

# Fluorophore-Appendant 5,5'-Bicalixarene Scaffolds for Host–Guest Sensing of Nitric Oxide

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Cite This: *Org. Lett.* 2020, 22, 9706–9711



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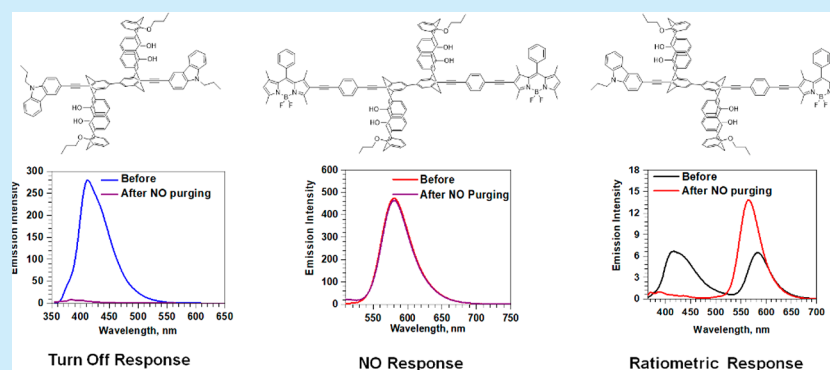
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**ABSTRACT:** Conjugated 5,5'-Bicalixarene scaffolds having fluorophores at the chain termini have been prepared and tested in the supramolecular detection of nitric oxide. Scaffolds bearing electron-rich fluorophores demonstrated a stronger turn-off response to the presence of NO than the fluorophore-free analogue in both organic and aqueous media, while no fluorescence quenching happened when the electron-deficient fluorophores were employed. Unprecedented ratiometric supramolecular sensing was observed when fluorophores of the opposite electronic demands were placed at the scaffold's termini.

Host–guest interactions involving soluble three-dimensional organic scaffolds have received considerable attention in the design of fluorescent molecular probes.<sup>1</sup> Usually, such probes involve a fluorescent guest molecule competing with the analyte for binding within the cavity of a supramolecular host.<sup>2,3</sup> Introduction of pendant fluorescent moieties at the periphery of a host molecule is another common technique.<sup>4</sup> In this case, the increased distance between the fluorophore and supramolecular receptor along with the increased flexibility typically result in a muted chemosensory response to the host–guest complexation. Interestingly, very few examples of probes for small molecules and ions based on host compounds with built-in fluorescent function have been reported in the literature.<sup>5</sup> For example, out of ca. 200 recently reviewed fluorescent calix[4]arene molecules only a handful have fluorescent motifs conjugated directly with the calixarene scaffold while the majority possess fluorophores attached as pendants at the phenolic moieties of the lower rim.<sup>6</sup> Such scarcity, which can be primarily attributed to synthetic challenges in assembling the corresponding 3D structures, significantly hinders applications of the host–guest calixarene complexation in fluorescent chemosensing. This is particularly true with regard to the detection of small gaseous compounds where no fluorescent probes have been reported despite the ability of such molecules to penetrate organic hosts, to the best of our knowledge.<sup>7</sup> At the beginning of the century, Rathore et

al. reported NO gas capture by the colored oxidized calixarene scaffolds leading to a significant color change.<sup>8,9</sup> Considering the importance of nitric oxide in a variety of biological processes,<sup>10</sup> we developed the fluorescent oxygen-depleted 5,5'-bicalixarene scaffolds<sup>11</sup> for reversible NO detection in solution.<sup>12</sup> Similarly constructed fluorophore units lacking the calixarene cavities showed no fluorescence changes in the presence of this gas, thus suggesting the unprecedented NO host–guest complexation mechanism operating in our systems.<sup>13</sup> While unresponsive to other common gases, such as O<sub>2</sub>, CO, CO<sub>2</sub>, and water or hydrocarbons, these earlier molecular systems operate via a turn-off mechanism at relatively short wavelengths and with moderate sensitivity. To address these issues, we now present a novel approach toward highly sensitive supramolecular host–guest detection at longer wavelengths including a ratiometric NO detection mechanism.

