

A Supramolecular Antibiotic Switch for Antibacterial Regulation

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Abstract: A supramolecular antibiotic switch is described that can reversibly “turn-on” and “turn-off” its antibacterial activity on demand, providing a proof-of-concept for a way to regulate antibacterial activity of biotics. The switch relies on supramolecular assembly and disassembly of cationic poly(phenylene vinylene) derivative (PPV) with cucurbit[7]uril (CB[7]) to regulate their different interactions with bacteria. This simple but efficient strategy does not require any chemical modification on the active sites of the antibacterial agent, and could also regulate the antibacterial activity of classical antibiotics or photosensitizers in photodynamic therapy. This supramolecular antibiotic switch may be a successful strategy to fight bacterial infections and decrease the emergence of bacterial resistance to antibiotics from a long-term point of view.

The problem of bacterial resistance to antibiotics has attracted more and more public attention in recent years, especially owing to the occurrence of multidrug-resistant bacterial strains.^[1–3] Resistance stems from the constant growth in the presence of antibiotics. The widespread use of successive generations of antibiotics in healthcare and agriculture accumulates a large amount of antibiotics in the environment that makes antibiotic resistance a serious public health threat.^[4,5] Intrinsic mutations and horizontal gene transfer among bacteria have been appreciated as important forces in antibiotic resistance.^[6,7] To address the challenges associated with bacterial resistance, great efforts have been made to develop new antibiotics or enhance the activity of existing antibiotics.^[8–11] However, the innate capacity of microbes to develop resistance at a rate that outpaces development of new antibiotics suggests that existing strategies for developing viable, long-term anti-microbial therapies are ultimately doomed to failure. Without alternative strategies, the acquisition of drug resistance by pathogenic microorganisms looms as possibly one of the most significant public health threats facing humanity in the 21st century.

Avoiding accumulation of active antibiotics in the environment will greatly decrease the emergence of bacterial resistance to antibiotics from a long-term point of view. In this regard, Mobashery and co-workers designed photodegrad-

able β -lactam antibiotics that could be light-inactivated after their pathogen treatment.^[12] Recently, Feringa and co-workers have reported photoswitchable antibiotic analogues with selective activation/inactivation upon light irradiation.^[13] These approaches provide unconventional strategies to control antibiotic activity and to avoid accumulation of the active antibiotic in the environment. However, they require complicated molecular design and synthesis, and caging the active sites of existing antibiotics with molecular photoswitch groups is not easy to be rationally predicted. In this work, we developed a supramolecular antibiotic switch to reversibly turn-on and turn-off antibacterial activity on demand by a supramolecular assembly and disassembly process. This strategy does not require any chemical modification on the active site of an existing antibacterial agent, making this approach simple, rapid, and efficient. This work offers a proof-of-concept to control antibacterial agent activity by simple supramolecular assembly and disassembly process, and could be very useful for enabling effective and precise pathogen treatment, as well as for avoiding accumulation of the active antibiotic in the environment.

The cationic poly(phenylene vinylene) (PPV) derivative with quaternary ammonium (QA) groups as side chain was chosen as our antibacterial agent.^[14] The biocidal activity of PPV depends on the penetration of the QA cationic groups into the cell membrane.^[15–18] The molecules cucurbit[7]uril (CB[7]) and amantadine (AD) are used to turn-on or turn-off the biocidal activity of PPV. As shown in Figure 1 a, cationic PPV could form a noncovalent complex with CB[7] that possesses a hydrophilic exterior and hydrophobic cavity for encapsulating QA molecules,^[19,20] and PPV/CB[7] complex reduce the biocidal activity of PPV owing to the encapsulation of QA groups (switch off). Upon adding AD, the more stable CB[7]/AD complex forms and releases PPV through competitive replacement.^[20] Thus the antibacterial activity of PPV is recovered (switch on).

