

Two-Component Dendritic Chain Reactions: Experiment and Theory

Eran Sella,[†] Ariel Lubelski,[‡] Joseph Klafter,[‡] and Doron Shabat^{*,†}*Department of Organic Chemistry and Department of Chemical Physics, School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel*

Received December 23, 2009; E-mail: chdoron@post.tau.ac.il

Abstract: New analytical diagnostic techniques that are based on signal-amplification mechanisms could significantly improve the sensitivity of detection of various analytes. We have developed a new approach to achieving exponential amplification of a diagnostic signal through a two-component dendritic chain reaction. The chain reaction generated the analyte of interest and thereby initiated additional diagnostic cycles. The system was designed for the detection of hydrogen peroxide and produced significantly larger intensity of diagnostic signal than a classic probe. In addition, a mathematical model that simulates the disassembly kinetics of one-component and two-component reactions was developed and shown to correlate well with the observed experimental data. The modularity and flexibility of a two-component detection system should allow extension to the detection of other analytes.

Introduction

The autoamplification mechanism is a well-known approach to achieving exponential growth of a desired target. Thus far, there are only a few examples of the use of exponential amplification methods to produce multiple copies of a target molecule. The most familiar is the polymerase chain reaction (PCR); this technique is utilized in molecular biology to amplify DNA exponentially by making copies of a specific region of a nucleic acid target.¹ Other examples include PCR-based techniques such as immuno-PCR,^{2,3} the amplification of an enantiomeric excess of chiral molecules,⁴ and self-replicating systems.⁵ Recently, two novel related approaches to achieving exponential amplification of diagnostic signals for the detection of specific analytes were reported in the literature. The first, developed by the Mirkin group, uses a cascade reaction involving a supramolecular allosteric catalyst that generates the analyte of interest.⁶ The generated analyte initiates additional reaction cycles and, consequently, produces exponential progress. The second approach, developed by our group, applies a distinctive dendritic chain reaction (DCR) that releases several reagent molecules upon reaction with an analyte.⁷ When the reagent molecules are free, they acquire the chemical reactivity of the analyte and therefore initiate additional reaction cycles

leading to exponential growth of the diagnostic signal.⁸ The DCR probe used in our report was based on an AB₃ self-immolative dendron, which is equipped with a trigger, one reporter, and two reagent end groups (Figure 1). Cleavage of the trigger by the analyte generates the release of one chromogenic reporter and two reagent molecules. The two free reagents then activate two additional dendrons by cleavage of their triggers. The process progresses exponentially until all dendrons have been disassembled. The preparation of the DCR probe demanded multistep synthesis with selective incorporation of the reagent units and the reporter on the dendritic platform.

A simpler option to obtaining DCR amplification would be to use the reagent component and the reporter component in the form of two different molecules (Figure 2). The first component (I) is based on an AB₂ self-immolative dendron⁹ equipped with two reagent units and a trigger designed to react with a specific analyte. The second component (II) is a probe composed of the same trigger attached to a reporter. The AB₂ self-immolative dendron acts as an amplifier moiety, and the other component acts as a probe that releases a chromogenic molecule to produce a diagnostic signal. The two-component dendritic chain reaction (2CDCR) mode of action is illustrated in Figure 2. An analyte molecule activates the trigger of the AB₂ self-immolative dendron component to release two reagent units. As a consequence of their release, the free reagents gain the chemical reactivity of the analyte of interest. Some of the reagent molecules now activate the triggers of additional AB₂ self-immolative dendrons, and some react with probe components to release reporter units. The process will progress until all of the dendritic molecules are disassembled. If more than two reagent units are released per molecule of analyte, then the signal obtained by the free reporter will grow exponentially. Here we report a simple approach to exponential signal

[†] Department of Organic Chemistry.[‡] Department of Chemical Physics.

- (1) Saiki, R. K.; Gelfand, D. H.; Stoffel, S.; Scharf, S. J.; Higuchi, R.; Horn, G. T.; Mullis, K. B.; Erlich, H. A. *Science* **1988**, *239*, 487–491.
- (2) Niemeyer, C. M.; Adler, M.; Wacker, R. *Trends Biotechnol.* **2005**, *23*, 208–216.
- (3) Sano, T.; Smith, C. L.; Cantor, C. R. *Science* **1992**, *258*, 120–122.
- (4) Soai, K.; Shibata, T.; Sato, I. *Acc. Chem. Res.* **2000**, *33*, 382–390.
- (5) Patzke, V.; von Kiedrowski, G. *ARKIVOC* **2007**, 293–310.
- (6) Yoon, H. J.; Mirkin, C. A. *J. Am. Chem. Soc.* **2008**, *130*, 11590–11591.
- (7) Sella, E.; Shabat, D. *J. Am. Chem. Soc.* **2009**, *131*, 9934–9936.

(8) Swiderska, M. A.; Reymond, J. L. *Nat. Chem.* **2009**, *1*, 527–528.(9) Erez, R.; Shabat, D. *Org. Biomol. Chem.* **2008**, *6*, 2669–2672.

