL) according to the above procedures, and then subjected to hairpins $H_{CC'}$ and $H_{DD'}$, 42 μL of 50 μM each, and 266 μL 1× CutSmart® Buffer, and allowed to equilibrate at 28 °C for 24 h to emerge CDN "Y". The generated CDN "Y" (300 μL) was treated with 20 μL *Hin*dIII and 280 μL 1× CutSmart® Buffer, annealed for 20 cycles at 40 °C for 1 min, then at 25 °C for 1 min and finally at 37 °C for 10 min, and allowed to equilibrate at 25 °C for 2 h to select DC'. Aliquots of 60 μL were withdrawn from the respective solutions and treated with sub5, sub6-noFQ, sub7-noFQ and sub8-noFQ (for CC'), or with sub5-noFQ, sub6, sub7-noFQ and sub8-noFQ (for CD'),

or with sub5-noFQ, sub6-noFQ, sub7 and sub8-noFQ (for DC'), or with sub5-noFQ, sub6-noFQ, sub7-noFQ and sub8 (for DD'), 3 μL of 100 μM each. Subsequently, the time-dependent fluorescence changes were followed (catalytic rate = d(*Δ*F)/dt). Using the appropriate calibration curves corresponding to the cleavage rates of the different substrates by the isolated constituents at various concentrations (Figures S6 and S7), we quantified the concentrations of the constituents in the different states.

For the hierarchical transformation of CDN "Z" into CDN "Z_E", and then into constituent FG', a mixture of E, F, G, E', F' and G', 2 μM each, in 1× CutSmart® Buffer, was annealed at 40 °C and then allowed to equilibrate at 25 °C for 2 h to yield CDN "Z". The prepared CDN "Z" (600 μL) was treated with 20 μL *Eco*RI and 540 μL 1× CutSmart® Buffer, annealed for 20 cycles at 40 °C for 1 min, then at 25 °C for 1 min and finally at 37 °C for 10 min, and allowed to equilibrate at 25 °C for 2 h to select CDN "Z_E". The survived CDN "Z_E" was subjected to 40 μL *Hin*dIII, annealed for 20 cycles at 40 °C for 1 min, then at 25 °C for 1 min and finally at 37 °C for 10 min, and allowed to equilibrate at 25 °C for 2 h to select FG'. For the hierarchical transformation of CDN "Z" into CDN "Z_H", and then into constituent FG', the prepared CDN "Z" (600 μL) was treated with 40 μL *Hin*dIII and 540 μL 1× CutSmart® Buffer, annealed for 20 cycles at 40 °C for 1 min, then at 25 °C for 1 min and finally at 37 °C for 10 min, and allowed to equilibrate at 25 °C for 2 h to yield CDN "Z_H". The selected CDN "Z_H" was subjected to 20 μL *Eco*RI, annealed for 20 cycles at 40 °C for 1 min, then at 25 °C for 1 min and finally at 37 °C for 10 min, and allowed to equilibrate at 25 °C for 2 h to select FG'. Aliquots of 60 μL were withdrawn from the respective solutions and treated with sub3, sub8-noFQ, sub1-noFQ, sub9-noFQ, sub10-noFQ, sub7-noFQ, sub4-noFQ, sub6-noFQ and sub2-noFQ (for EE'), or with sub3-noFQ, sub8, sub1-noFQ, sub9-noFQ, sub10-noFQ, sub7-noFQ, sub4-noFQ, sub6-noFQ and