First, ¹H NMR, dynamic light scattering (DLS), and fluorescence titration experiments were carried out to confirm the noncovalent binding of CB[7] to PPV. ¹H NMR spectroscopy can be used to study polymer structural properties and molecular interactions. As shown in Figure 1 b, upon adding CB[7] to the aqueous solution of PPV, all of the proton signals of CB[7] can be clearly detected, while the proton peaks of the quaternary ammonium groups in PPV (3.7–3.2 ppm) showed a 0.5 ppm shift toward higher field. Furthermore, the characteristic signals of the aromatic protons in the PPV backbone (7.2–7.8 ppm) in D₂O are broadened into the baseline upon CB[7] binding. These observations imply the formation of PPV/CB[7] complex and that CB[7] may wrap around PPV QA molecules. PPV is a rigid-rod-like molecule in which the backbone and alkyl side chain are

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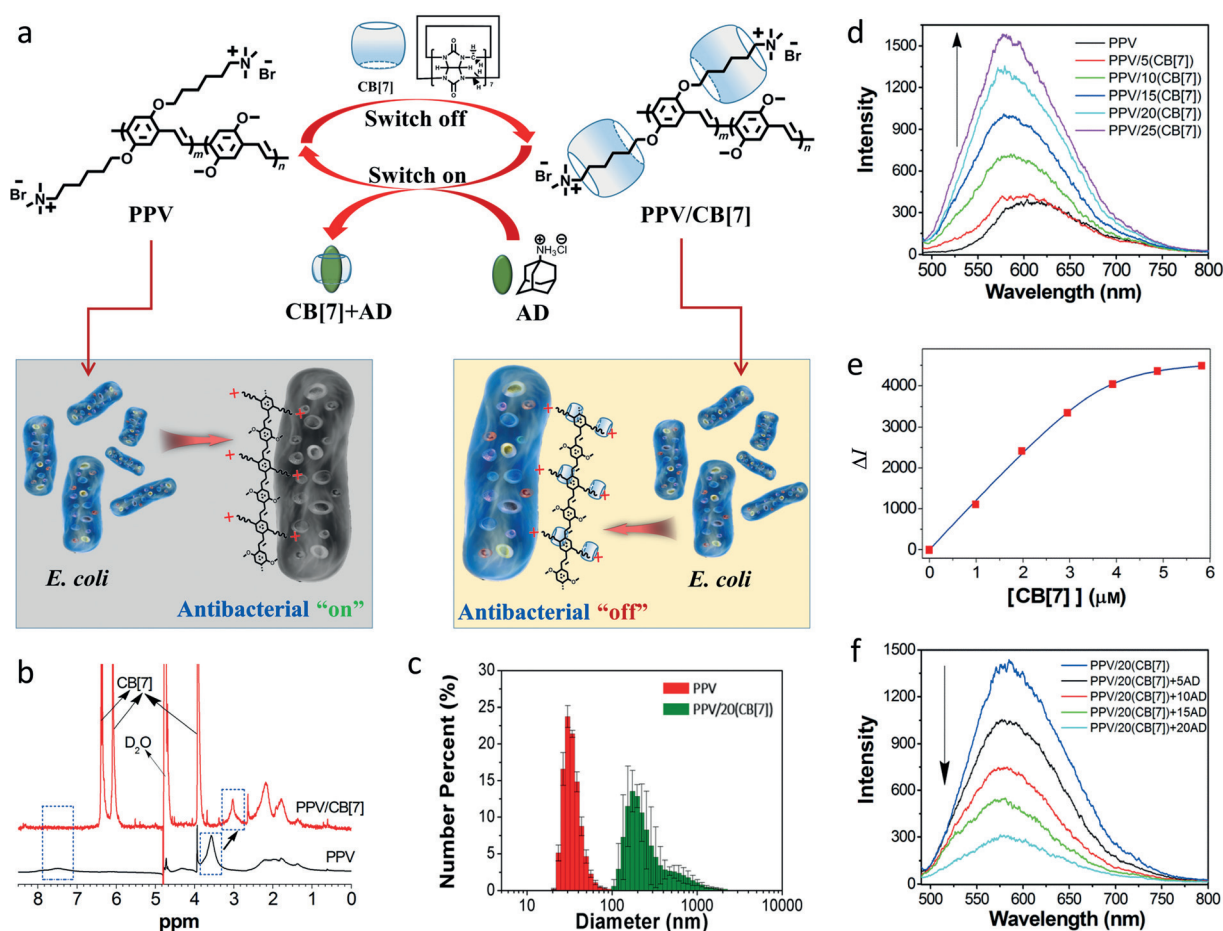


