Molecular Machines Hot Paper

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An Autonomously Reciprocating Transmembrane Nanoactuator

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Abstract: *Biological molecular machines operate far from equilibrium by coupling chemical potential to repeated cycles of dissipative nanomechanical motion. This principle has been exploited in supramolecular systems that exhibit true machine behavior in solution and on surfaces. However, designed membrane-spanning assemblies developed to date have been limited to simple switches or stochastic shuttles, and true machine behavior has remained elusive. Herein, we present a transmembrane nanoactuator that turns over chemical fuel to drive autonomous reciprocating (back-and-forth) nanomechanical motion. Ratcheted reciprocating motion of a DNA/PEG copolymer threaded through a single α-hemolysin pore was induced by a combination of DNA strand displacement processes and enzyme-catalyzed reactions. Ion-current recordings revealed saw-tooth patterns, indicating that the assemblies operated in autonomous, asymmetric cycles of conformational change at rates of up to one cycle per minute.*

Efforts to mimic the nanomechanical motions of natural molecular machines have developed from two-state switchable molecules to mechanically interlocked supramolecular systems that may operate free in solution or anchored to a surface.[1] The realization of emergent behavior in such compartmentalized systems that operate "far from equilibrium" is an important step in developing a degree of control over matter comparable to that seen in living systems.[1a,g,2] DNA-based[3] and wholly synthetic shuttles and walkers that mimic the processivity of polymerases[4] and even the ribosome protein factory have been demonstrated.[5] Repetitive mechanical cycles in response to photoirradiation or electrical stimulation have also been achieved,[6] whereas arrays of molecular shuttles or rotary motors have been shown to be capable of performing macroscopic work.[7] Stoddart and co-workers have recently developed a supramolecular system in which up to two macrocycles can be "pumped" onto a molecular thread, forming a mechanically interlocked rotaxane[8] in response to repeated switching of the redox conditions.[1g] In nature, lipid membranes facilitate compartmentalization, allowing potential gradients to be coupled to cycles of conformational change in membrane-spanning molecular machines. For example, transmembrane pumps[9] couple rotational[10] or reciprocating (back-and-forth) nanomechanical cycles[11] to transmembrane potentials.[11a,12]

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Both passive and stimuli-responsive nanomechanical gating of transmembrane channels has been demonstrated in synthetic systems[13] and re-engineered nanopore proteins.[14] Similarly, the stochastic walking of a small molecule bound to the inside of an individual mutant protein pore[15] and the mediated transport of DNA through a nanopore have been reported.[16] However, the realization of true machine behavior[1a,d] (e.g., the autonomous conversion of potential energy into nanomechanical motion) in a designed transmembrane system has yet to be demonstrated.[1c]

Herein, we have constructed a transmembrane supramolecular assembly (Figure 1) that operates as a prototypical transmembrane molecular machine by turning over chemical fuels to generate reciprocating nanomechanical motion across a lipid membrane under an applied potential. The nanomechanical operation of the device was interrogated at the single-molecule level by monitoring stepwise changes in the transmembrane ion current flowing through a nanopore (Figure 1, Figure 2, and Figure 3).

We sought to integrate the lessons learned from biological, synthetic, and DNA-based nanomachines to construct a transmembrane molecular machine. We prepared a mechanically interlocked transmembrane rotaxane by capturing a streptavidin-capped DNA–polyethylene glycol (DNA–PEG) copolymer molecule (from the trans well) inside a single α-HL nanopore under an applied transmembrane potential of −140 mV (Figure 1a).[17] A single-stranded DNA primer present on the opposite (grounded) side of the bilayer was hybridized to the thread sequence protruding from the pore (Figure 1b). We reasoned that reciprocating (back-and-forth) nanomechanical motion could be induced in the captured DNA thread by repeated cycles of enzyme-catalyzed primer degradation followed by strand displacement[18] (Figure 1b). Hence, stepwise degradation of the rotaxane-bound primer should result in the movement of the threaded DNA strand in the direction of the externally applied transmembrane voltage of +25 mV, while also exposing a toehold binding site on the thread strand for an incoming primer 7 fuel molecule. Strand displacement of the degraded rotaxane-bound primer by a primer 7 molecule pulls the threaded DNA strand against the applied field (as the nanopore is too small to permit the threading of double-stranded DNA),[19] thereby constituting a power stroke. The nanomechanical cycle depicted in Figure 1b mimics biological transmembrane machines, which also couple transmembrane potentials through repetitious, asymmetric cycles of nanomechanical conformational change powered by the turnover of phosphate-based chemical fuels.[20]