sub2-noFQ (for EF'), or with sub3-noFQ, sub8-noFQ, sub1, sub9-noFQ, sub10-noFQ, sub7-noFQ, sub4-noFQ, sub6-noFQ and sub2-noFQ (for EG'), or with sub3-noFQ, sub8-noFQ, sub1-noFQ, sub9, sub10-noFQ, sub7-noFQ, sub4-noFQ, sub6-noFQ and sub2-noFQ (for FE'), or with sub3-noFQ, sub8-noFQ, sub1-noFQ, sub9-noFQ, sub10, sub7-noFQ, sub4-noFQ, sub6-noFQ and sub2-noFQ (for FF'), or with sub3-noFQ, sub8-noFQ, sub1-noFQ, sub9-noFQ, sub10-noFQ, sub7, sub4-noFQ, sub6-noFQ and sub2-noFQ (for FG'), or with sub3-noFQ, sub8-noFQ, sub1-noFQ, sub9-noFQ, sub10-noFQ, sub7-noFQ, sub4, sub6-noFQ and sub2-noFQ (for GE'), or with sub3-noFQ, sub8-noFQ, sub1-noFQ, sub9-noFQ, sub10-noFQ, sub7-noFQ, sub4-noFQ, sub6 and sub2-noFQ (for GF'), or with sub3-noFQ, sub8-noFQ, sub1-noFQ, sub9-noFQ, sub10-noFQ, sub7-noFQ, sub4-noFQ, sub6-noFQ and sub2 (for GG'), 3 μL of 100 μM each. Subsequently, the time-dependent fluorescence changes were followed (catalytic rate = d(*Δ*F)/dt). Using the appropriate calibration curves corresponding to the cleavage rates of the different substrates by the isolated constituents at various concentrations (Figures S9 and S10), we quantified the concentrations of the constituents in the different states.

Quantitative evaluation of the concentrations of the constituents of the CDN systems by gel electrophoresis. Native polyacrylamide gel electrophoresis experiments were performed using the polyacrylamide gels (12%, acrylamide/bis-acrylamide 19:1) with a gel thickness of 1.5 mm. The samples were separated upon applying a potential of 100 V at 15 °C for 40 h for the CDN systems.

The separation of the CDN “X” system is taken as an example. The samples before (t = 0 h) and after (t = 24 h) the formation of CDN “X”, and after the treatment of the resulting CDN “X” with *Eco*RI and the selection of constituent BA' were loaded in the gel and the individual constituents AA', AB', BA', BB', and $H_{BB'}$ at identical concentrations, 1.0 μM, were loaded as references in predefined

lanes (Note that in order to enhance the separation of the mixture, modified A' (A'_m) instead of A' was used, whereas the modified tethers do not participate in the equilibrium of the network). The separated bands of the systems were stained with GelRed nucleic acid gel stain and the intensities of the different bands were quantitatively analyzed by the ImageJ software by comparing the intensities of the respective separated bands to the intensities generated by the individual reference constituents at the known concentrations (1 μM).