Figure 1. a) Supramolecular assembly of PPV with CB[7] and disassembly of PPV with CB[7] mediated by AD molecule for reversible control of antibacterial activity of PPV. b) ^1H NMR spectra of PPV in D_2O before and after adding CB[7]. c) Size distribution histograms of PPV in water before and after adding CB[7] resulting from dynamic light scattering measurement. d) Fluorescence spectra of PPV in the water upon adding CB[7]. [PPV] = $2\ \mu\text{M}$ in repeated units (RUs); [CB[7]] = 0–50 μM . Excitation wavelength: 478 nm. e) The nonlinear least-squares analysis of fluorescence intensity changes (ΔI) at 581 nm against the concentration of CB[7] to calculate the binding constant ($K_a = 8.5 \times 10^6\ \text{L mol}^{-1}$) between PPV and CB[7]. f) Fluorescence spectra of PPV/20(CB[7]) in the water upon adding different concentrations of AD. [PPV] = $2\ \mu\text{M}$ in RUs; [CB[7]] = 40 μM ; [AD] = 0–40 μM . Excitation wavelength: 478 nm.

hydrophobic moieties, while the cationic charged quaternary amines control electrostatic interactions. The resulting amphiphilic characteristics lead to aggregated structures in aqueous solution.^[21] Dynamic light scattering (DLS) was carried out to show the aggregate size change of PPV in aqueous solution upon binding CB[7]. As shown in Figure 1c, PPV alone forms dispersed aggregates with an average diameter of about 100 nm, while the average diameter of PPV/20(CB[7]) complex was much larger (100–1000 nm) owing to the encapsulation of QA groups by CB[7]. The data further corroborate the interactions between PPV and CB[7]. To better understand the interactions between PPV with CB[7], we examined PPV in water with varying amounts of CB[7] using fluorescence spectroscopy. In this experiment, a series of solutions composed of different ratios of PPV to CB[7] (PPV/CB[7] = 1:0, 1:5, 1:10, 1:15, 1:20, and 1:25) were prepared. As shown in Figure 1d and Figure S1a (Supporting Information), the emission intensity of PPV increases gradually with the addition of CB[7] accompanied by a blue-shift of the emission peak, while the shapes and intensities of the spectra show

negligible changes (Supporting Information, Figure S2). Moreover, a linear enhancement was observed at the wavelength of 581 nm for PPV emission. The increase of fluorescence intensity illustrates that the assembling of CB[7] and PPV changes the spatial conformation and reduces the aggregation of PPV. As the positively charged conjugated polymers can form aggregates in aqueous solution, the interactions between molecular chains increase the non-radioactive energy consumption and their fluorescence intensity decreases significantly through fluorescence self-quenching.^[22,23] Thus in the desired supramolecular complex, CB[7] has the effect of remarkably reducing the polymer self-quenching.

To understand the mechanism of reversible antibacterial control, zeta potential (ζ) measurements were conducted to investigate the interactions between the Gram-negative bacteria *E. coli* and PPV in the cases of assembly and disassembly with CB[7]. As shown in Figure 2a, the potentials of *E. coli* exhibit remarkable changes with the addition of PPV/CB[7] complex, and the PPV bound with more CB[7] leads to