It has previously been shown that the movement of biopolymers threaded through nanopores can be monitored by studying the stepwise changes in the transmembrane ion current under an applied potential.[17,21] DNA blocks the ion

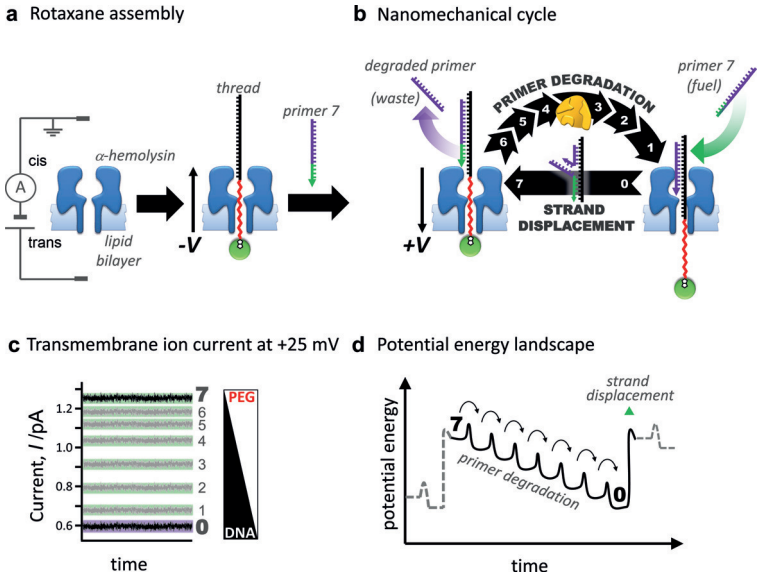

Figure 1. a) Experimental setup and transmembrane rotaxane assembly. A DNA–PEG copolymer strand was threaded from the trans well under a transmembrane potential of −140 mV into a single α-hemolysin (α-HL) protein nanopore inserted in a lipid bilayer. The voltage was reduced to −28 mV to allow a primer present on the opposite side of the bilayer (in the cis well) to form the interlocked assembly. The structures and sequences of the DNA strands are given in the Supporting Information. b) Asymmetric nanomechanical cycle obtained under an applied potential of +25 mV with respect to the ground electrode, in which an enzyme (yellow) degrades the primer to reveal a toehold binding site on the thread strand for an incoming primer 7 fuel strand. Subsequent DNA strand invasion and displacement of the degraded primer by the primer 7 fuel constitutes a power stroke that pulls the thread against the applied potential, completing the nanomechanical cycle. c) The ion current flowing through the nanopore was dependent on the length of the primer at a potential of +25 mV owing to the different PEG/DNA ratios threaded through the pore in each nanomechanical state (DNA blocks the ion current more than PEG). Intermediate current states were observed for intermediate primer lengths (Figures 2 and S4). d) Potential energy profile corresponding to the proposed nanomechanical cycle.

current more than PEG; thus, in accord with previous works, a low current was recorded in state 0 (low PEG/DNA ratio), whereas a high current was observed in state 7 (high PEG/DNA ratio; Figure 1c). Furthermore, the ion current can reveal changes in the nanomechanical state of the assembly with single-nucleotide resolution (states 1 to 6 in Figure 1c; see also the Supporting Information Figure S4).[17]

Initial attempts used exonuclease III (Figure S5a) or KF(exo-) in the presence of pyrophosphate[22] ($P_2O_7^{4-}$; Figure S7) to degrade the 3′ end of the rotaxane-bound primer as part of the cycle shown in Figure 1b. However, the corresponding ion-current recordings did not yield the desired behavior (see Figures S5b, S8 and the associated discussion). Pleasingly, the addition of Mn^{2+} to the reaction buffer and the replacement of $P_2O_7^{4-}$ with $V_2O_7^{4-}$ in the presence of DNA polymerase allowed true machine behavior to be realized in the transmembrane assembly.