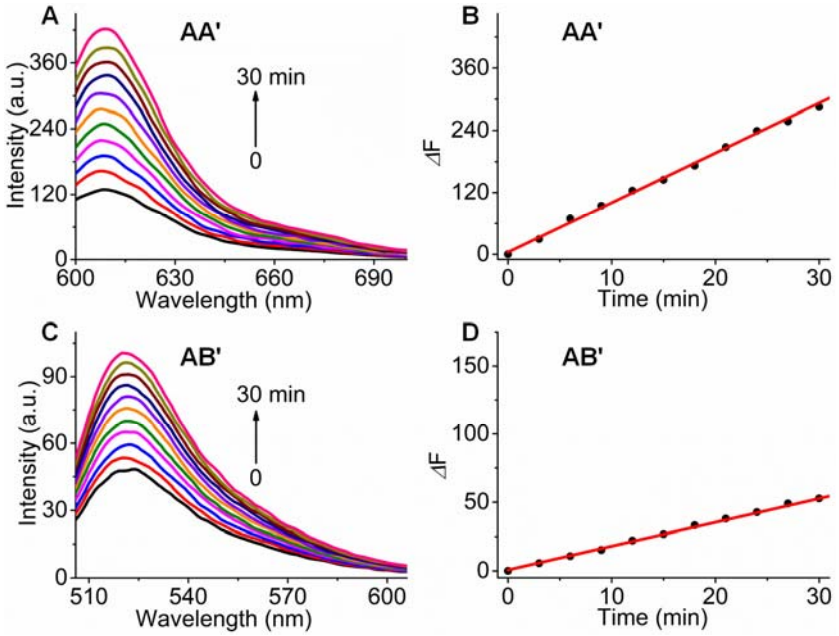


Figure S1. (A) Time-dependent fluorescence spectra of the fluorophore-modified fragmented strand of the substrate generated upon the catalytic cleavage of the corresponding fluorophore/quencher-functionalized substrate by the Mg^{2+}-ion-dependent DNAzyme reporter unit associated with constituent AA' in CDN "X". (B) Time-dependent fluorescence changes generated by the Mg^{2+}-ion-dependent DNAzyme reporter unit associated with constituent AA' in CDN "X" derived from the data shown in (A). (C) Time-dependent fluorescence spectra of the fluorophore-modified fragmented strand of the substrate generated upon the catalytic cleavage of the corresponding fluorophore/quencher-modified substrate by the Mg^{2+}-ion-dependent DNAzyme reporter unit associated with constituent AB' in CDN "X". (D) Time-dependent fluorescence changes generated by the Mg^{2+}-ion-dependent DNAzyme reporter unit associated with constituent AB' in CDN "X" derived from the data shown in (C).

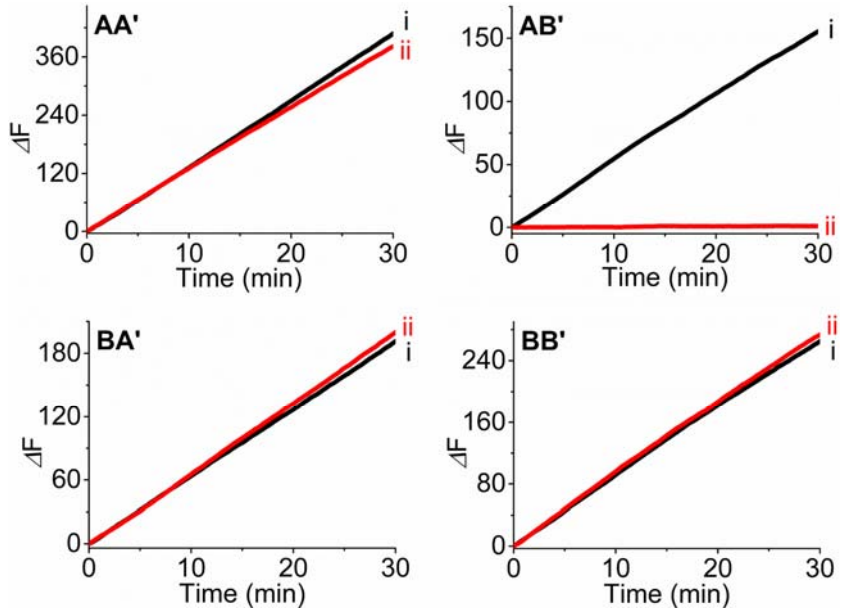


Figure S2. Time-dependent fluorescence changes generated by the Mg^{2+}-ion-dependent DNAzyme reporter units associated with intact constituents AA', AB', BA' and BB' at a concentration of 1 μM, respectively: (i) Before the treatment of the intact constituents with endonuclease *Eco*RI. (ii) After subjecting the intact constituents to endonuclease *Eco*RI (0.33 U μL^{-1}), annealing for 20 cycles at 40 °C for 1 min, then at 25 °C for 1 min and finally at 37 °C for 10 min, and equilibrating at 25 °C for 2 h.