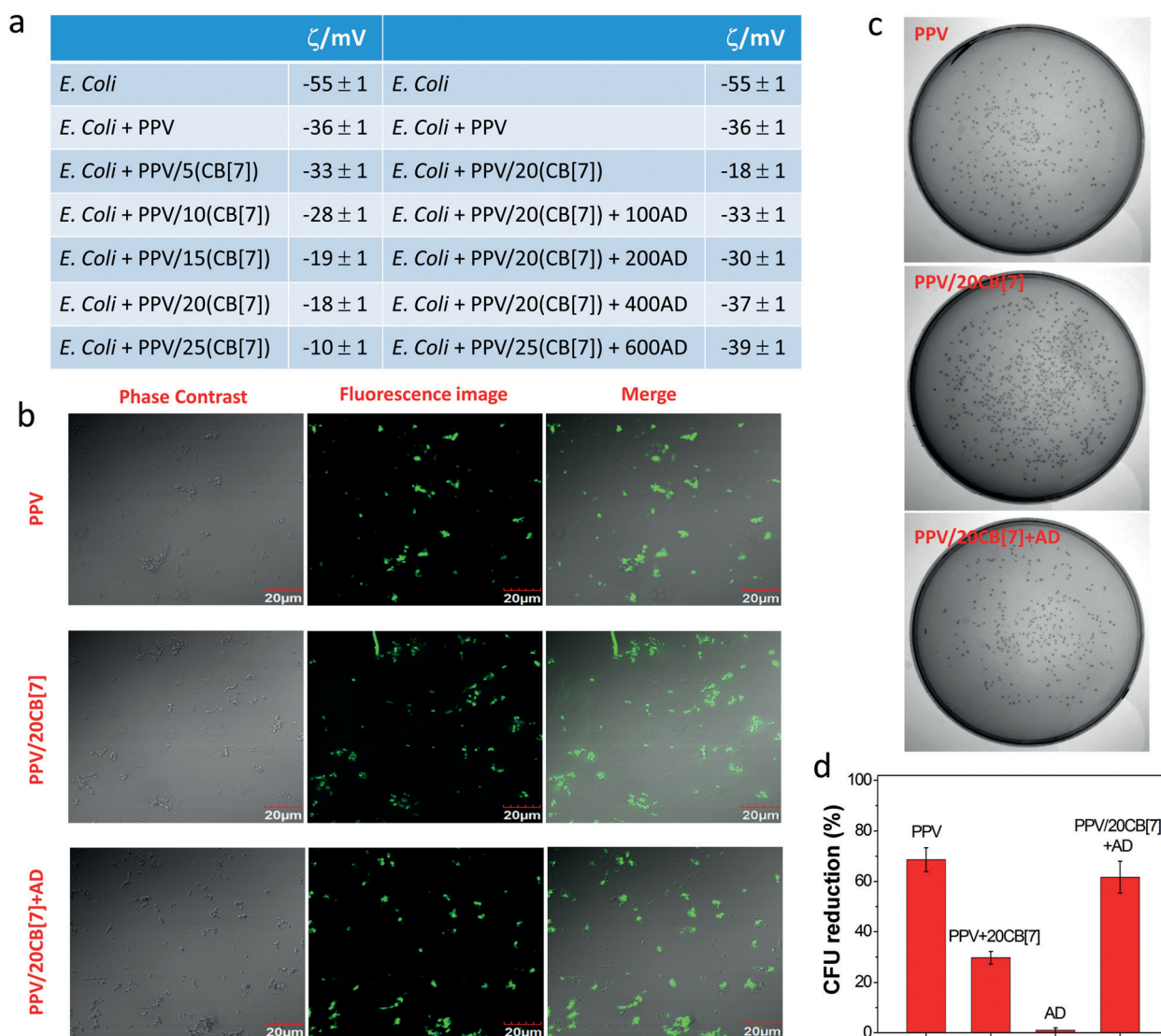


Figure 2. a) ζ potentials of *E. coli* upon the addition of PPV and PPV/CB[7] complexes (molar ratios: PPV/CB[7] = 1:5, 1:10, 1:15, 1:20, 1:25), also the ζ potentials of *E. coli*/PPV/CB[7] upon the addition of AD (molar ratios: PPV/CB[7] = 1:20, CB[7]/AD = 1:5, 1:10, 1:20, 1:24). Measurements were performed in water at 25 °C. [PPV] = 5 μ M in RUs. b) Confocal laser scanning microscopy images of *E. coli* with PPV, and PPV/CB[7] complex before and after addition of AD. Scale bars: 20 μ m. c) Colony forming units (CFU) for *E. coli* treated with PPV, and PPV/CB[7] complex before and after addition of AD on LB agar plate in dark. d) Antibacterial activity of PPV, and PPV/CB[7] complex before and after addition of AD in dark. PPV/CB[7] = 1:20; CB[7]/AD = 1:5; and [PPV] = 5 μ M in RUs.

