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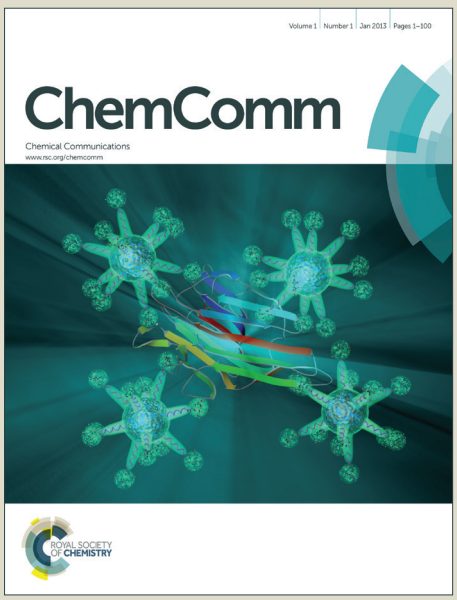

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Single molecule analysis of the self-assembly process operated by host-guest interactions†

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The self-assembly process from 1:1 to 1:2 complex operated by *para*-sulfonatocalix[6]arenes (SC6) as host and methyl viologen (MV^{2+}) as guest was analyzed at single molecule level through an *α*-hemolysin nanopore. Especially, the assembled structure of the complex has been real-time discriminated in the mixture of SC6 and MV^{2+}.

Supramolecular chemistry based on the weak and non-covalent interactions has become an important role in the bottom-up nanofabrication of molecular devices as well as a perfect bridge to tightly combine biology and chemistry.[1-8] Among all non-covalent interactions, the study of host-guest interaction is one of the most popular research fields which facilitate constructing a diversity of functional materials.[9-10] The host-guest recognition motif endows the materials with highly controlled and cooperative manner.[11-13] Depending on the different ratio of host and guest monomers, host-guest system can be assembled into various topological features which further provide a range of emerging applications including sensing, drug and gene delivery, diagnostics, coating and tissue engineering.[14-15] For instance, the interaction of a homoditopic monomer containing two bis(m-phenylene)-32-crown-10 units and a complementary homoditopic monomer containing two paraquat units produced both cyclic and linear species. The complexes of 2:2 self-assembled into cyclic species.[16] Thus, the understanding of the self-assembly process operated by the ratio of host to guest molecule is one of the key points to exquisitely design self-assembled host-guest structures. The precise analysis of each assembled complex will illuminate the construction of sophisticated and organized architectures from host-guest recognitions. However, the studies of host-guest supramolecules were usually based on the detections at micro-level.[17-21] Therefore, it is necessary to analyze the self-assembly process induced by every single monomer as well as discriminate the assembled stucture of an individual complex.

α-Hemolysin (*α*-HL) nanopore, a mushroom-shaped transmembrane channel,[22] has been proven as an ultra-sensitive system for the characterization of individual molecules, such as ssDNA,[23-27] peptides,[28-30] proteins,[31-33] ions[34-36] and host-guest molecules[37-40] at single molecule level. Previous studies in our group introduced calix[4]arene-based host-guest interactions into *α*-HL nanopore for the purpose of developing a functionalized nanopore biosensor.[38] By real-time monitoring the inhibitions, this calix[4]arene-functionalized nanopore biosensor has achieved to monitor the individual motion of light-regulated molecular machines. In order to further analyze the self-assembly process induced by each monomer via *α*-HL nanopore, here we employed *para*–sulfonatocalix[6]arenes (SC6) as host and methyl viologen (MV^{2+}) as guest. As shown in Fig.1, two different kinds of complexes, 1:1 and 1:2 complex, are self-assembled by SC6 and MV^{2+}.[41-44] In this proof of concept study, the self-assembled process from 1:1 to 1:2 complex was analyzed at single molecule level through an *α*-HL nanopore. Furthermore, every individual assembled structure of 1:2 complex has been real-time discriminated in the mixture of SC6 and MV^{2+}.