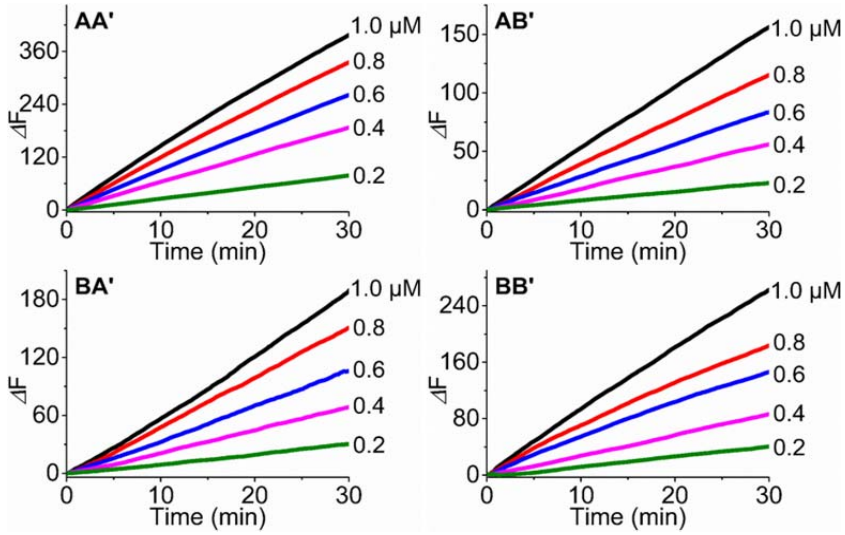


Figure S3. Time-dependent fluorescence changes generated from the cleavage of the fluorophore/quencher-modified substrates by the respective DNAzyme reporter units associated with the individual constituents of CDN "X" at variable concentrations. It should be noted that hairpin $H_{BB'}$ reveals similar catalytic activity as that of constituent BB'.

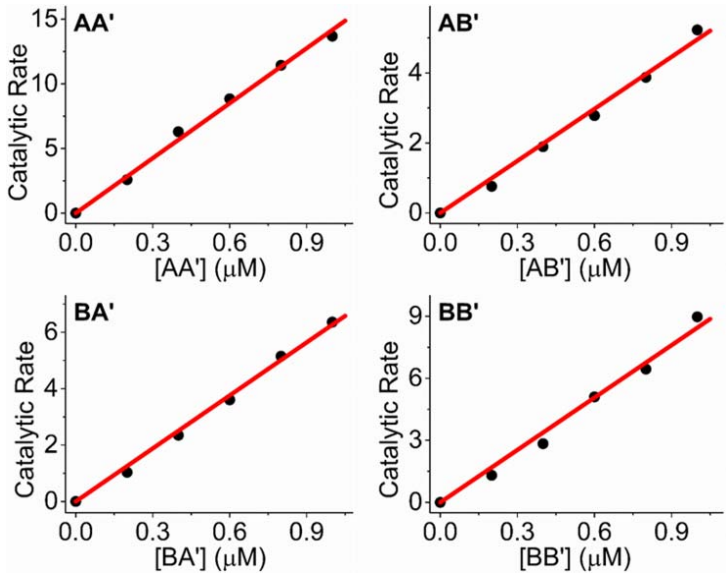
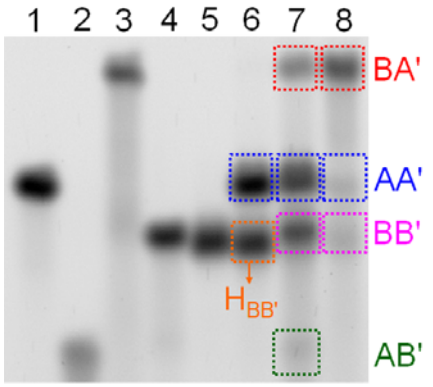


Figure S4. Corresponding calibration curves of the catalytic rates of the constituents of CDN "X" as a function of their concentrations, derived from the data shown in Figure S3.



	Lane							
	1	2	3	4	5	6	7	8
BA'	-	-	5336	-	-	118	1717	5211
AA'	8065	-	-	-	-	7973	5886	583
BB'	-	-	-	6553	-	-	4904	607
$H_{BB'}$	-	-	-	-	6734	6571	-	-
AB'	-	3866	-	-	-	0	918	0

Figure S5. Electrophoretic quantitative separation of the constituents involved in the emergence and selection processes associated with the CDN "X" system. Lanes 1–5, control bands corresponding to the individual structures: Lane 1 – AA'; Lane 2 – AB'; Lane 3 – BA'; Lane 4 – BB'; Lane 5 – $H_{BB'}$. Lane 6 – Separated bands observed upon mixing AA' with $H_{BB'}$ at time t = 0 h. Lane 7 – Separated bands associated with the constituents of CDN "X" generated upon subjecting AA' to $H_{BB'}$ for 24 h. Lane 8 – Separated bands corresponding to the constituents after the *Eco*RI-stimulated selection of BA' from CDN "X". The right table presents the software-integrated intensities of the color-developed images of the bands, used to quantitatively evaluate the contents of the separated constituents.