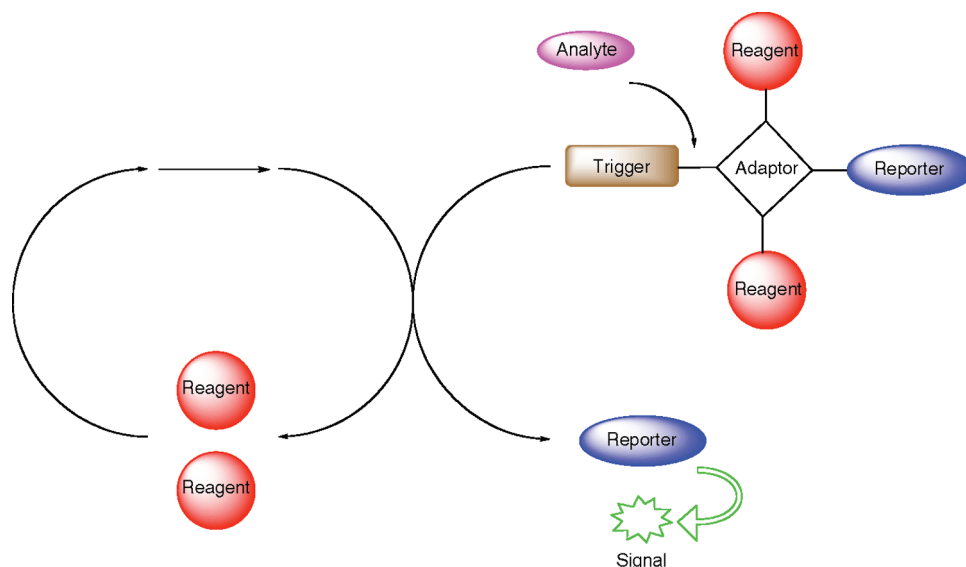


Figure 1. Amplification cycle produced by a dendritic chain reaction with an AB₃ self-immolative dendron.

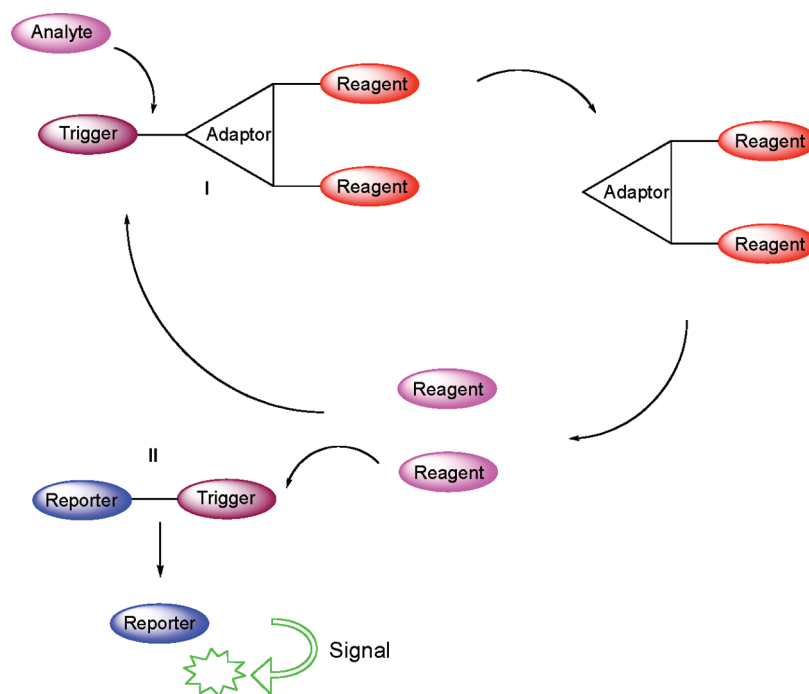


Figure 2. Graphical illustration of a two-component dendritic chain reaction.

amplification based on a two-component dendritic chain reaction and describe a mathematical model of the kinetic disassembly behavior observed.

Results

On the basis of the above illustration, we designed self-immolative AB₂ dendron **1** to detect the analyte, hydrogen peroxide, and probe **2** containing a reporter unit (Figure 3). Dendron **1** was composed of two choline units and phenylboronic acid as a trigger, which is cleaved upon reaction with hydrogen peroxide.¹⁰ Probe **2** is composed of the 5-amino-2-nitrobenzoic acid reporter attached to the phenylboronic acid trigger. The two-component DCR amplification cycle of dendron

1 and probe **2** is illustrated in Figure 3. Cleavage of the trigger of dendron **1** by a hydrogen peroxide molecule will generate the release of the two choline molecules. The two free choline units will be oxidized by choline oxidase (COX) present in solution to produce four molecules of hydrogen peroxide that then activate two AB₂ dendrons and two probe molecules. Choline is initially oxidized to betain aldehyde and then to betain; each step produces one molecule of hydrogen peroxide. The rate of disassembly should exponentially increase until all of the reporter molecules have been released. The signal can be detected with a spectrophotometer by monitoring the yellow color of the released 5-amino-2-nitrobenzoic acid.

Self-immolative AB₂ dendron **1** was synthesized as described in Scheme 1. Phenolic dendritic core **1b** was prepared as

(10) Lo, L.-C.; Chu, C.-Y. *Chem. Commun.* **2003**, 2728–2729.