In the first step, SC6 was driven into the *trans* side of *α*-HL nanopore at holding potential of -60 mV. A previous study in our group showed that *para*–sulfonatocalix[4]arene (SC4) could bind *α*-HL nanopore as the guest to efficiently induce the long-lived close-states, due to the strong host-guest interactions between the positive residues (probably Lys131 and Lys147) and SC4.[38] Because the binding constant of SC6:Lys is about 94 M^{-1}, nearly two orders of magnitude smaller than that of SC4: Lys (Ka = 753 M^{-1}) at pH = 8 [45], SC6 only induced short-lived blockages with duration time (τ_{off}) of 0.15 ± 0.01 ms at -60 mV (Fig. 2a and Fig. S1). About 90% of blockages fall into Distribution I which is bounded within the logarithm value of τ_{off} ranging from -1 to -0.6 and I/I_0 from 0.2 to 0.85, where I is defined as the blockage current produced by the analyte and I_0 as the open pore current (Fig. 2b). This behavior of SC6 was retained even at holding

potential of -100 mV and a high concentration of 200 μM (Fig. S2). Therefore, SC6 neither acts as a host to alter the magnitude of ion conductance of *α*-HL like calix[4]arene[38] nor lodges in the lumen of the channel like *β*-

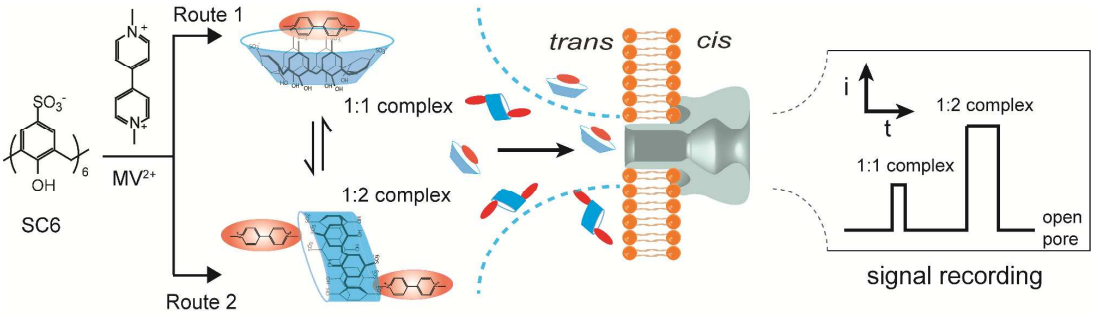

Fig. 1 Detection of the self-assembly process induced by SC6 and MV^{2+} at single molecule level using an *α*-HL nanopore. 1:1 and 1:2 complex are self-assembled by SC6 and MV^{2+} at the ratio of 1:1 and 1:2, respectively. These two complexes generate distinguishable blockages. The structures of 1:1 and 1:2 complex are depicted based on previous study.[41-44] Counterions are omitted for clarity.

cyclodextrin[37, 46]. Both the translocation and bumping of SC6 cause these characteristic short-lived blockages in Distribution I.

In the next step, the single molecule analysis of 1:1 and 1:2 complexes self-assembled by SC6 and MV^{2+} was carried out via *α*-HL nanopore, respectively. To ensure the formation of 1:1 complex, we mixed the SC6 and MV^{2+} at ratio of $[MV^{2+}]/[SC6]$ = 0.2. The blockages of the mixtures were recorded at -60 mV for 14 minutes. Similar to SC6 alone, the mixture of $[MV^{2+}]/[SC6]$=0.2 generated the short-lived blockages which located in the Distribution I (Fig. 2c-d). However, the mixture of $[MV^{2+}]/[SC6]$=0.2 dramatically increased the frequency of the blockages compared to SC6 alone (Fig. 2a and 2c). The cumulative number of blockages in Distribution I per unit time reveals a linear growth with slop of 94.1 ± 14.1 events/min for the mixture, which is about twice as large as SC6 alone (47.6 ± 5.2 events/min, Fig. 3a, Fig. S3 and Table S1). Since MV^{2+} is too small to induce any blockages (Fig. S3), the dramatical increase of the blockages in Distribution I were ascribed to the 1:1 complex assembled in the mixture of $[MV^{2+}]/[SC6]$=0.2. As shown in Fig. S4, the value of τ_{off} for 1:1 complex is about 0.16 ± 0.01 ms, which is comparable to that for SC6 alone (0.15 ± 0.01 ms). These above results indicate that 1:1 complex is much more prone to traverse the *α*-HL as well as bounce against the *trans*-side opening of the pore than SC6.