The autonomous operation of the nanomechanical cycle was monitored in a single transmembrane rotaxane under these conditions and revealed a saw-tooth pattern in the transmembrane ion current (Figure 2) that mirrored the changes in the potential energy landscape of the assembly (Figure 1d). Five cycles composed of 24 nanomechanical state changes over five minutes of operation were observed owing to changes in the DNA/PEG ratio threaded through the pore, commensurate with changes in primer length. The ion-current traces indicate that the bases were removed from the rotaxane-bound primer in a stepwise fashion by DNA polymerase catalyzed pyrovanadolysis (downward steps in Figure 2a, iv; Figure S12).[23] It should be noted that the overhanging 3′ end of the thread strand is not a substrate for DNA polymerase, precluding its undesirable degradation. Consistent with previous observations,[17] the binding of DNA polymerase to the 3′ end of the rotaxane-bound primer also resulted in transient spikes in the current signal (Figure 2a, iii) owing to changes in the PEG/DNA ratio threaded through the pore. Power strokes owing to strand displacement of the degraded primer at the end of each nanomechanical cycle resulted in large upward steps in the ion current as the assembly was returned to the initial state (Figure 2a, ii).

Data collated from 17 cycles across four experiments (Figure S13) revealed the most common dwell time in each state as approximately 15 seconds (Figure 3a). Base removal by pyrovanadolysis usually proceeded base by base, although up to four bases were occasionally removed during a single enzyme-binding event (Figure 3b). Furthermore, most power strokes occurred after the primer strand had been degraded by seven bases (Figure 3c). However, the kinetics of strand displacement were not tightly coupled to the kinetics of primer degradation, and thus it was possible for the primer to be degraded beyond state 0 before strand displacement occurred, or for the power stroke to occur prematurely. Examples of such events can be seen in Figure S13 (top, displacement from state −1) and the third cycle shown in Figure 2a (displacement from state 1). Transient spikes as the current briefly returned to state 7 were also observed. These likely correspond to toehold-binding events without strand displacement (Figure 2a,i and Figure S13). There were 15 failed compared to 16 successful strand displacements across the 17 cycles studied. Indeed, such non-ideal behavior is characteristic of natural biological molecular machines,[1c] and to be expected when observing any molecular machine at the single-molecule level; 33% of the steps exhibited by ATP synthase under a proton gradient are retrograde,[24] whereas single-base mutations introduced by DNA polymerases during DNA replication may approach 5% in the absence of subsequent proof-reading processes.[25]

Our nanomechanical device constitutes a true machine that operates by an information ratchet mechanism,[1a,26] as strand displacement can only occur after primer degradation has revealed a long enough region of the thread to serve as a toehold for the incoming fuel strand.[18a] Strand displacement actuates the threaded DNA–PEG copolymer by approximately 2.4 nm (seven bases of ca. 0.34 nm each) plus