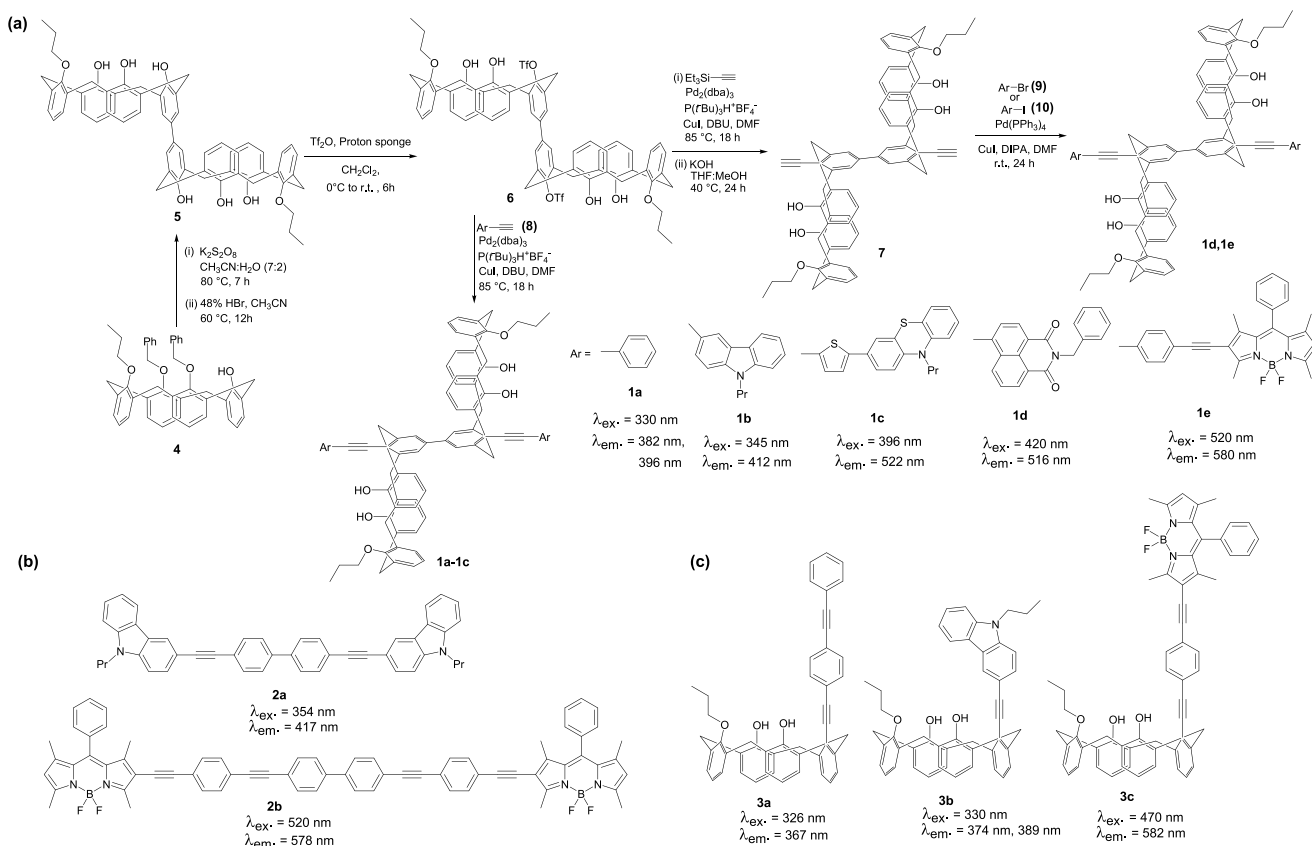
As in the original systems the fluorescence results from extended conjugation of unsaturated hydrocarbon groups (e.g.,