To further identify 1:2 complex self-assembled by SC6 and MV^{2+}, large amounts of MV^{2+} (5 equiv) was added into SC6 (1 equiv). Surprisingly, the mixture of $[MV^{2+}]/[SC6]$=5 causes a new region of long-lived blockages with large current amplitudes, where logarithm values of τ_{off} are above -0.6 and I/I_0 are above 0.6 (Fig. 2e-f). The population of the blockages in this region was assigned as Distribution II. When ratio of $[MV^{2+}]$ to [SC6] increased from 0.2 to 5, the event frequencies of Distribution I decreased from 94.1 ± 14.1 events/min to 54.8 ± 6.6 events/min while that of Distribution II increased from 5.3 ± 0.6 events/min to 24.6 ± 2.5 events/min (Fig. 2, Table S1). This result indicates that the long-lived blockages in Distribution II could be ascribed to the formation of 1:2 complex which is self-assembled by SC6 and MV^{2+} at the ratio of 1:2. As shown in Fig.2c-f and Fig. S4, the τ_{off} of the long-lived blockages in Distribution II induced by 1:2 complex is about 0.28 ± 0.02 ms, which was larger than that of short-lived blockages in Distribution I generated by 1:1 complex (0.16 ± 0.01 ms). Meanwhile, the

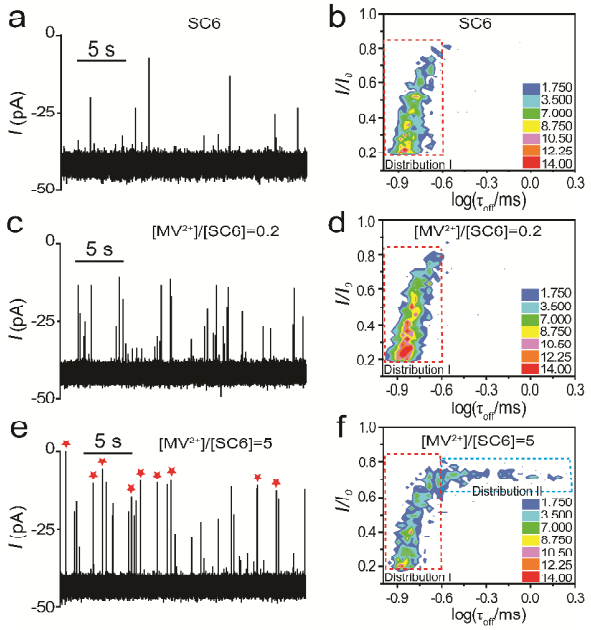

Fig. 2 The current traces and 2D counter plots for SC6 (a, b) and the mixtures of $[MV^{2+}]/[SC6]$ = 0.2 (c, d) and 5 (e, f). The blockages used in the 2D counter plots were obtained by continuously recording for 14 min during the nanopore experiments. The current traces were recorded in solutions containing 1.0 M KCl buffered with 10 mM Tris-HCl (pH=8.0) at -60 mV. The concentrations of SC6 in the mixtures were kept at 150 μM. The red stars indicate the capturing of 1:2 complex by an *α*-HL nanopore.