more positive potential shift (from -55 ± 1 to -10 ± 1 mV). Previous reports have shown that cationic conjugated oligomers/polymers with QA groups as side chains could bind and insert into the negatively charged membrane of bacteria by electrostatic and hydrophobic interactions.^[14,25] Inserting into the membrane by hydrophobic interactions does not affect the zeta potential of bacteria, but binding by electrostatic interactions leads to a remarkable positive potential shift. Thus the encapsulation of QA groups in PPV by CB[7] prevents cationic groups from inserting into the membrane of *E. coli* and the electrostatic interactions play a main role, possibly explaining the positive potential shift of *E. coli*. In a reversible way, by adding AD the ζ potentials of *E. coli* binding with PPV/CB[7] complex almost recover to that of *E. coli* binding with PPV alone as a result of the release of

PPV through competitive replacement. These results verify again that the reversible complex is controllable. Confocal laser scanning microscopy (CLSM) was utilized to directly visualize the interactions between *E. coli* and PPV in the cases of assembly and disassembly with CB[7]. There is no obvious difference of fluorescence intensity in the CLSM images for *E. coli*/PPV and *E. coli*/PPV/CB[7] (Figure 2b). These observations illustrate that both PPV and PPV/CB[7] could bind to *E. coli* membrane, but they have different interactions. The antibacterial activities of PPV and PPV/CB[7] complex toward *E. coli* were further explored. PPV alone exhibits high killing efficiency (70%) towards *E. coli*, while killing efficiency less than 30% was obtained for PPV/CB[7] complex (Figure 2c,d). Upon disassembly of PPV/CB[7] complex by AD, the killing efficiency recovers to more than