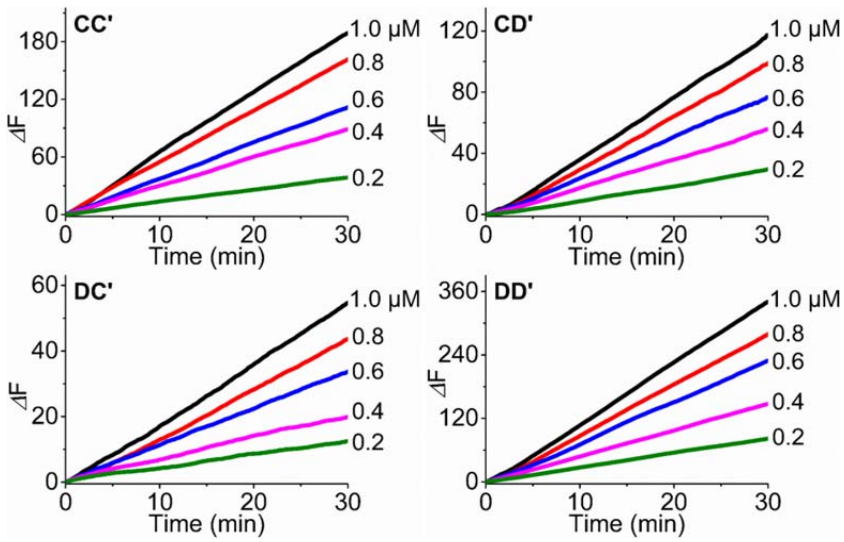


Figure S6. Time-dependent fluorescence changes generated from the cleavage of the fluorophore/quencher-modified substrates by the respective DNAzyme reporter units associated with the individual constituents of CDN "Y" at variable concentrations. It should be noted that hairpins $H_{CC'}$ and $H_{DD'}$ reveal similar catalytic activities as those of constituents CC' and DD', respectively.

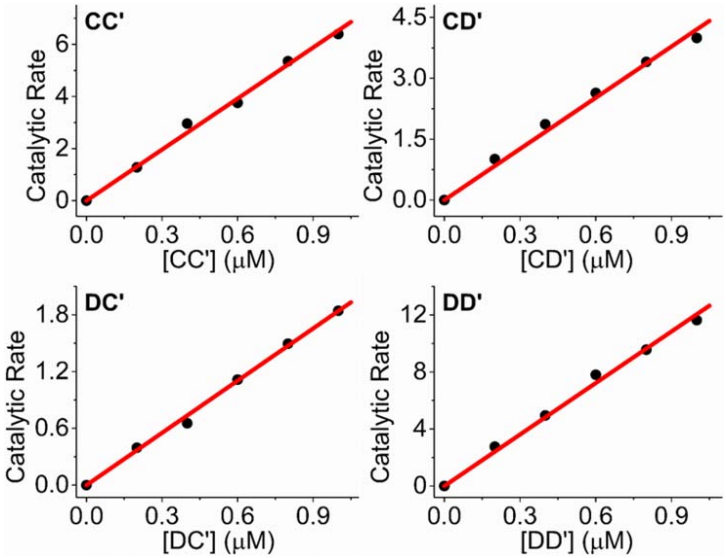


Figure S7. Corresponding calibration curves of the catalytic rates of the constituents of CDN "Y" as a function of their concentrations, derived from the data shown in Figure S6.