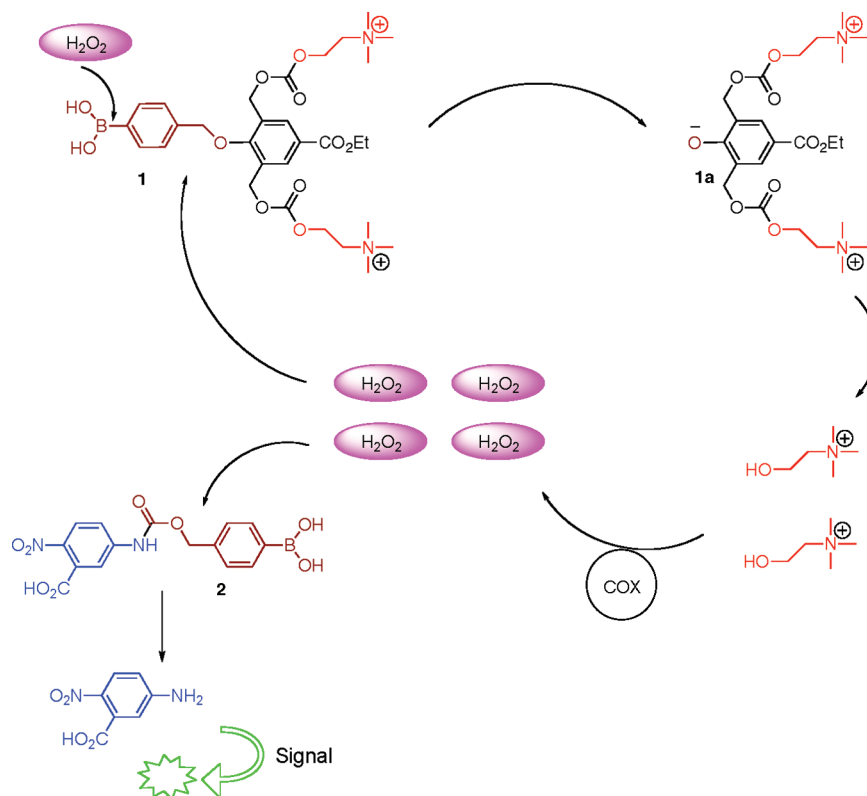
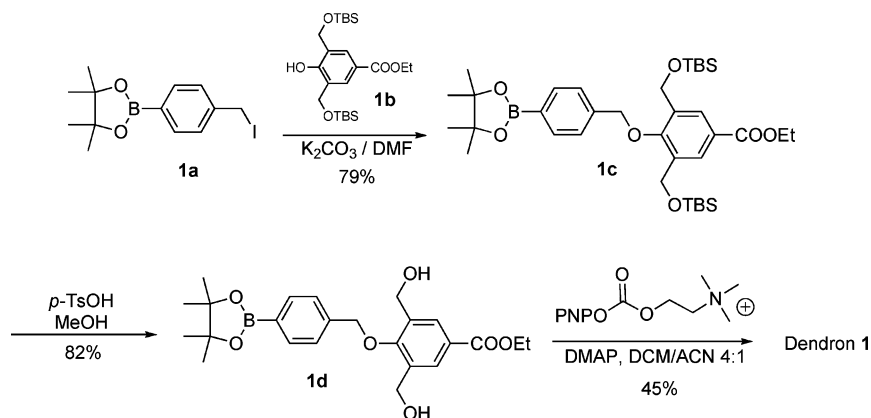


Figure 3. Two-component DCR system to detect hydrogen peroxide. The choline reagent units and 5-amino-2-nitrobenzoic acid reporter are indicated.

Scheme 1. Chemical Synthesis of Dendron 1



previously described.¹¹ Etherification of the phenol group of **1b** by benzyl iodide **1a** and potassium carbonate generated ether **1c**. The latter was deprotected with *p*-TsOH to give diol **1d**, which was further treated with 2 equiv of *p*-nitrophenyl-choline carbonate in the presence of DMAP to afford dendron **1**.

The synthesis of probe **2** was performed using the synthesis strategy shown in Scheme 2. Commercially available 5-amino-2-nitrobenzoic acid was protected with allyl alcohol to give ester **2a**. The reaction of **2a** with triphosgene generated the isocyanate derivative, which was immediately reacted with alcohol **2b** to afford carbamate **2c**. Deprotection of the allyl ester by Pd(PPh₃)₄ in the presence of benzylamine produced desired probe **2**.