Received: November 11, 2020

Published: December 7, 2020

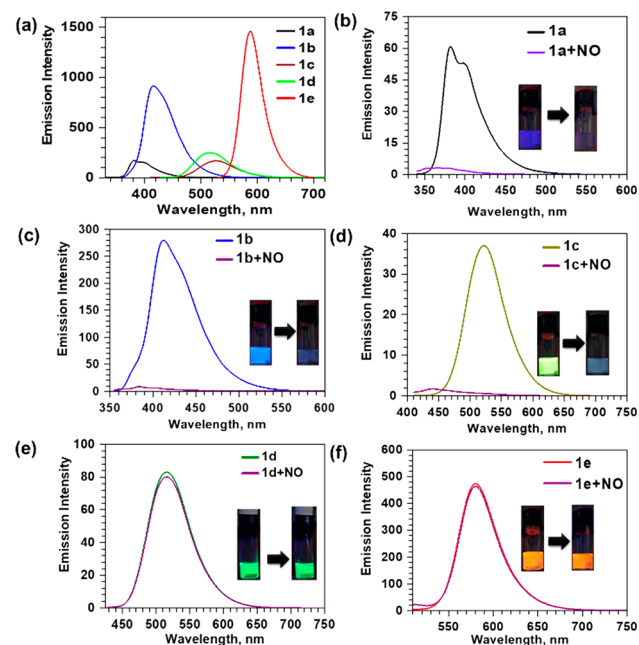


## Scheme 1. Synthesis of Fluorescent 5,5'-Bicalixarene Scaffolds and Structures of Model Fluorophores



compound **1a**, Scheme 1), the ability to affect their photophysical properties, such as emission wavelength and intensity, is limited. We hypothesized that an introduction of strongly emitting fluorophores at the termini of the conjugated 5,5'-bicalixarene scaffolds would greatly affect both the photophysics of the systems and their sensitivity toward guest molecules. To validate this, we prepared a series of 5,5'-bicalixarenes **1b–e** using the synthetic routes shown in Scheme 1. Although the synthesis of a common precursor **5** has been reported earlier, the yields were generally low (20%). Because of the higher electron density of the protected phenolic rings in compound **4**, the original oxidative coupling using  $\text{FeCl}_3$  as the oxidant<sup>11d</sup> gave mixtures of products and required tedious column purification. Using a two-step bromination/Ni-assisted Ullmann-type coupling was a cleaner route but required stoichiometric quantities of  $\text{Ni}(\text{COD})_2$ .<sup>12b</sup> We have found that the direct oxidation of **4** with potassium persulfate using the protocol recently reported for phenols oxidative coupling<sup>14</sup> gave the desired **5** in a 71% yield (Scheme 1a). The selective introduction of the triflate group followed by the modified protocol of the original Sonogashira-type cross-coupling<sup>15</sup> gave the oxygen depleted bicalixarene scaffolds which were converted to the final products. To verify the potential direct interactions of NO gas with the fluorophores, not involving host–guest complexation, we also prepared compounds **2** lacking bicalixarene binding sites (Scheme 1b). Finally, we synthesized compounds **3** bearing a single calixarene cavity with an attached fluorophore (Scheme 1c).<sup>16</sup>

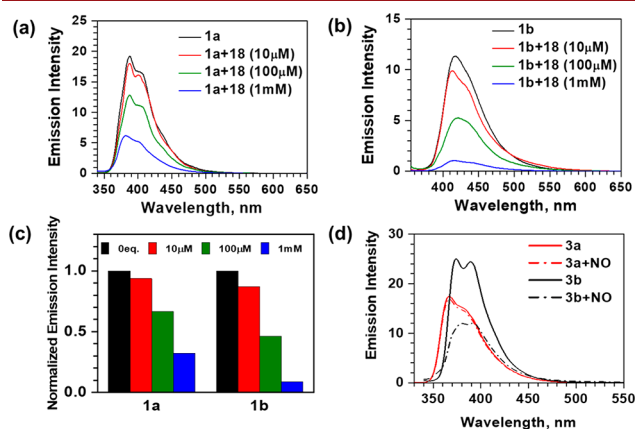
All compounds bearing fluorophores (**1b–e**) showed fluorescence at longer wavelengths compared with either **1a** or unmodified parent fluorophores (Scheme 1 and Figure 1a).



**Figure 1.** (a) Emission spectra of **1a–1e** ( $10 \mu\text{M}$  in  $\text{CH}_2\text{Cl}_2$ ). (b–f) Emission spectra of **1a–1e** ( $1 \mu\text{M}$  in  $\text{CH}_2\text{Cl}_2$ ) before and after passing NO gas for 10 min (insets: photographs taken upon excitation with a 365 nm UV lamp).