peak current of Distribution II has a larger value of I/I_0=0.73 ± 0.02 than that of Distribution I which is 0.25 ± 0.02. These dramatical differences are due to the structural difference between assembled binary 1:1 complex and ternary 1:2 complex (see ESI). SC6 adopts cone conformation as it forms binary complexes and the 1,2,3-alternate conformation as it forms ternary complex (Fig.1).[41-44] On account of the large volume, 1:2 complex blocked the majority of the ionic current through the *α*-HL, leading to the blockages in Distribution II. As shown in Fig. 2e, the assembled structure of each 1:2 complex could be readily discriminated in the mixture by *α*-HL nanopore via recognizing the blockages of Distribution II.

By analyzing the mixture of 1:1 and 1:2 complex, the self-assembly process was further investigated. The mixtures were prepared by incubating SC6 with MV^{2+} at the ratio of $[MV^{2+}]$/[SC6] ranging from 0.2 to 5 for at least 20 min, respectively. Then, the mixtures were injected into the *trans* chamber. The final concentrations of SC6 in the mixtures were kept at 150 μM and the potential was held at -60 mV. As shown in Fig. 3a-b and Fig. S5, the events frequencies for the mixtures were carried out by counting the number of events for a time-interval of one minute. Since $[MV^{2+}]$/[SC6] increased gradually from 0.2 to 5, the event frequencies of Distribution I decreased exponentially while that of Distribution II obviously increased (Fig. 3c). When the ratio of $[MV^{2+}]$/[SC6] exceeded 2, the frequency of both Distribution I and Distribution II gradually reached the saturation. These results demonstrate the excessive amount of $[MV^{2+}]$ poises the self-assembly process in favor of 1:2 complex. Moreover, the concentrations of 1:2 complex in the mixtures could be calculated based on the event frequencies of Distribution II (Fig. 3d and Fig. S6). The traditional ^{1}H NMR titration studies suggest that MV^{2+} is encapsulated into the cavity of SC6 to form 1:1 and 1:2 complex (Fig. S7-S9), which confirms our findings.

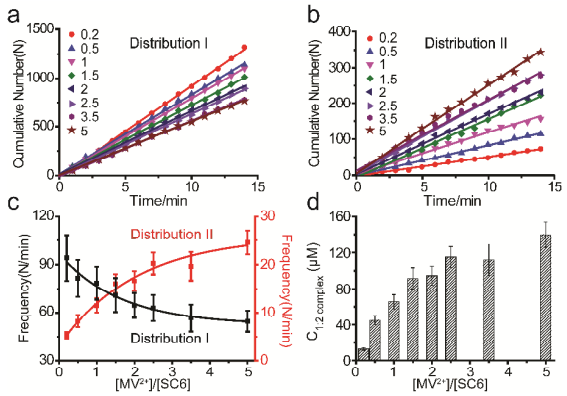

Fig. 3 The cumulative event number *versus* detection time (min) for blockages in Distribution I (a) and Distribution II (b) induced by the mixtures with $[MV^{2+}]$/[SC6] = 0.2, 0.5, 1, 1.5, 2, 2.5, 3.5, 5. The number of events was counted for a time-interval of one minute. The blockages were recorded within 14 min. The slops of fitted lines represented the event frequencies of Distribution I or Distribution II. (c) The event frequencies of Distribution I and Distribution II for the mixtures. The event frequency changes of Distribution I correspond to the left vertical coordinate and that of Distribution II correspond to the right one. (d) Concentration of 1:2 complex *versus* the ratio of $[MV^{2+}]$/[SC6]. Experiments were carried out with SC6 fixed at 150 μM in *trans* chamber containing 1.0 M KCl buffered with 10 mM Tris-HCl (pH=8.0) at potential of -60 mV. Error bars for all plots were based on three separated experiments.