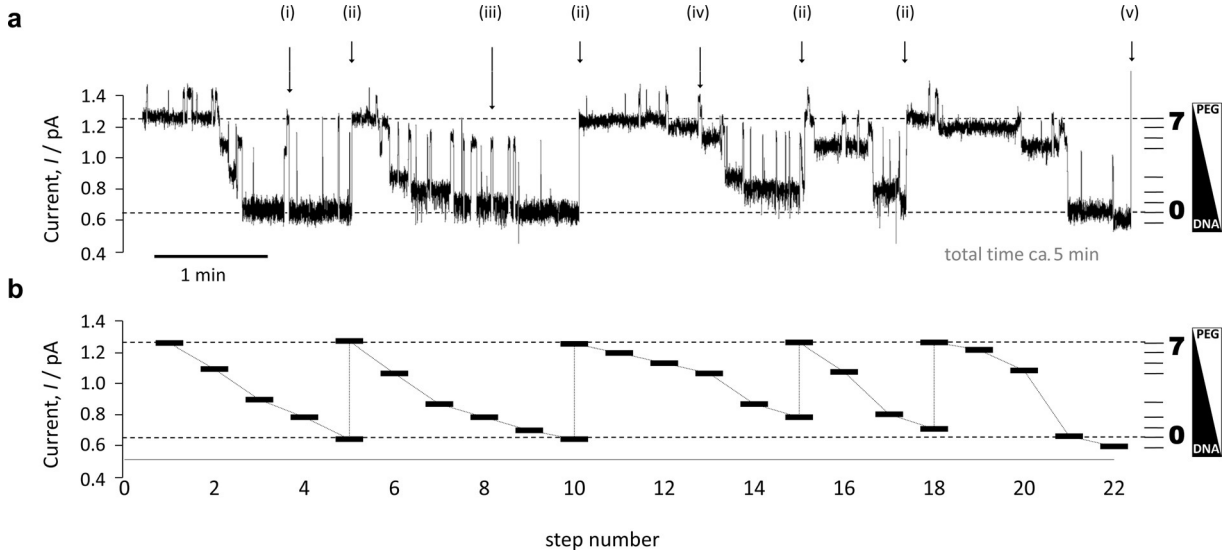

Figure 2. a) Continuous real-time ion-current trace of a single transmembrane assembly showing five asymmetric cycles of a single rotaxane driven by DNA hybridization and pyrovanadolysis during autonomous operation. i) Failed strand displacement event. ii) Strand displacement by the full-length primer fuel. iii) An enzyme binding event without pyrovanadolysis. iv) An enzyme binding event that resulted in pyrovanadolysis. v) Disassembly of the rotaxane after the rotaxane-bound primer has been degraded to state −1. b) Average currents extracted from the raw data correspond to nanomechanical states associated with changes in primer length during operation. Additional experiments are described in the Supporting Information. At the start of the experiment, the buffer in each well contained KCl (150 mM), Tris-HCl (33 mM), and $MgCl_2$ (8.3 mM) at pH 8. Furthermore, the live well contained the streptavidin-capped DNA–PEG copolymer (800 nM), while the cis well contained primer 7 (5 μM). Once a rotaxane had been formed, $MnCl_2$ was added to each well to a final concentration of 1 mM, while $Na_4V_2O_7$ and KF(exo-) DNA polymerase were added to the cis well to give final concentrations of 2 mM and 7 nM, respectively. The experiment was conducted at 21 ± 2 °C.

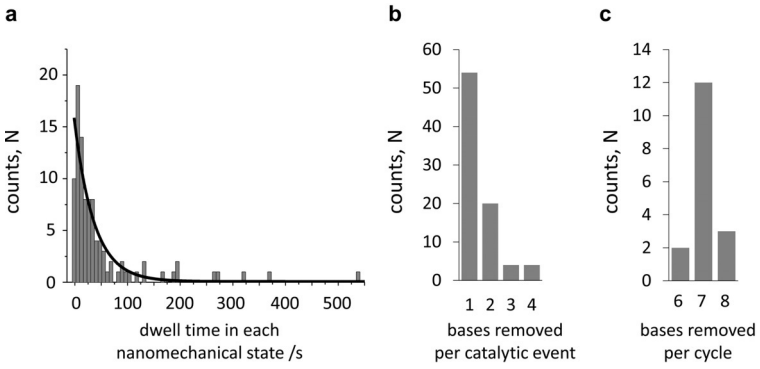

Figure 3. a) Histogram of the observed dwell times in each nanomechanical state fitted to a single exponential decay (black line), giving the most probable dwell time as 15.2 s. b) The number of bases removed in enzyme-binding events in which pyrovanadolysis occurred. c) The number of bases removed per cycle. Data collated from four experiments over 17 cycles.

an additional 4 nm owing to the double-stranded region of the rotaxane in state 7 residing mostly outside the pore vestibule, whereas it mostly resides inside the vestibule in state 0 (Figure S4). Thus, the turnover of the primer chemical fuel (involving the cleavage of up to seven phosphate bonds) results in an asymmetric reciprocating actuation of about 6 nm. Furthermore, the approach of driving the DNA polymerase in reverse using Mn^{2+} and pyrovanadate might be useful to control DNA threading in nanopore sequencing.[14e,f,21b]

We also examined a complementary mode of reciprocating operation that employed KF(exo-) to extend the primer and a nicking enzyme to excise a short extended region. However, whereas nicking enzymes have proven useful in the operation of DNA walkers,[4d] the efficiency of this mode was limited by the low activity of the nicking enzymes examined (Figures S16–S18).