To evaluate the two-component DCR technique, dendron **1** and probe **2** were incubated with various amounts of hydrogen peroxide in the presence of COX, and the release of the 5-amino-

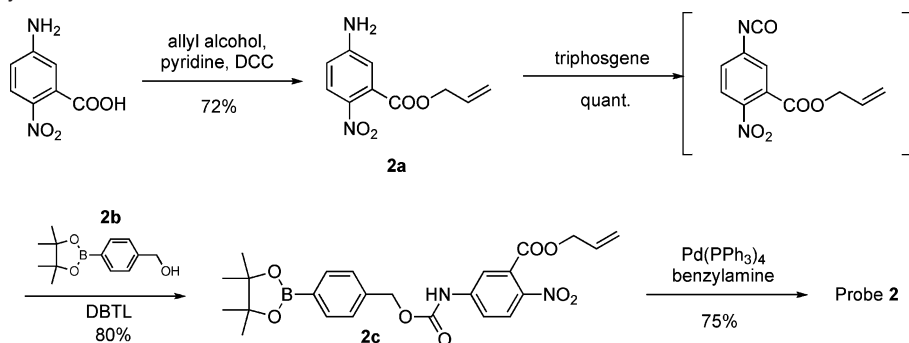
2-nitro-benzoic acid reporter was monitored at a wavelength of 405 nm (Figure 4).

When 1.0 equiv of hydrogen peroxide (vs dendron **1**) was used, the system reached complete disassembly within 30 min. As expected, disassembly was slower when less hydrogen peroxide was used (Figure 4). The exponential progress of the system disassembly is demonstrated by the sigmoidal plots obtained for reaction with various equivalents of H₂O₂. The background signal obtained because of spontaneous hydrolysis is also amplified; therefore, the sensitivity of this system is limited, and detection of the analyte can be observed down to 5 μM H₂O₂.

Next, we evaluated the 2CDCR system disassembly using various ratios of dendron **1** versus probe **2**. The results are presented in Figure 5. When a high ratio of dendron **1** versus probe **2** was used (2:1), system disassembly occurred rapidly

(11) Haba, K.; Popkov, M.; Shamis, M.; Lerner, R. A.; Barbas, C. F., III; Shabat, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 716–720.

Scheme 2. Chemical Synthesis of Probe 2



(within 60 min). At ratios of dendron **1** versus probe **2** of 1:1 and 1:2, disassembly was slower (100 and 200 min, respectively).

The diagnostic activity of probe **2** is based on the release of a chromogenic molecule. In principle, it is possible to use other probes in the 2CDCR technique that have different modes of action. As a representative example, we tested a fluorogenic probe composed of phenylboronic acid (as a trigger for hydrogen peroxide) linked to 7-hydroxycoumarin **4a**. Upon reaction with

hydrogen peroxide, probe **4** releases free 7-hydroxycoumarin that can be detected using fluorescence spectroscopy (Scheme 3).

Probe **4** was evaluated together with dendron **1** in the 2CDCR assay for the detection of hydrogen peroxide. An exponential increase in the fluorogenic signal was observed in a manner similar to that obtained with probe **2** (data not shown). The increased sensitivity of the 2CDCR technique is best demonstrated when the background noise is subtracted from the measured signal. In Figure 6, we presented this data for probes **2** and **4**. The net signal obtained using the 2CDCR approach with probes **2** and **4** (for 0.01 equiv of hydrogen peroxide per probe) in the presence of dendron **1** is significantly larger than that observed in the absence of dendron **1**.

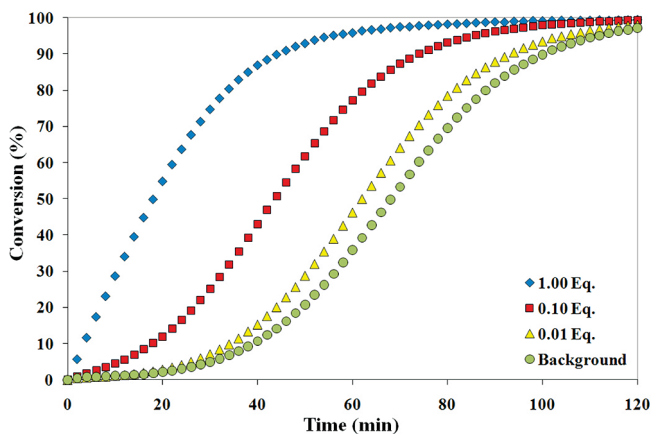


Figure 4. Release of 5-amino-2-nitrobenzoic acid from probe **2** (500 μ M) in the presence of dendron **1** (500 μ M) and choline oxidase (0.3 mg/mL) in PBS (pH 7.4) upon addition of buffer only (background) or the indicated equivalents of H_2O_2 . The reaction progress was monitored at 405 nm for the indicated time period.

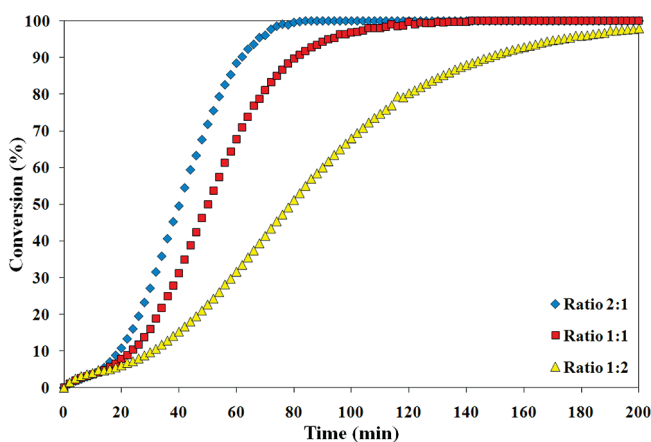


Figure 5. Release of 5-amino-2-nitrobenzoic acid from probe **2** in the presence of choline oxidase upon addition of H_2O_2 (0.01 equiv vs probe **2**) when various ratios of dendron **1** and probe **2** are applied. The reaction progress was monitored at 405 nm for the indicated time period.