In summary, we investigated self-assembly process operated by host-guest interactions at single molecule level by an *α*-HL nanopore biosensor. The 1:1 and 1:2 complex self-assembled by SC6 and MV^{2+} were discriminated in the mixtures of $[MV^{2+}]$/[SC6] based on their characteristic blockage distributions. Especially, each 1:2 complex could be real-time recognized in the mixtures due to its unique blockage. Furthermore, nanopore-based single molecule study has achieved to monitor the process that 1:1 complex self-assembled into 1:2 complex. The present study reveals that *α*-HL nanopore could be used as a general single molecule method for understanding the self-assembled mechanism of host-guest supramolecules. Studies toward probing and analyzing more sophisticated topological features of self-assembly process in supramolecular system are currently under further investigation in our laboratory.

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Notes and references

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1 J. M. Lehn, *Angew. Chem. Int. Ed.*, 1988, **27**, 89.
2 V. Balzani, A. Credi, F. M. Raymo and J. F. Stoddart, *Angew. Chem. Int. Ed.*, 2000, **39**, 3348.
3 V. Balzani, A. Credi and M. Venturi, *Molecular devices and machines*, Wiley-Vch Weinheim, Germany, 2004.
4 H. Tian and Q. C. Wang, *Chem. Soc. Rev.*, 2006, **35**, 361.
5 E. R. Kay, D. A. Leigh and F. Zerbetto, *Angew. Chem. Int. Ed.*, 2007, **46**, 72.
6 D. A. Uhlenheuer, K. Petkau and L. Brunsveld, *Chem. Soc. Rev.*, 2010, **39**, 2817.
7. B. Shi, P. Zhang, T. Wei, H. Yao, Q. Lin and Y. Zhang, *Chem. Commun.*, 2013, **49**, 7812.