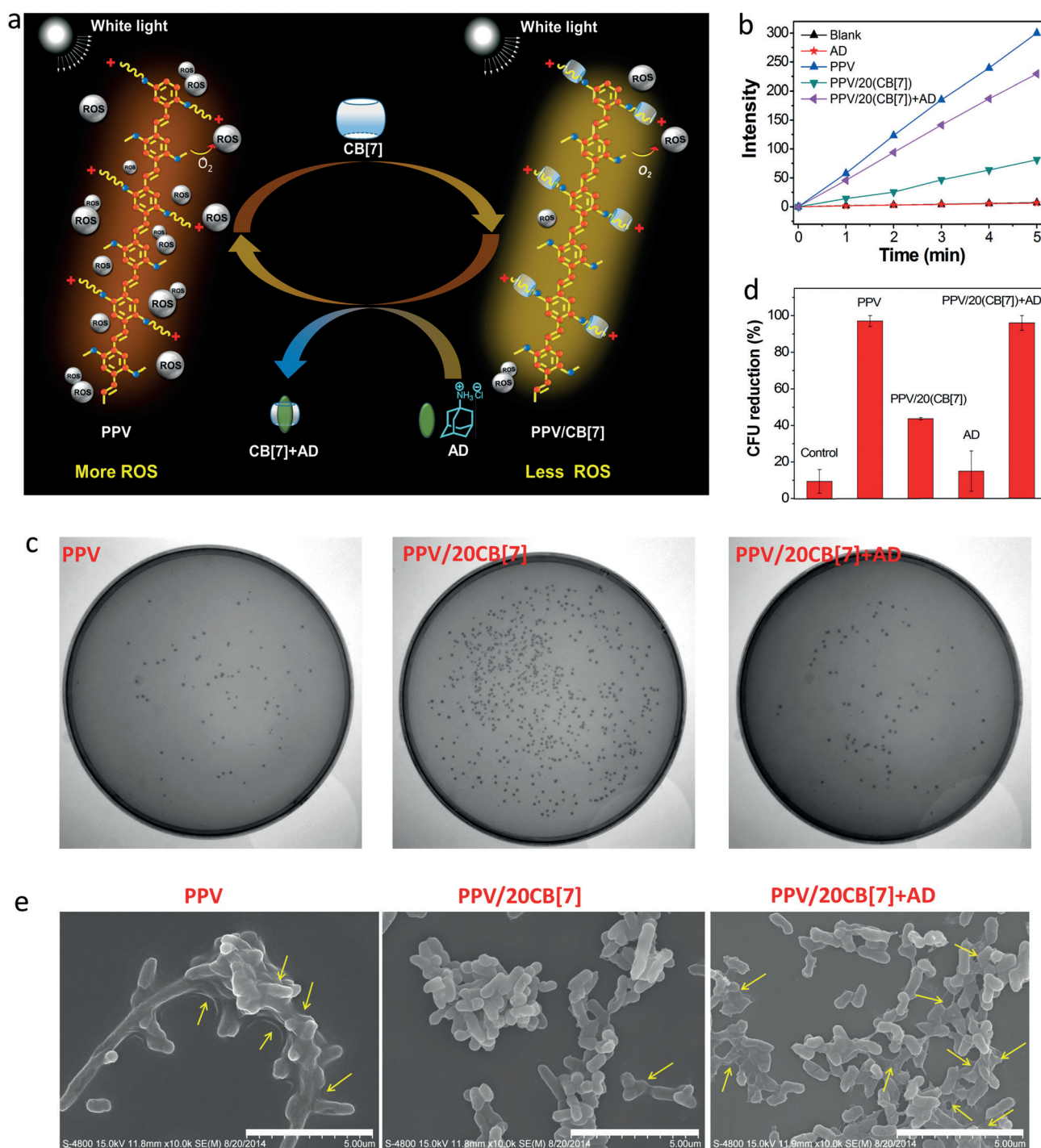


Figure 3. a) Supramolecular assembly and dis-assembly of PPV with CB[7] for reversible control of ROS generation of PPV. b) Fluorescence intensity of DCFH in the presence of PPV and PPV/CB[7] complex (PPV/CB[7] = 1:20) as well as PPV/CB[7] upon the addition of AD upon white light irradiation (0–5 min) with an excitation of 488 nm. c) Colony-forming units (CFU) for *E. coli* treated with PPV and PPV/CB[7] complex before and after addition of AD on LB agar plate under white light. d) Antibacterial activity of PPV and PPV/CB[7] complex before and after addition of AD under white light. e) SEM images of *E. coli* treated with PPV and PPV/CB[7] complex before and after addition of AD under white light. The yellow arrows indicate the collapse and split of membranes. White light irradiation (75 mWcm^{-2}) for 7 min for c–e. For b–d: PPV/CB[7] = 1:20, CB[7]/AD = 1:5, [PPV] = $5 \mu\text{M}$ in RUs. Scale bars: $5 \mu\text{m}$.

60%, which is nearly the same as that of PPV alone. The growth of bacterial cells is not affected by AD as well as the blank group without any treatments (Supporting Information, Figure S3), indicating that AD has no obvious influence on *E.*

coli. Furthermore, PPV is still able to be turned-on and turned-off over long periods of time (Supporting Information, Figure S6). The switchable activity of killing bacteria was demonstrated by adding AD and CB[7] into the PPV/CB[7]

complex solution sequentially and repeatedly (Supporting Information, Figure S8). These results demonstrate that the antibacterial activity of cationic PPV is reversibly regulated by simple supramolecular assembly and dis-assembly process.