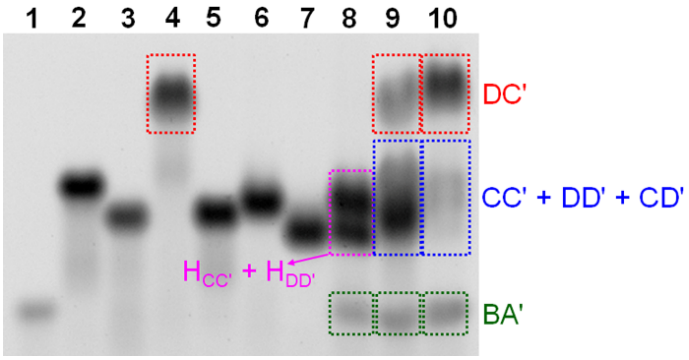


Figure S8. Electrophoretic separation of the constituents involved in the cascaded emergence and selection processes associated with the CDN "Y" system. Lanes 1–7, control bands corresponding to the individual structures: Lane 1 – BA'; Lane 2 – CC'; Lane 3 – CD'; Lane 4 – DC'; Lane 5 – DD'; Lane 6 – $H_{CC'}$; Lane 7 – $H_{DD'}$. Lane 8 – Separated bands observed upon treatment of the survived BA' with hairpins $H_{CC'}$ and $H_{DD'}$ at time t = 0 h. Lane 9 – Separated bands associated with the constituents of CDN "Y" generated upon subjecting the survived BA' to $H_{CC'}$ and $H_{DD'}$ for 24 h. Lane 10 – Separated bands corresponding to the constituents after the *Hin*dIII-induced survival of DC' from CDN "Y". Comparison of the separated bands in lanes 8 to 10 to the reference bands provided in lanes 1–7 allows us to draw the following conclusions: (i) Upon the survived BA' with hairpins $H_{CC'}$ and $H_{DD'}$ at time t = 0 h (lane 8), no band of constituent DC' and only two bands corresponding to $H_{CC'}$ and $H_{DD'}$ are observed; (ii) Upon subjecting the survived BA' to $H_{CC'}$ and $H_{DD'}$ for 24 h (lane 9), a band corresponding to constituent DC' is observed, and using the ImageJ software to compare the intensity of the band to that of the individual reference constituent at the known concentration (1 μM), the concentration of DC' is evaluated to be 0.40 μM; (iii) After the *Hin*dIII-induced survival of DC' from CDN "Y" (lane 10), an intensified band corresponding to DC' is observed with the concentration of 0.94 μM, quantified by the ImageJ software, and the intensities of the bands corresponding to CC', CD' and DD' are very low as compared to those in CDN "Y" (lane 9). These results are qualitatively consistent with the results derived from the catalytic functions of the DNAzyme reporter units.