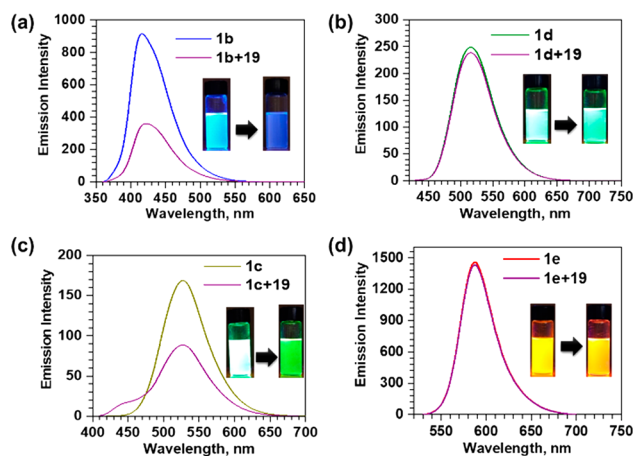
Surprisingly, while the fluorescence of compounds **1a–c** was completely quenched upon passing NO gas through their  $\text{CH}_2\text{Cl}_2$  solutions, compounds **1d,e** did not show changes in their emission spectra under the same conditions (Figure 1b–f).

The fluorescence quenching for **1a–c** was irreversible, and no fluorescence was observed after applying vacuum or passing  $N_2$  through their solutions for over 15 min. The  $^1H$  NMR spectrum of **1b** showed some signal broadening<sup>12b</sup> but no indication of a chemical reaction with the fluorophore. To further compare the NO sensing properties of the original “all carbon” 5,5'-bicalixarene scaffold with the fluorophore-decorated one, we performed quantitative studies using a soluble source of NO, diethylammonium (Z)-1-(*N,N*-diethylamino) diazen-1-ium-1,2-diolate (DEA NONOate, **18**). As compounds **1** are not soluble in water, the measurements were done in a micellar solution of sodium dodecyl sulfate (SDS). Under unoptimized conditions, half of the original fluorescence of **1b** ( $1 \mu M$ ) was quenched upon stirring with a  $100 \mu M$  solution of **18**,<sup>17</sup> while roughly 66% of the original fluorescence remained for **1a**. Moreover, with a 1 mM solution of **18**, more than 90% of the fluorescence was quenched for **1b** while only 68% was quenched for **1a** (Figure 2a–c). Noteworthy, compound **3b**, representing the model



**Figure 2.** Effect of the fluorescent scaffold design on the detection of NO in aqueous (a–c) and organic phase (d). Concentration of **1a**, **1b**, **3a**, and **3b** is  $1 \mu M$ .

electron-rich monocalixarene “half” of **1b**, showed very limited response to NO gas, while the monocalixarene **3a**<sup>18</sup> did not show fluorescence changes in the presence of NO (Figure 3d). Finally, the corresponding conjugated fluorophores lacking the



**Figure 3.** Emission spectra of **1b–e** ( $10 \mu M$  in  $CHCl_3:CH_3CN$ , 9:1) before and after addition of 50 equiv of *N*-methylpyridinium triflate (**19**).

calixarene scaffolds did not undergo quenching under the reported conditions (Figure S7), ruling out the direct interactions between the fluorophores and NO and testifying to the importance of the overall design of **1** for the observation of the NO sensing behavior.

The lack of the fluorescence response to NO gas from compounds **1d,e** can be reasonably attributed to the electron-deficient nature of the BODIPY and naphthalimide dyes, especially when compared with the strongly responding **1b,c** bearing electron-rich fluorophores. Such electronic deficiency can negatively affect both the postulated host–guest complexation properties between the bicalixarene and NO, and fluorescence quenching of the dye by the encapsulated guest. To gain further insight into the host–guest chemistry of compounds **1**, we performed complexation studies between **1** and the *N*-methylpyridinium cation, a common guest for calixarene compounds.<sup>11,19</sup> The fluorescence signals of **1a–c** decreased noticeably upon mixing with *N*-methylpyridinium triflate (NMPT, **19**) in a 9:1 chloroform–acetonitrile solution. Compounds **1b,c** showed significantly higher sensitivity than the unsubstituted **1a** with the limit of detection (LOD) for **1b** being more than 20 times lower than that of **1a** ( $1.93 \times 10^{-7} M$  vs  $4.27 \times 10^{-6} M$ , Figure S5). These data correlate with the stronger binding of **19** within the electron-rich cavity of **1b** ( $K_a = 102 \times 10^3 M^{-1}$ ) compared to **1a** ( $K_a = 6.3 \times 10^3 M^{-1}$ ). Importantly, similar to NO, no changes in the emission spectra were observed for **1d,e** when an excess of the pyridinium salt was added (Figure 3).