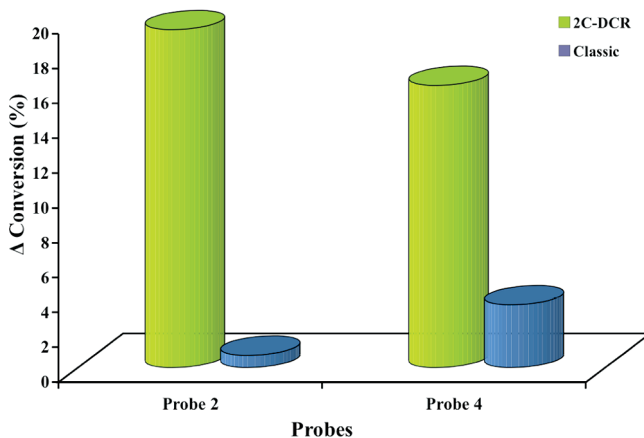


Figure 6. Comparison of signals measured (at a time point of 50 min) in the presence of dendron **1** (green) vs signals measured in the absence of dendron **1** (blue). The background signal observed at 50 min was subtracted from the measured values.

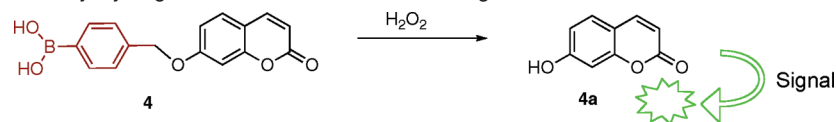
Theoretical Studies

To analyze the one-component DCR and the two-component DCR disassembly behavior, we composed mathematical equations that describe the kinetics of both systems. Figure 7 shows the chemical structure of dendron **3**, used in the DCR, and the chemical structures of dendron **1** and probe **2**, used in 2CDCR approach.

Initially, we modeled the kinetics of the one-component dendritic chain reaction obtained by the disassembly of molecules such as dendron **3**



where AB_2X is the dendritic molecule that produces the chain reaction, X is the reporter molecule whose concentration can be measured using a spectrophotometer, B is the reagent

Scheme 3. Activation of Probe **4** by Hydrogen Peroxide to Release a Fluorogenic Molecule

molecule (in our case, the choline molecule), and n_D is the number of hydrogen peroxide molecules that are generated per one molecule of dendron **3**. DCR probe **3** is composed of two choline molecules, a reporter molecule, and a trigger that reacts with hydrogen peroxide. This reaction imitates the disassembly of the dendritic molecule. The chemical reaction in eq 1 represents the net reaction equation (the whole process involving the reaction of choline with the COX enzyme that generates two hydrogen peroxide molecules).

We described the reaction in eq 1 as a simple first-order reaction:

$$\frac{d[AB_2X]}{dt} = -K[AB_2X][H_2O_2] \quad (2)$$

The relatively complicated reaction sequence is simplified and characterized by a single kinetic constant (K) because the oxidation step by COX is relatively fast and thus does not affect the reaction between the dendritic probe and hydrogen peroxide.

The following equations are valid at all times during the course of the reaction

$$[AB_2X] + [X] = [AB_2X]_0 \quad (3)$$

$$[H_2O_2] = [H_2O_2]_0 + (n_D - 1)([AB_2X]_0 - [AB_2X]) \quad (4)$$

where $[H_2O_2]_0$ and $[AB_2X]_0$ are the initial concentrations of hydrogen peroxide and the dendritic probe, respectively, and $[AB_2X]$, $[X]$, and $[H_2O_2]$ are the concentrations of the dendritic probe, the reporter molecule, and hydrogen peroxide, respectively, as functions of time.

We substitute eq 4 into eq 2 to obtain a simple differential equation

$$\frac{d[AB_2X]}{dt} = -K[AB_2X]([H_2O_2]_0 + (n_D - 1)([AB_2X]_0 - [AB_2X])) \quad (5)$$

that can be rewritten as

$$\frac{d[AB_2X]}{dt} = -K[AB_2X](b - a[AB_2X]) \quad (6)$$

where $a = n_D - 1$ and $b = [H_2O_2]_0 + (n_D - 1)[AB_2X]_0$. By integrating eq 6, we obtain the following result:

$$[AB_2X] = \left([AB_2X]_0 + \frac{[H_2O_2]_0}{n_D - 1} \right) \times \left(\frac{[AB_2X]_0}{[AB_2X]_0 + \frac{[H_2O_2]_0}{n_D - 1} \exp(Kbt)} \right) \quad (7)$$

This result is valid only for circumstances when the initial concentration of hydrogen peroxide ($[H_2O_2]_0$) is not zero. From

eq 7 combined with eq 3, we obtain the concentration of the released reporter molecule as a function of time:

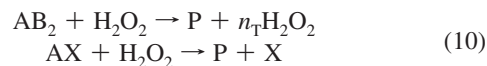
$$[X] = [AB_2X]_0 - \left([AB_2X]_0 + \frac{[H_2O_2]_0}{n_D - 1} \right) \times \left(\frac{[AB_2X]_0}{[AB_2X]_0 + \frac{[H_2O_2]_0}{n_D - 1} \exp(Kbt)} \right) \quad (8)$$

The signal that we obtained in the experiment is the normalized reporter concentration percentage, meaning the reporter concentration from eq 8 divided by the reporter concentration at very long times multiplied by 100. This limit of long reaction time can be easily obtained from eq 8, where the reporter concentration reaches $[X] = [AB_2X]_0$ and the experimental signal takes the form

$$S_D(t) = \frac{100[H_2O_2]_0(1 + \exp(-Kbt))}{[H_2O_2]_0 + (n_D - 1)[AB_2X]_0 \exp(-Kbt)} \quad (9)$$

Figure 8 shows the obtained correlations between the calculated signal function from eq 9 to the experimental data for various concentrations of hydrogen peroxide.

Next we modeled the kinetics of the two-component dendritic chain reaction, which can be described by the following pair of chemical equations



where AB_2 is a dendritic molecule composed of a trigger and a reagent molecule (choline) such as compound **1**, AX is a molecule composed of a trigger and a reporter part such as probe **2**, n_T is the number of hydrogen peroxide molecules that are generated per molecule of AB_2 using the two-component dendritic system, and P represents all the other products irrelevant to the kinetic model. Here, as in the previous case, we can write an equation that describes the dependency among the concentrations:

$$[H_2O_2] - [H_2O_2]_0 = (n_T - 1)([AB_2]_0 - [AB_2]) - ([AX]_0 - [AX]) \quad (11)$$

We rewrite this equation to obtain the concentration of hydrogen peroxide

$$[H_2O_2] = a + [AX] - (n_T - 1)[AB_2] \quad (12)$$

where a contains all of the constants:

$$a = (n_T - 1)[AB_2]_0 - [AX]_0 + [H_2O_2]_0 \quad (13)$$

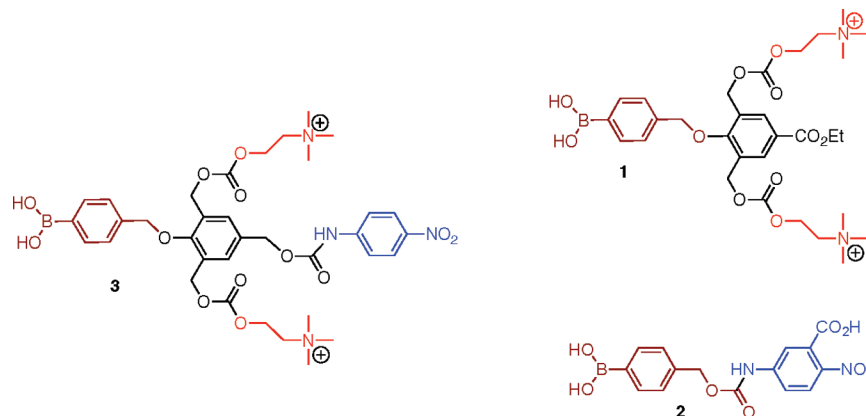


Figure 7. Chemical structures of DCR probe dendron **3**, dendron **1**, and probe **2**.

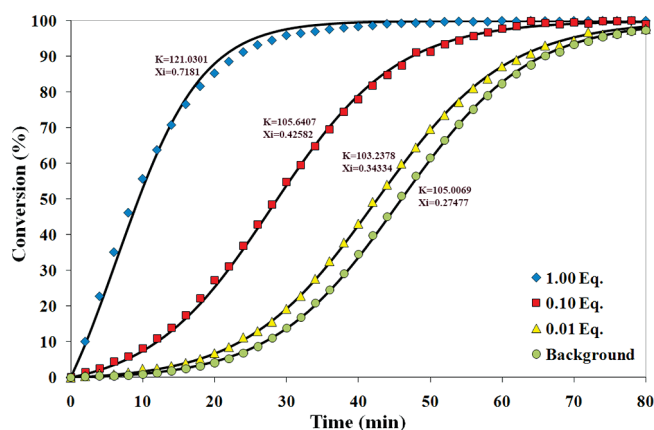


Figure 8. Release of 4-nitroaniline from probe **3** under DCR conditions. Correlation between calculated values obtained from eq 9 (solid line) and experimental data observed by the disassembly behavior of DCR probe **3** (dotted plot). Concentrations were 350 μ M for probe **3** and 0.3 mg/mL for choline oxidase in PBS (pH 7.4). Hydrogen peroxide concentrations were 350, 35, 3.5, and 0 μ M. From these correlations, we obtained the kinetic constant (K). Xi is a measure of the fit (square deviation). The background experiment was correlated numerically using a different procedure than the correlation to eq 9 because eq 9 is valid only for an initial hydrogen peroxide concentration other than zero.