In summary, we have developed a supramolecular nanoactuator that dissipates the energy from chemical fuel by inducing reciprocating (back-and-forth) motions of a DNA–PEG copolymer molecule threaded through a transmembrane protein nanopore. Our assembly shares several characteristics with biological transmembrane machines. It is mechanically interlocked[28] and operates under a transmembrane potential[11,20] while turning over nucleic acid fuel molecules (see also Figure S15). Most importantly, it utilizes information ratchet mechanisms to organize non-directional chemical processes to elicit cycles of directional nanoactuation despite the constant non-directional thermal forces experienced at nanometer length scales.[27] The asymmetry of the conformational cycle was confirmed by the saw-tooth patterns in the ion current flowing through a single nanopore assembly (equivalent to an oscillating potential). Similarly, biological nanomechanical transmembrane pumps also operate autonomously with an oscillating potential that emerges from asymmetric cycles of conformational change driven by the turnover of chemical fuels and/or the exploitation of non-

fluctuating potential gradients.[10,11] This contrasts with the pumping that has been demonstrated in synthetic systems that require external (chemical or electromagnetic) oscillating potentials.[1e,g,29] Indeed, the grand aim of using biologically inspired autonomous mechanisms to achieve transmembrane pumping in a fully synthetic molecular machine has yet to be demonstrated. Hence, we hope that our system, which autonomously transduces chemical potential into cycles of reciprocating nanomechanical motion, will serve as an early prototype in the development of synthetic transmembrane architectures that approach the sophistication and functionality of biological molecular machines.[2a]

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[1] a) E. R. Kay, D. A. Leigh, F. Zerbetto, *Angew. Chem. Int. Ed.* **2007**, *46*, 72 – 191; *Angew. Chem.* **2007**, *119*, 72 – 196; b) M. G. L. van den Heuvel, C. Dekker, *Science* **2007**, *317*, 333 – 336; c) S. Erbas-Cakmak, D. A. Leigh, C. T. McTernan, A. L. Nussbaumer, *Chem. Rev.* **2015**, *115*, 10081 – 10206; d) W. R. Browne, B. L. Feringa, *Nat. Nanotechnol.* **2006**, *1*, 25 – 35; e) D. A. Leigh, J. K. Y. Wong, F. Dehez, F. Zerbetto, *Nature* **2003**, *424*, 174 – 179; f) J. D. Badjić, V. Balzani, A. Credi, S. Silvi, J. F. Stoddart, *Science* **2004**, *303*, 1845 – 1849; g) C. Cheng, P. R. McGonigal, S. T. Schneebeli, H. Li, N. A. Vermeulen, C. Ke, J. F. Stoddart, *Nat. Nanotechnol.* **2015**, *10*, 547 – 553; h) E. R. Kay, D. A. Leigh, *Angew. Chem. Int. Ed.* **2015**, *54*, 10080 – 10088; *Angew. Chem.* **2015**, *127*, 10218 – 10226.

[2] a) E. Mattia, S. Otto, *Nat. Nanotechnol.* **2015**, *10*, 111 – 119; b) M. N. Chatterjee, E. R. Kay, D. A. Leigh, *J. Am. Chem. Soc.* **2006**, *128*, 4058 – 4073.

[3] a) N. C. Seeman, *Trends Biochem. Sci.* **2005**, *30*, 119 – 125; b) S.-S. Jester, M. Famulok, *Acc. Chem. Res.* **2014**, *47*, 1700 – 1709; c) F. Wang, C.-H. Lu, I. Willner, *Chem. Rev.* **2014**, *114*, 2881 – 2941.

[4] a) S. Venkataraman, R. M. Dirks, P. W. K. Rothemund, E. Winfree, N. A. Pierce, *Nat. Nanotechnol.* **2007**, *2*, 490 – 494; b) S. F. J. Wickham, J. Bath, Y. Katsuda, M. Endo, K. Hidaka, H. Sugiyama, A. J. Turberfield, *Nat. Nanotechnol.* **2012**, *7*, 169 – 173; c) M. von Delius, E. M. Geertsema, D. A. Leigh, *Nat. Chem.* **2010**, *2*, 96 – 101; d) J. Bath, S. J. Green, A. J. Turberfield, *Angew. Chem. Int. Ed.* **2005**, *44*, 4358 – 4361; *Angew. Chem.* **2005**, *117*, 4432 – 4435; e) S. Green, J. Bath, A. Turberfield, *Phys. Rev. Lett.* **2008**, *101*, 237801.

[5] a) B. Lewandowski, G. De Bo, J. W. Ward, M. Papmeyer, S. Kuschel, M. J. Aldegunde, P. M. E. Gramlich, D. Heckmann, S. M. Goldup, D. M. D'Souza, A. E. Fernandes, D. A. Leigh, *Science* **2013**, *339*, 189 – 193; b) Y. He, D. R. Liu, *Nat. Nanotechnol.* **2010**, *5*, 778 – 782.