Water-soluble conjugated polymers can sensitize the surrounding oxygen to produce reactive oxygen species (ROS) upon light illumination for killing bacteria.^[26–28] As shown in Figure 3a, the cationic PPV could efficiently generate ROS, while the PPV/CB[7] complex produced less ROS because encapsulated PPV is prevented from contacting the surrounding oxygen. Subsequently, the PPV switch system was examined to turn-on and turn-off its antibacterial activity under white light irradiation. The ROS production ability of PPV and PPV/CB[7] complex was probed by DCFH-DA that could be converted into highly fluorescent 2,7-dichlorofluorescein (DCF, $\lambda_{em} = 524$ nm) in the presence of ROS.^[29] For PPV, the fluorescent intensity at 524 nm increases dramatically as light irradiation time increases (0–5 min), generating ROS from the sensitized PPV (Figure 3b). For PPV/CB[7], the fluorescence intensity at 524 nm increased more slowly than that for PPV (the intensity for PPV is 4 times higher than that for PPV/CB[7] after 5 min irradiation). With the addition of competitive AD into the solution of PPV/CB[7] complex, the ROS generation ability recovered closely to that of initial PPV. The antibacterial experiments of PPV and PPV/CB[7] complex were performed under white light (75 mWcm⁻²). Furthermore, bacterial cell growth is not affected by AD as well as the blank group without any treatment, indicating that AD has no obvious influence on *E. coli* under white light irradiation (Supporting Information, Figure S4). More than 95% of *E. coli* are killed by PPV, but only about 40% by PPV/CB[7] complex (Figure 3c,d). As expected, the bacterial killing efficiency recovers to more than 95% upon the addition of AD into PPV/CB[7] complex. Thus, the antibacterial activity of cationic PPV is reversibly regulated by simple assembly and dis-assembly process simply using CB[7] and AD molecules. To acquire further evidence of antibacterial activity of PPV switch toward *E. coli* in the dark and under white light irradiation, scanning electron microscopy (SEM) was employed to visualize the morphological changes of bacteria. The clear edges and the surface integrity of bacterial cells treated with AD under white light were observed as well as the blank group without any treatment (Supporting Information, Figure S5), which indicates that AD and white light have no obvious influence on *E. coli*. For PPV-treated *E. coli*, collapsed, split, and merged membranes were seen under white light (Figure 3e). However, for PPV/CB[7] under white light irradiation, the bacteria have clear edges and surface integrity because of the prevention of ROS release and the insertion of QA groups into bacterial membrane from PPV/CB[7] complex. Upon disassembling PPV/CB[7] by AD, the bacterial membrane morphology exhibits collapse and then merge again under white light (Figure 3e). Note that PPV and PPV/CB[7] complex do not exhibit obvious cytotoxicity on mammalian cells under the antimicrobial condition by the MTT assay (Supporting Information, Figure S7).

In summary, we have successfully constructed a simple and novel antibiotic switch based on supramolecular self-

assembly and dis-assembly between PPV and CB[7] for reversible turn-on and turn-off of antibacterial activity. This switch concept works for classical cationic antibacterial agents, as well as for photosensitizers in photodynamic therapy. This strategy does not require any chemical modification on the active site of existing antibacterial agent, making this approach simple, rapid, and efficient. Such a supramolecular antibiotic switch system could be applied to other macromolecular cationic antibiotics. This work will provide a simple, effective, and fast way to control the activity of antimicrobial molecule in the future and have a long-term impact on fighting bacterial infections and drug-resistance.