We then formulate simple kinetic equations for the two reactants (AB_2 and AX):

$$\begin{aligned}\frac{d[\text{AB}_2]}{dt} &= -K[\text{AB}_2][\text{H}_2\text{O}_2] \\ \frac{d[\text{AX}]}{dt} &= -K[\text{AX}][\text{H}_2\text{O}_2]\end{aligned}\quad (14)$$

It is important to note that these two equations use the same kinetic constant (K) because the reaction of hydrogen peroxide with the trigger is identical. (The relatively fast enzymatic step that is included in the first reaction is assumed not to change the kinetic constant.) By multiplying the first equation by $(n_T - 1)$ and subtracting the second equation from the first, we obtain the following equation:

$$\frac{d([\text{AX}] - (n_T - 1)[\text{AB}_2])}{dt} = -K([\text{AX}] - (n_T - 1)[\text{AB}_2])[\text{H}_2\text{O}_2] \quad (15)$$

We substitute eq 12 into eq 15 and obtain

$$\frac{d([\text{AX}] - (n_T - 1)[\text{AB}_2])}{dt} = -K([\text{AX}] - (n_T - 1)[\text{AB}_2])(a + [\text{AX}] - (n_T - 1)[\text{AB}_2]) \quad (16)$$

By marking

$$y = [\text{AX}] - (n_T - 1)[\text{AB}_2] \quad (17)$$

and substituting it into eq 16, we obtain a simple differential equation

$$\frac{dy}{dt} = -Ky(a + y) \quad (18)$$

that resembles eq 6. By solving this equation, we obtain

$$y(t) = [\text{AX}] - (n_T - 1)[\text{AB}_2] = \frac{ay_0 \exp(-Kat)}{y_0 + a - y_0 \exp(-Kat)} \quad (19)$$

The next step is to obtain the concentrations of $[\text{AX}]$ and $[\text{AB}_2]$ as functions of time. We return to kinetic eq 14 and divide each equation by $[\text{AX}]$ and $[\text{AB}_2]$, respectively, to obtain

$$\begin{aligned}\frac{d[\text{AB}_2]}{[\text{AB}_2]} &= -K[\text{H}_2\text{O}_2] dt \\ \frac{d[\text{AX}]}{[\text{AX}]} &= -K[\text{H}_2\text{O}_2] dt\end{aligned}\quad (20)$$

By integrating these two equations, we obtain

$$\begin{aligned}\log\left(\frac{[\text{AB}_2]}{[\text{AB}_2]_0}\right) &= -\int_0^t K[\text{H}_2\text{O}_2] dt \\ \log\left(\frac{[\text{AX}]}{[\text{AX}]_0}\right) &= -\int_0^t K[\text{H}_2\text{O}_2] dt\end{aligned}\quad (21)$$

From eq 21, we observe that

$$\frac{[\text{AB}_2]}{[\text{AB}_2]_0} = \frac{[\text{AX}]}{[\text{AX}]_0} \quad (22)$$

We rewrite eq 22 as

$$[AB_2] = \frac{[AB_2]_0}{[AX]_0} [AX] \quad (23)$$

By substituting eq 23 into eq 19, we obtain

$$[AX] = \frac{((n_T - 1)[AB_2]_0 - [AX]_0 + [H_2O_2]_0)[AX]_0 \exp(-Kat)}{[H_2O_2]_0 - ([AX]_0 - (n_T - 1)[AB_2]_0) \exp(-Kat)} \quad (24)$$

The next step is to calculate the reporter concentration as a function of time. We use the following equation, which is valid at all times:

$$[AX] + [X] = [AX]_0 \quad (25)$$

By combining with eq 24, dividing by the reporter concentration at long times, and multiplying by 100, we finally obtain the signal value, which is the normalized concentration of the reporter:

$$S_T(t) = \frac{100[H_2O_2]_0(1 - \exp(-Kat))}{[H_2O_2]_0 - ([AX]_0 - (n_T - 1)[AB_2]_0) \exp(-Kat)} \quad (26)$$

Figure 9 shows correlations of the calculated signals obtained from eq 26 versus the measured experimental data for different ratios of dendron **1** and probe **2**.

Discussion

Most signal-amplification methods are based on a linear increase in a measurable signal.^{12–17} In contrast, the described approach progresses through an exponential pathway. The two-component DCR approach is based on disassembly mechanisms of a dendritic amplifier moiety and a chromogenic probe.^{18–21} Both are equipped with identical triggering systems designed for activation by a specific analyte. The amplifier component releases reagent units that acquire the reactivity of the analyte, thereby initiating amplification cycles, and the probe component generates a chromogenic signal. The synthesis of this two-component amplification system was rather simple in comparison with synthesis of our initial DCR system. In addition, the two-component system provides additional flexibility. Once the amplifier component is in hand, the diagnostic assay can be performed in combination with different types of probe molecules. We demonstrated this versatility by the synthesis and use of two different probe molecules. The first was based on the chromogenic reporter, 5-amino-2-nitrobenzoic acid, which generates a visible spectroscopic signal when its amine functional group is free. The second probe contained 7-hydroxycoumarin, which produces a fluorogenic spectroscopic