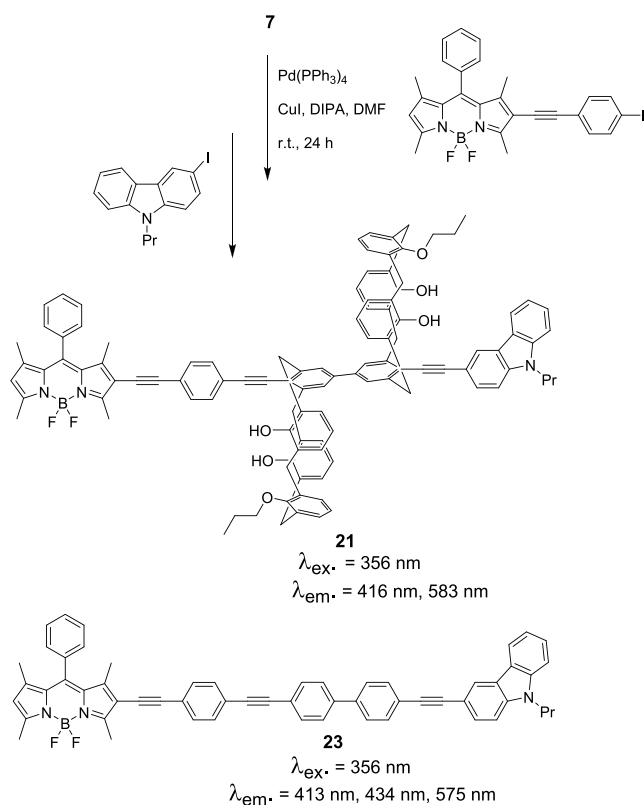
The host–guest complexation between **1** and **19** was also studied by the  $^1H$  NMR spectroscopy. The NMR analysis of 1:1 mixtures of **1** and **19** in 9:1  $CDCl_3/CD_3CN$  showed strong upfield shifts of the guest cation signals for **1a–c** (1.5–2 ppm for *N*-Me group, Figure S8) and only minor changes for **1d,e** (<0.5 ppm for *N*-Me group, Figure S9).<sup>16</sup> Considering that the same bicalixarene receptor group is present in all compounds **1**, such a difference in complexation behavior can be attributed to the difference in the electronic properties of the dyes at the chain termini; electron-deficient BODIPY and naphthalimide strongly decrease the electron density within the binding pocket and weaken the host–guest interactions.

Preliminary DFT studies at the B3LYP-D3/6-31G level of theory<sup>20</sup> on **1b**, **1d**, and NO (uB3LYP-D3/6-31G) were performed, and the frontier molecular orbitals of these molecules were extracted. These calculations revealed that the **1b** and **1d** respective LUMOs have higher energy than those of NO, thus potentially allowing electron transfer from their excited states to the NO guest (Figures S78 and S79). However, while in **1b** the LUMO is localized within the bicalixarene receptor, and in **1d** it is localized at the peripheral fluorophore. Thus, we assume that the host–guest complexation between **1d** and NO within the bicalixarene scaffold would have no effect on the fluorescence of that molecule.

The diminished complexation properties and lack of the fluorescence response of the bicalixarenes decorated with electron-poor fluorophores led us to explore the possibility of ratiometric sensing via host–guest interactions.<sup>21</sup> We hypothesized that a bicalixarene core bearing donor and acceptor fluorophores at opposing terminal positions of a rigid conjugated chain would demonstrate through-bond intramolecular energy transfer. The host–guest interaction with such a cavity would primarily take place within the electron-rich part, thus reducing the energy transfer between the two termini and restoring the fluorescence of the electron-poor fluorophore.