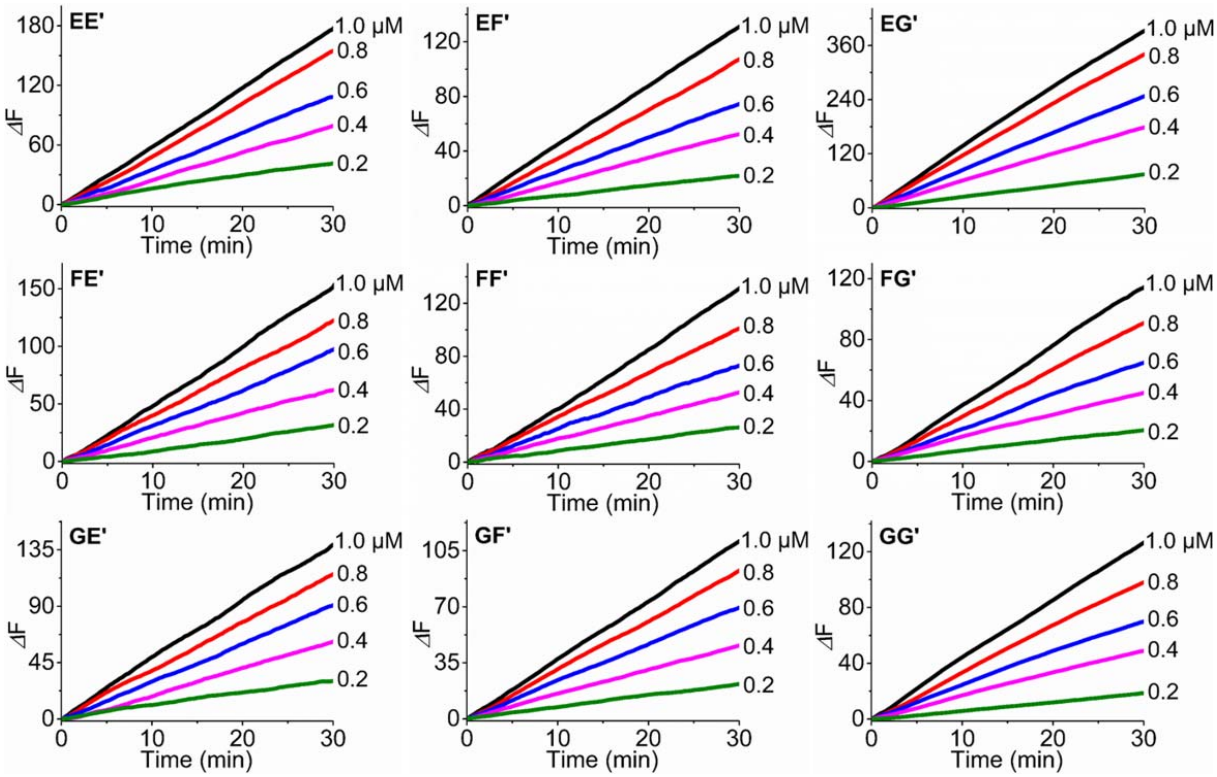


Figure S9. Time-dependent fluorescence changes generated from the cleavage of the fluorophore/quencher-modified substrates by the respective DNAzyme reporter units associated with the individual constituents of CDN "Z" at variable concentrations.

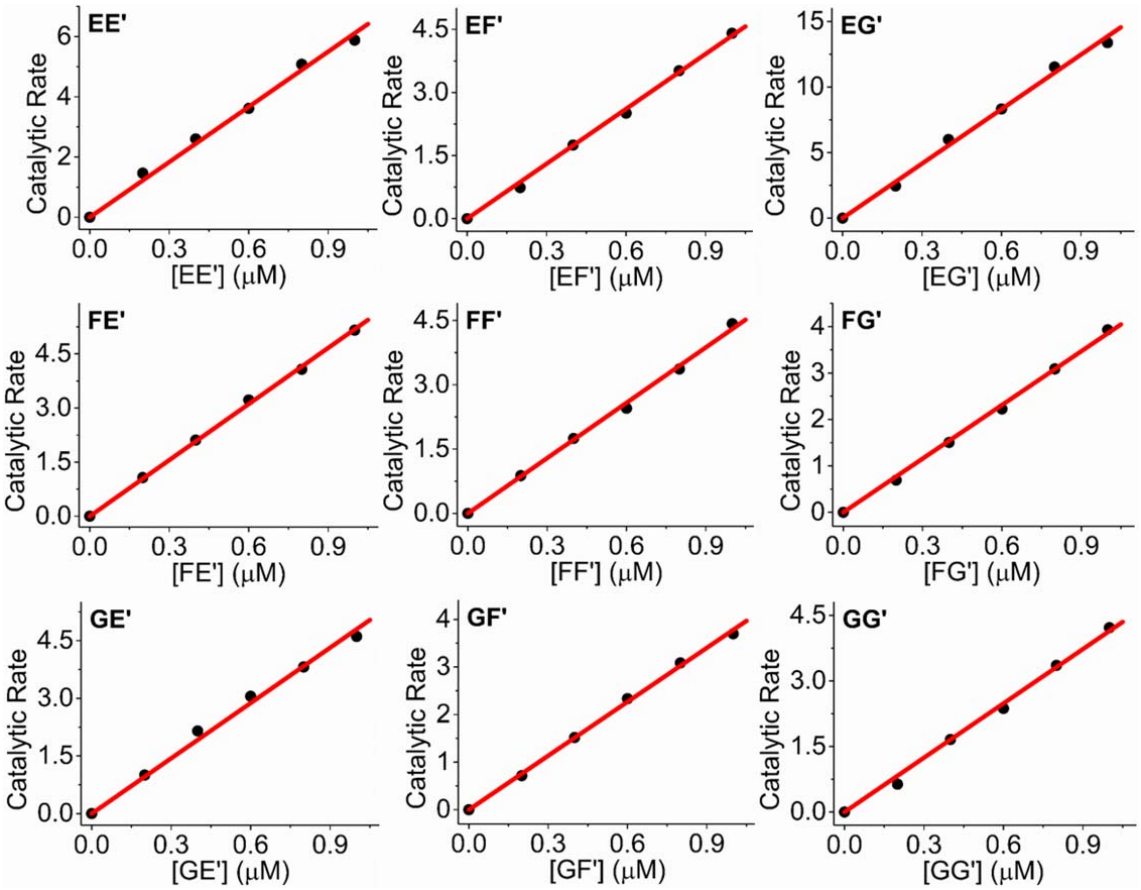


Figure S10. Corresponding calibration curves of the catalytic rates of the constituents of CDN "Z" as a function of their concentrations, derived from the data shown in Figure S9.