[6] a) G. Ragazzon, M. Baroncini, S. Silvi, M. Venturi, A. Credi, *Nat. Nanotechnol.* **2015**, *10*, 70 – 75; b) T. Kudernac, N. Ruangsupapichat, M. Parschau, B. Macia, N. Katsonis, S. R. Harutyunyan, K.-H. Ernst, B. L. Feringa, *Nature* **2011**, *479*, 208 – 211; c) H. L. Tierney, C. J. Murphy, A. D. Jewell, A. E. Baber, E. V. Iski, H. Y. Khodaverdian, A. F. McGuire, N. Klebanov, E. C. H. Sykes, *Nat. Nanotechnol.* **2011**, *6*, 625 – 629; d) N. Ruangsupapichat, M. M. Pollard, S. R. Harutyunyan, B. L. Feringa, *Nat. Chem.* **2011**, *3*, 53 – 60.

[7] a) R. Eelkema, M. M. Pollard, J. Vicario, N. Katsonis, B. S. Ramon, C. W. M. Bastiaansen, D. J. Broer, B. L. Feringa, *Nature* **2006**, *440*, 163 – 163; b) J. Berná, D. A. Leigh, M. Lubomska, S. M. Mendoza, E. M. Perez, P. Rudolf, G. Teobaldi, F. Zerbetto, *Nat. Mater.* **2005**, *4*, 704 – 710; c) B. K. Juluri, A. S. Kumar, Y. Liu, T. Ye, Y.-W. Yang, A. H. Flood, L. Fang, J. F. Stoddart, P. S. Weiss, T. J. Huang, *ACS Nano* **2009**, *3*, 291 – 300; d) S. Iamsaard, S. J. Aßhoff, B. Matt, T. Kudernac, J. J. L. M. Cornelissen, S. P. Fletcher, N. Katsonis, *Nat. Chem.* **2014**, *6*, 229 – 235; e) X. Shen, C. Viney, E. R. Johnson, C. Wang, J. Q. Lu, *Nat. Chem.* **2013**, *5*, 1035 – 1041; f) G. Du, E. Moulin, N. Jouault, E. Buhler, N. Giuseppone, *Angew. Chem. Int. Ed.* **2012**, *51*, 12504 – 12508; *Angew. Chem.* **2012**, *124*, 12672 – 12676.

[8] C. Dietrich-Buchecker, M. C. Jimenez-Molero, V. Sartor, J. P. Sauvage, *Pure Appl. Chem.* **2003**, *75*, 1383 – 1393.

[9] D. C. Gadsby, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 344 – 352.

[10] H. Noji, R. Yasuda, M. Yoshida, K. Kinosita, *Nature* **1997**, *386*, 299 – 302.

[11] a) R. G. Efremov, R. Baradaran, L. A. Sazanov, *Nature* **2010**, *465*, 441 – 445; b) C. Lee, H. J. Kang, C. von Ballmoos, S. Newstead, P. Uzdavinys, D. L. Dotson, S. Iwata, O. Beckstein, A. D. Cameron, D. Drew, *Nature* **2013**, *501*, 573 – 577.

[12] a) E. Richard, C. Miller, *Science* **1990**, *247*, 1208 – 1210; b) S. Geibel, J. H. Kaplan, E. Bamberg, T. Friedrich, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 964 – 969.

[13] a) M. R. Ghadiri, J. R. Granja, L. K. Buehler, *Nature* **1994**, *369*, 301 – 304; b) S. Matile, A. Vargas Jentzsch, J. Montenegro, A. Fin, *Chem. Soc. Rev.* **2011**, *40*, 2453 – 2474; c) M. Langecker, V. Arnaut, T. G. Martin, J. List, S. Renner, M. Mayer, H. Dietz, F. C. Simmel, *Science* **2012**, *338*, 932 – 936.