While changes in energy transfer in rigid donor–acceptor (D–A) compounds upon analyte complexation have been used in the design of ratiometric fluorescent probes, including for NO detection,<sup>22</sup> we are unaware of such a mechanism employed in combination with the host–guest complexation. To verify this hypothesis, we prepared the D–A bicalixarene **21** (Scheme 2)

**Scheme 2. Synthesis of Fluorescent Donor–Acceptor 5,5'-Bicalixarene Scaffold 21**

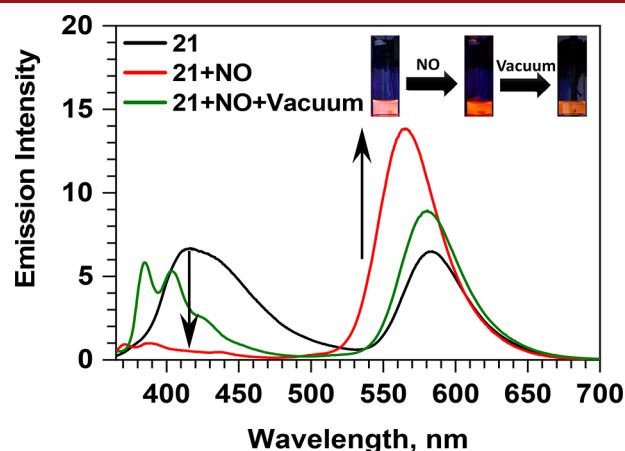


decorated with carbazole and BODIPY dyes at the terminal positions of the conjugated chain. The synthesis was accomplished in two steps starting from the precursor **7** (Scheme 2). Introducing the electron-withdrawing BODIPY fragment at the first step under the Pd-catalyzed conditions allowed the isolation of a monosubstituted bicalixarene fragment **20**<sup>16</sup> which was reacted with the iodocarbazole derivative under the same conditions to give **21** in a 28% yield over two steps. The substitution order was important, as attempts to selectively attach a single carbazole fragment were unsuccessful, giving instead **1b** as the major product.

Compound **21** shows dual fluorescence signals at 416 and 583 nm with significantly lower intensity than the parent fluorophores or the corresponding conjugated building blocks. Furthermore, the ratio between the signals is strongly dependent on the solvent polarity. Gratifyingly, the addition of **19** to a solution of **21** in a dichloroethane (DCE)–CH<sub>3</sub>CN 9:1 mixture led to a strong decrease in the intensity of the carbazole-derived signal at 416 nm and concomitant increase in the intensity of the BODIPY-derived signal at 570 nm (Figure S4). This observed blue shift further supports a decrease of internal energy transfer interaction between the conjugated termini of the molecule. The <sup>1</sup>H NMR spectra showed a very strong upfield shift of the signals of **19** (2.5 ppm for N-Me group, Figure S10), thus suggesting

that the guest is primarily interacting with the carbazole-substituted half of the bicalixarene molecule.

To our delight, passing NO gas through a solution of **21** in DCE also resulted in a strong ratiometric response (Figure 4).



**Figure 4.** Fluorescence studies of compound **21** (1  $\mu\text{M}$  in CH<sub>2</sub>Cl<sub>2</sub>).

Furthermore, the presumed weaker binding properties of **21** as compared to **1b** allowed the reversal of the ratiometric response. After the evaporation of a NO@**21** solution and addition of new solvent, the excitation spectrum showed a pattern similar to that of **21** (Figure 4). Although the BODIPY-related part of the spectrum was nearly restored, the carbazole-centered emission remained blue-shifted and showed fine structure, the origin of which is presently unknown.

We prepared fluorescent bicalixarene receptors that demonstrate enhanced sensitivity toward NO gas. Scaffolds bearing electron-rich fluorophores show a strong turn-off response to NO at longer wavelengths than the parent bicalixarenes lacking fluorophore units, while no such response was observed in the case of the electron-poor analogues possessing the same binding site. A combination of donor and acceptor fluorophores at the opposing termini of the bicalixarene receptor allowed reversible ratiometric detection of NO gas. Further studies on the nature of NO–calixarene interactions and applications of the host–guest complexation mechanism for nitric oxide sensing are in progress in our laboratories.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.orglett.0c03764>.

Complete experimental and computational details (PDF)

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## Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This work was supported by the Israel Science Foundation (Grant 696/16) and Pazy Foundation (Grant 5100011590).

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