Table S1. Concentrations of the constituents involved in the emergence and selection processes associated with the CDN "X" system: (i) Before (t = 0 h) and (ii) after (t = 24 h) the formation of CDN "X"; (iii) After treatment of the resulting CDN "X" with *Eco*RI to stimulate the survival of BA'.

	Concentration (μM)			
	[AA']	[AB']	[BA']	[BB']
i[a]	0.97 ± 0.04	0.02 ± 0.01	0.04 ± 0.02	0.98 ± 0.04
i[b]	(0.99)	(0)	(0.02)	(0.98)
ii[a]	0.68 ± 0.03	0.32 ± 0.02	0.30 ± 0.03	0.69 ± 0.03
ii[b]	(0.73)	(0.24)	(0.32)	(0.75)
iii[a]	0.05 ± 0.01	0.01 ± 0.01	0.94 ± 0.04	0.09 ± 0.02
iii[b]	(0.07)	(0)	(0.98)	(0.09)

[a]Data provided by the catalytic DNAzyme reporter units. [b]Data provided by quantitative gel electrophoresis. Standard deviations were derived from $N = 3$ experiments.

Table S2. Concentrations of the constituents included in the cascaded emergence and selection processes associated with the CDN "Y" system: (i) Before (t = 0 h) and (ii) after (t = 24 h) the emergence of CDN "Y"; (iii) After subjecting the resulting CDN "Y" to *Hin*dIII to stimulate the selection of DC'.[a]

	Concentration (μM)			
	[CC']	[CD']	[DC']	[DD']
i	0.96 ± 0.04	0.04 ± 0.01	0.06 ± 0.01	0.97 ± 0.04
ii	0.65 ± 0.03	0.34 ± 0.03	0.36 ± 0.02	0.66 ± 0.02
iii	0.13 ± 0.02	0.01 ± 0.01	0.90 ± 0.03	0.10 ± 0.01

[a]Standard deviations were derived from $N = 3$ experiments.

Table S3. Concentrations of the constituents involved in the hierarchical selection processes associated with the [3×3] CDN system: (i) CDN "Z"; (ii) After treatment of CDN "Z" with *Eco*RI to stimulate the survival of [2×2] CDN "Z_E"; (iii) After subjecting the resulting CDN "Z_E" to *Hin*dIII to induce the selection of constituent FG'; (iv) After treatment of CDN "Z" with *Hin*dIII to stimulate the selection of [2×2] CDN "Z_H"; (v) After subjecting the resulting CDN "Z_H" to *Eco*RI to induce the selection of constituent FG'.[a]

	Concentration (μM)								
	[EE']	[EF']	[EG']	[FE']	[FF']	[FG']	[GE']	[GF']	[GG']
i	0.42 ± 0.03	0.24 ± 0.02	0.33 ± 0.03	0.30 ± 0.02	0.31 ± 0.02	0.42 ± 0.03	0.33 ± 0.02	0.40 ± 0.04	0.26 ± 0.02
ii	0.04 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.28 ± 0.03	0.06 ± 0.01	0.64 ± 0.03	0.60 ± 0.03	0.08 ± 0.02	0.27 ± 0.03
iii	0.09 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.88 ± 0.04	0.03 ± 0.01	0.15 ± 0.03	0.11 ± 0.02
iv	0.12 ± 0.02	0.63 ± 0.04	0.29 ± 0.02	0.07 ± 0.01	0.27 ± 0.02	0.62 ± 0.03	0.03 ± 0.01	0.16 ± 0.03	0.06 ± 0.01
v	0.09 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.86 ± 0.03	0.04 ± 0.01	0.12 ± 0.02	0.13 ± 0.02

[a]Standard deviations were derived from $N = 3$ experiments.