[14] a) A. Koçer, M. Walko, W. Meijberg, B. L. Feringa, *Science* **2005**, *309*, 755 – 758; b) D. Wendell, P. Jing, J. Geng, V. Subramaniam, T. J. Lee, C. Montemagno, P. Guo, *Nat. Nanotechnol.* **2009**, *4*, 765 – 772; c) S. Biswas, K. Kinbara, T. Niwa, H. Taguchi, N. Ishii, S. Watanabe, K. Miyata, K. Kataoka, T. Aida, *Nat. Chem.* **2013**, *5*, 613 – 620; d) J. J. Kasianowicz, E. Brandin, D. Branton, D. W. Deamer, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13770 – 13773; e) D. Branton, D. W. Deamer, A. Marziali, H. Bayley, S. A. Benner, T. Butler, M. Di Ventra, S. Garaj, A. Hibbs, X. Huang, S. B. Jovanovich, P. S. Krstic, S. Lindsay, X. S. Ling, C. H. Mastrangelo, A. Meller, J. S. Oliver, Y. V. Pershin, J. M. Ramsey, R. Riehn, G. V. Soni, V. Tabard-Cossa, M. Wanunu, M. Wiggin, J. A. Schloss, *Nat. Biotechnol.* **2008**, *26*, 1146 – 1153; f) E. A. Manrao, I. M. Derrington, A. H. Laszlo, K. W. Langford, M. K. Hopper, N. Gillgren, M. Pavlenok, M. Niederweis, J. H. Gundlach, *Nat. Biotechnol.* **2012**, *30*, 349 – 353.

[15] G. S. Pulcu, E. Mikhailova, L.-S. Choi, H. Bayley, *Nat. Nanotechnol.* **2015**, *10*, 76 – 83.

[16] L. Franceschini, M. Soskine, A. Biesemans, G. Maglia, *Nat. Commun.* **2013**, *4*, 2415.

[17] a) S. L. Cockroft, J. Chu, M. Amorin, M. R. Ghadiri, *J. Am. Chem. Soc.* **2008**, *130*, 818 – 820; b) J. Chu, M. González-López, S. L. Cockroft, M. Amorin, M. R. Ghadiri, *Angew. Chem. Int. Ed.* **2010**, *49*, 10106 – 10109; *Angew. Chem.* **2010**, *122*, 10304 – 10307.

[18] a) D. Y. Zhang, G. Seelig, *Nat. Chem.* **2011**, *3*, 103 – 113; b) R. R. F. Machinek, T. E. Ouldridge, N. E. C. Haley, J. Bath, A. J. Turberfield, *Nat. Commun.* **2014**, *5*, 5324.

[19] L. Song, M. R. Hobaugh, C. Shustak, S. Cheley, H. Bayley, J. E. Gouaux, *Science* **1996**, *274*, 1859 – 1865.

[20] J. Weber, *Nat. Chem. Biol.* **2010**, *6*, 794 – 795.

[21] a) J. Nivala, D. B. Marks, M. Akeson, *Nat. Biotechnol.* **2013**, *31*, 247 – 250; b) G. M. Cherf, K. R. Lieberman, H. Rashid, C. E. Lam, K. Karplus, M. Akeson, *Nat. Biotechnol.* **2012**, *30*, 344 – 348.
[22] Q. Liu, S. S. Sommer, *Nucleic Acids Res.* **2002**, *30*, 598 – 604.
[23] B. Akabayov, A. W. Kulczyk, S. R. Akabayov, C. Theile, L. W. McLaughlin, B. Beauchamp, A. M. van Oijen, C. C. Richardson, *J. Biol. Chem.* **2011**, *286*, 29146 – 29157.
[24] R. Watanabe, K. V. Tabata, R. Iino, H. Ueno, M. Iwamoto, S. Oiki, H. Noji, *Nat. Commun.* **2013**, *4*, 1631.
[25] M. F. Goodman, B. Tippin, *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 101 – 109.
[26] V. Serreli, C.-F. Lee, E. R. Kay, D. A. Leigh, *Nature* **2007**, *445*, 523 – 527.
[27] R. D. Astumian, *Phys. Chem. Chem. Phys.* **2007**, *9*, 5067.
[28] C. A. Schalley, K. Beizai, F. Vögtle, *Acc. Chem. Res.* **2001**, *34*, 465 – 476.
[29] a) Z. Siwy, A. Fuliński, *Phys. Rev. Lett.* **2002**, *89*, 198103; b) V. Bermudez, N. Capron, T. Gase, F. G. Gatti, F. Kajzar, D. A. Leigh, F. Zerbetto, S. Zhang, *Nature* **2000**, *406*, 608 – 611.

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