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- [1] P. A. Smith, F. E. Romesberg, *Nat. Chem. Biol.* **2007**, *3*, 549–556.
- [2] Q. Zhang, G. Lambert, D. Liao, H. Kim, K. Robin, C.-K. Tung, N. Pourmand, R. H. Austin, *Science* **2011**, *333*, 1764–1767.
- [3] R. Chait, A. Craney, R. Kishony, *Nature* **2007**, *446*, 668–671.
- [4] J. L. Martinez, *Science* **2008**, *321*, 365–367.
- [5] J. Carlet, P. Collignon, D. Goldmann, H. Goossens, I. C. Gyssens, S. Harbarth, V. Jarlier, S. B. Levy, B. N'Doye, D. Pittet, R. Richtmann, W. H. Seto, J. W. M. van der Meer, A. Voss, *Lancet* **2011**, *378*, 369–371.
- [6] J. L. Martinez, F. Baquero, D. I. Andersson, *Nat. Rev. Microbiol.* **2007**, *5*, 958–965.
- [7] J. Davies, *Science* **1994**, *264*, 375–382.
- [8] S. Zhitni, E. D. Brown, *Nature* **2009**, *458*, 39–40.
- [9] L. Ejim, M. A. Farha, S. B. Falconer, J. Wildenhain, B. K. Coombes, M. Tyers, E. D. Brown, G. D. Wright, *Nat. Chem. Biol.* **2011**, *7*, 348–350.
- [10] D. J. Payne, M. N. Gwynn, D. J. Holmes, D. L. Pompliano, *Nat. Rev. Drug Discovery* **2007**, *6*, 29–40.
- [11] Y.-C. Liu, Y.-S. Li, S.-Y. Lyu, L.-J. Hsu, Y.-H. Chen, Y.-T. Huang, H.-C. Chan, C.-J. Huang, G.-H. Chen, C.-C. Chou, M.-D. Tsai, T.-L. Li, *Nat. Chem. Biol.* **2011**, *7*, 304–309.
- [12] W. Lee, Z.-H. Li, S. Vakulenko, S. Mobashery, *J. Med. Chem.* **2000**, *43*, 128–132.
- [13] W. A. Velema, J. P. van der Berg, M. J. Hansen, W. Szymanski, A. J. M. Driessen, B. L. Feringa, *Nat. Chem.* **2013**, *5*, 924–928.
- [14] H. Yuan, Z. Liu, L. Liu, F. Lv, Y. Wang, S. Wang, *Adv. Mater.* **2014**, *26*, 4333–4338.
- [15] L. D. Lu, F. H. Rininsland, S. K. Wittenburg, K. E. Achyuthan, D. W. McBranch, D. G. Whitten, *Langmuir* **2005**, *21*, 10154–10159.
- [16] C. Zhu, L. Liu, Q. Yang, F. Lv, S. Wang, *Chem. Rev.* **2012**, *112*, 4687–4735.
- [17] Y. Wang, Y. Tang, Z. Zhou, E. Ji, G. P. Lopez, E. Y. Chi, K. S. Schanze, D. G. Whitten, *Langmuir* **2010**, *26*, 12509–12514.

- [18] G. N. Tew, R. W. Scott, M. L. Klein, W. F. Degrado, *Acc. Chem. Res.* **2010**, *43*, 30–39.
- [19] K. Liu, Y. Liu, Y. Yao, H. Yuan, S. Wang, Z. Wang, X. Zhang, *Angew. Chem. Int. Ed.* **2013**, *52*, 8285–8289; *Angew. Chem.* **2013**, *125*, 8443–8447.
- [20] K. Kim, N. Selvapalam, Y. H. Ko, K. M. Park, D. Kim, J. Kim, *Chem. Soc. Rev.* **2007**, *36*, 267–279.
- [21] S. Wang, G. C. Bazan, *Chem. Commun.* **2004**, 2508–2509.
- [22] D. T. McQuade, A. E. Pullen, T. M. Swager, *Chem. Rev.* **2000**, *100*, 2537–2574.
- [23] B. Liu, G. C. Bazan, *Chem. Mater.* **2004**, *16*, 4467–4476.
- [24] Y. Liu, B.-H. Han, S.-X. Sun, T. Wada, Y. Inoue, *J. Org. Chem.* **1999**, *64*, 1487–1493.
- [25] C. Zhu, Q. Yang, L. Liu, F. Lv, S. Li, G. Yang, S. Wang, *Adv. Mater.* **2011**, *23*, 4805–4810.
- [26] S. Chemburu, T. S. Corbitt, L. K. Ista, E. Ji, J. Fulghum, G. P. Lopez, K. Ogawa, K. S. Schanze, D. G. Whitten, *Langmuir* **2008**, *24*, 11053–11062.
- [27] H. Yuan, B. Wang, F. Lv, L. Liu, S. Wang, *Adv. Mater.* **2014**, *26*, 6978–6982.
- [28] C. Xing, Q. Xu, H. Tang, L. Liu, S. Wang, *J. Am. Chem. Soc.* **2009**, *131*, 13117–13124.
- [29] L. Bourré, S. Thibaut, A. Briffaud, N. Rousset, S. Eléouet, Y. Lajat, T. Patrice, *J. Photochem. Photobiol. B* **2002**, *67*, 23–31.

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