8. Q. Lin, B. Sun, Q. Yang, Y. Fu, X. Zhu, Y. Zhang and T. Wei, *Chem. Commun.*, 2014, 54, 10669.
9 S. Dong, B. Zheng, F. Wang and F. Huang, *Acc. Chem. Res.*, 2014, **47**, 1982.
10 S. Ghosh and L. Isaacs, *J. Am. Chem. Soc.*, 2010, **132**, 4445.
11 D. J. Cram and J. M. Cram, *Science*, 1974, **183**, 803.
12 H. J. Schneider, *Angew. Chem. Int. Ed.*, 1991, **30**, 1417.
13 J. Hu and S. Liu, *Acc. Chem. Res.*, 2014, **47**, 2084.
14 B. Zheng, F. Wang, S. Dong and F. Huang, *Chem. Soc. Rev.*, 2012, **41**, 1621.
15 D. S. Guo and Y. Liu, *Chem. Soc. Rev.*, 2012, **41**, 5907.
16 F. Huang, D. S. Nagvekar, X. Zhou and H. W. Gibson, *Macromolecules*, 2007, **40**, 3561.
17 M. Xue, Y. Yang, X. Chi, Z. Zhang and F. Huang, *Acc. Chem. Res.*, 2012, **45**, 1294.
18 R. Fang, Y. Liu, Z. Wang and X. Zhang, *Polym. Chem.*, 2013, **4**, 900.
19 X. Ma and H. Tian, *Acc. Chem. Res.*, 2014, **47**, 1971.
20 D. H. Qu, F. Y. Ji, Q. C. Wang and H. Tian, *Adv. Mater.*, 2006, **18**, 2035.
21 D. H. Qu, Q. C. Wang, X. Ma and H. Tian, *Chem. -Eur. J.*, 2005, **11**, 5929.
22 L. Song, M. R. Hobaugh, C. Shustak, S. Cheley, H. Bayley and J. E. Gouaux, *Science*, 1996, **274**, 1859.
23 J. J. Kasianowicz, E. Brandin, D. Branton and D. W. Deamer, *Proc. Natl. Acad. Sci.*, 1996, **93**, 13770.
24 Y. L. Ying, D. W. Li, Y. Li, J. S. Lee and Y. T. Long, *Chem. Commun.*, 2011, **47**, 5690.
25 Y. L. Ying, H. Y. Wang, T. C. Sutherland and Y. T. Long, *Small*, 2011, **7**, 87.
26 Y. Wang, D. Zheng, Q. Tan, M. X. Wang and L. Q. Gu, *Nat. Nanotechnol.*, 2011, **6**, 668.
27 Y. L. Ying, J. Zhang, R. Gao and Y. T. Long, *Angew. Chem. Int. Ed.*, 2013, **52**, 13154.
28 L. Mereuta, I. Schiopu, A. Asandei, Y. Park, K. S. Hahm and T. Luchian, *Langmuir*, 2012, **28**, 17079.
29 H. Y. Wang, Y. L. Ying, Y. Li, H.-B. Kraatz and Y. T. Long, *Anal. Chem.*, 2011, **83**, 1746.
30 H. Y. Wang, Z. Gu, C. Cao, J. Wang and Y. T. Long, *Anal. Chem.*, 2013, **85**, 8254.
31 L. Payet, M. Martinho, M. Pastoriza-Gallego, J.-M. Betton, L. Auvray, J. Pelta and J. Mathé, *Anal. Chem.*, 2012, **84**, 4071.
32 D. Rotem, L. Jayasinghe, M. Salichou and H. Bayley, *J. Am. Chem. Soc.*, 2012, **134**, 2781
33 J. Nivala, D. B. Marks and M. Akeson, *Nat. Biotechnol.*, 2013, **31**, 247.
34 S. Wen, T. Zeng, L. Liu, K. Zhao, Y. L. Zhao, X. J. Liu and H. C. Wu, *J. Am. Chem. Soc.*, 2011, **133**, 18312.
35 C. Yang, L. Liu, T. Zeng, D. W. Yang, Z. Y. Yao, Y. L. Zhao and H. C. Wu, *Anal. Chem.*, 2013, **85**, 7302.
36 Y. Liu, Y. L. Ying, H. Y. Wang, C. Cao, D. W. Li, W. Q. Zhang and Y. T. Long, *Chem. Commun.*, 2013, **49**, 6584.
37 L. Q. Gu, O. Braha, S. Conlan, S. Cheley and H. Bayley, *Nature*, 1999, **398**, 686.
38 Y. L. Ying, J. Zhang, F. N. Meng, C. Cao, X. Yao, I. Willner, H. Tian and Y. T. Long, *Sci. Rep.*, 2013, **3**, 1662.
39 W. W. Li, T. D. W. Claridge, Q. Li, M. R. Wormald, B. G. Davis and H. Bayley, *J. Am. Chem. Soc.*, 2011, **133**, 1987.
40 P. A. Gurnev, D. Harries, V. A. Parsegian and S. M. Bezrukov, *Chem. Phys. Chem.*, 2009, **10**, 1445.
41 R. Castro, L. A. Godínez, C. M. Criss and A. E. Kaifer, *J. Org. Chem.*, 1997, **62**, 4928.
42 N. Douteau-Guével, F. Perret, A. W. Coleman, J.-P. Morel and N. Morel-Desrosiers, *J. Chem. Soc., Perkin Trans. 2*, 2002, 524.
43 S. A. Fernandes, L. F. Cabeça, A. J. Marsaioli and E. de Paula, *J. Incl. Phenom. Macrocycl. Chem.*, 2007, **57**, 395.
44 Y. Sueishi, N. Inazumi and T. Hanaya, *J. Phys. Org. Chem.*, 2005, **18**, 448.
45 N. Douteau-Guével, A. W. Coleman, J. P. Morel and N. Morel Desrosiers, *J. Chem. Soc., Perkin Trans. 2*, 1999, 629.
46 L. Q. Gu, S. Cheley and H. Bayley, *Science*, 2001, **291**, 636.