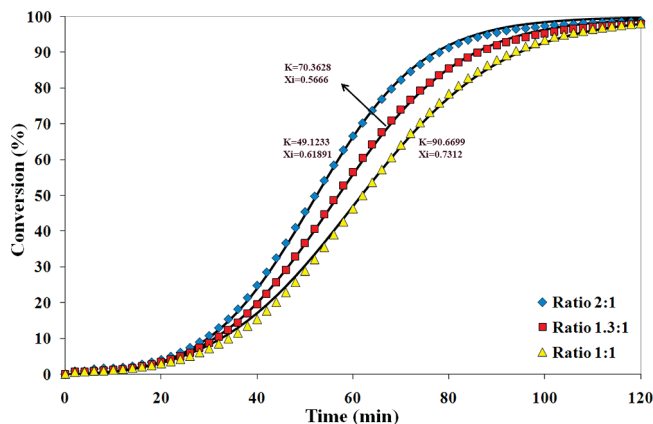


Figure 9. Correlations among calculated values obtained from eq 26 (solid line) and the experimentally measured disassembly behavior of dendron **1** and probe **2** (dotted plot) for various ratios of dendron **1** vs probe **2**. The various correlations provided the kinetic constant (K). χ_i is the value of the fit quality (square deviation).

signal upon its release. Another important feature of the 2CDCR system is the option to use various ratios of the two components. This option allows control of the amplification rate as demonstrated by our experiments using different ratios of dendron **1** and probe **2** (Figure 5).

The two-component DCR approach offers considerable advantages compared to diagnostic assays based on the stoichiometric reaction between the analyte and the probe. In the reaction demonstrated in this study, analyte, hydrogen peroxide, and a phenylboronic acid-based probe react stoichiometrically. As demonstrated in Figure 6, the net signal obtained for the two-component DCR assay is significantly larger than the signal produced by analogous classic probe behavior.

Currently, the two factors that limit the detection sensitivity of the 2CDCR assay are the minor amount of spontaneous hydrolysis of dendritic compound **1** and its disassembly rate. To prepare an ideal dendritic compound, one should design a molecule that is very stable to spontaneous hydrolysis yet can undergo rapid disassembly upon relation to the analyte. This assignment should be possible because the 2CDCR system is modular. Various reporter molecules may be used, as demonstrated, and any analyte of interest that has a cleavage reactivity toward a specific trigger could be incorporated. This modularity should allow the design of an ideal system. Once an efficient system is in hand, the 2CDCR-based assay could be extended to the detection of other analytes and biocatalysts by coupling it with another probe activity. This option was previously demonstrated with the one-component DCR approach for the detection of the biocatalytic activity of enzyme penicillin-G-amidase.⁷

We were able to develop simple mathematical equations that model the kinetic behavior of the one-component and two-component dendritic chain reactions. The calculated plots obtained from the equations showed good to excellent correlations with the experimental results. This mathematical model should assist in predicting the kinetic disassembly of other DCR systems once the individual constants are calculated and introduced into the corresponding equations.

Conclusions

We have demonstrated a new approach to achieving exponential amplification of a diagnostic signal through a two-component dendritic chain reaction. The amplification

- (12) Masar, M. S., III; Gianneschi, N. C.; Oliveri, C. G.; Stern, C. L.; Nguyen, S. T.; Mirkin, C. A. *J. Am. Chem. Soc.* **2007**, *129*, 10149–10158.
- (13) Nam, J. M.; Thaxton, C. S.; Mirkin, C. A. *Science* **2003**, *301*, 1884–1886.
- (14) Sagi, A.; Weinstein, R.; Karton, N.; Shabat, D. *J. Am. Chem. Soc.* **2008**, *130*, 5434–5435.
- (15) Thomas, S. W.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339–1386.
- (16) Wu, Q.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 14682–14683.
- (17) Zhu, L.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2006**, *45*, 1190–1196.
- (18) Amir, R. J.; Pessah, N.; Shamis, M.; Shabat, D. *Angew. Chem., Int. Ed.* **2003**, *42*, 4494–4499.
- (19) Sagi, A.; Segal, E.; Satchi-Fainaro, R.; Shabat, D. *Bioorg. Med. Chem.* **2007**, *15*, 3720–3727.
- (20) Sella, E.; Shabat, D. *Chem. Commun.* **2008**, 5701–5703.
- (21) Shamis, M.; Shabat, D. *Chem.—Eur. J.* **2007**, *13*, 4523–4528.

occurred through a distinctive chain reaction that generated the analyte of interest, thereby initiating additional diagnostic cycles. The system was designed for the detection of hydrogen peroxide, and we demonstrated that it produced a significantly higher diagnostic signal than a classic probe. The versatility of the two-component amplification system was exemplified by the use of two different spectroscopic probes that generated either a chromogenic or a fluorogenic signal. A mathematical model that simulates the disassembly kinetics of the one-component and the two-component systems was developed and was shown to correlate well with the observed experimental data. Additional 2CDCR systems with superior detection sensitivity for the identification of

other analytes are currently being developed and will be reported in due course.

Acknowledgment. D.S. thanks the Israel Science Foundation (ISF) and the Binational Science Foundation (BSF) for financial support.

Supporting Information Available: Full experimental details, characterization data of all new compounds, and spectroscopic assay conditions. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JA910839N