THE JOURNAL OF PHYSICAL CHEMISTRY Letters

Letter

pubs.acs.org/JPCL

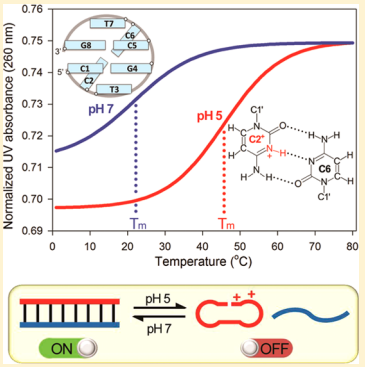
An Extraordinarily Stable DNA Minidumbbell

Pei Guo and Sik Lok Lam*

Department of Chemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

Supporting Information

ABSTRACT: The minidumbbell (MDB) is a new type of native DNA structure. At neutral pH, two TTTA or CCTG repeats can fold into the highly compact MDB with a melting temperature of ~22 °C. Owing to the relatively low thermodynamic stability, MDBs have been proposed to be the structural intermediates that lead to efficient DNA repair escape and thus repeat expansions. In this study, we reveal that two CCTG repeats can also form an extraordinarily stable MDB with a melting temperature of ~46 °C at pH 5.0. This unusual stability predominantly results from the formation of a three hydrogen bond C^+·C mispair between the two minor groove cytosine residues. Due to the drastic stability change, the CCTG MDB, when combined with its complementary sequence, shows instant and complete structural conversions when the pH switches between 5.0 and 7.0, making the system serve as a simple and efficient pH-controlled molecular switch.



Non-B DNA structures such as hairpins, triplexes, and quadruplexes have been shown to participate in many biological processes.[1,2] The minidumbbell (MDB) is a new form of non-B structures formed by native DNA sequences.[3−5] The reported MDB formed by two TTTA or CCTG repeats has a melting temperature (T_m) of ~22 °C at pH 7.0. This relatively low thermodynamic stability makes the MDBs formed in a repeat tract convert to other structures easily, providing a potential pathway for efficient DNA repair escape and thus TTTA and CCTG repeat expansions in *Staphylococcus aureus* and myotonic dystrophy type 2 patients, respectively.[3,4,6,7] In addition to their biological significance, the unique structural features of non-B DNAs have also made them become fascinating materials in the fields of nanotechnology, biotechnology, and material science in recent years.[2,8] As the MDBs have the advantage of being small and compact in size, they can be a promising candidate in constructing simple and efficient molecular systems. Therefore, an understanding of the detailed structures and thermodynamic stabilities of MDBs is essential in advancing their applications.

The reported TTTA and CCTG MDBs both contain two type II tetraloops in which the first (L1/L1′) and fourth loop residues (L4/L4′) form L1-L4/L1′-L4′ loop-closing base pair, the second loop residues (L2 and L2′) sit in the minor groove, and the third loop residues (L3 and L3′) stack on L1-L4 and L1′-L4′, respectively (Figure 1A,B). These MDBs show extensive stabilizing loop−loop interactions,[5] making them easily distinguishable from larger dumbbell structures in which there are no loop−loop interactions.[9−11] In particular, the two L2 and L2′ residues have been found to participate in most of the loop−loop interactions,[5] as they not only stack (Figure 1A) or form a mispair (Figure 1B) with each other, but also form hydrogen bonds with the L1-L4/L1′-L4′ base pair and/or the phosphodiester backbone of the other loop. In the CCTG MDB, the two minor groove cytosine residues form a C·C mispair with a pairing geometry containing one/two hydrogen bond(s) or Na^+-mediated electrostatic interactions.[5] Although C·C is known to be the least stable DNA mispair at neutral pH,[12] it can form a more stable C^+·C or C·C^+ mispair upon protonating one of the cytosine residues at acidic pH.[13] Therefore, we speculate that the stability of the CCTG MDB can be improved by the formation of a $C2^+$·C6 or C2·$C6^+$ mispair in the minor groove. In this study, we aim to determine the effects of pH on the structure and thermodynamic stability of the CCTG MDB using high resolution nuclear magnetic resonance (NMR) and ultraviolet (UV) absorption spectroscopy.

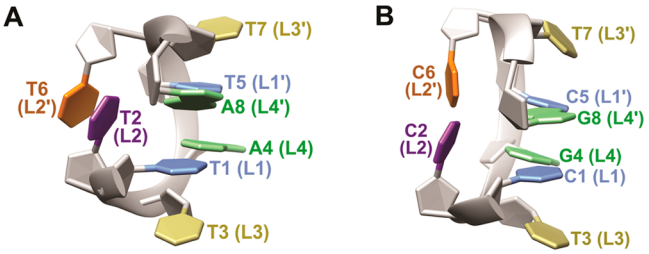


Figure 1. Averaged structures of (A) TTTA MDB (PDB ID: 5GWQ) and (B) CCTG MDB (PDB ID: 5GWL). The two minor groove residues T2 and T6 stack with each other in the TTTA MDB, whereas C2 and C6 form a mispair in the CCTG MDB.

First, we used the temperature-dependent UV absorbance data to determine the thermodynamic stabilities of the CCTG MDBs at different pH values. Table 1 summarizes the extracted thermodynamic parameters, including the T_m, Gibbs free energy change ($\Delta G°$), changes in enthalpy ($\Delta H°$), and entropy

Received: June 29, 2017
Accepted: July 11, 2017
Published: July 11, 2017

DOI: 10.1021/acs.jpclett.7b01666
J. Phys. Chem. Lett. 2017, 8, 3478−3481

Table 1. Thermodynamic Parameters of the CCTG MDB at Different pH Values[a]

pH	T_m (°C)	$\Delta H°$ (kcal·mol^{-1})	$\Delta S°$ (cal·K^{-1}·mol^{-1})	$\Delta G°$ (kcal·mol^{-1})
8.0	17 ± 2	−19 ± 1	−64 ± 3	0.5 ± 0.1
7.0	22.1 ± 0.4	−21 ± 1	−72 ± 4	0.20 ± 0.04
5.0	45.9 ± 0.3	−26 ± 1	−82 ± 4	−1.7 ± 0.1

[a]The uncertainties were determined from the standard deviations of three replicate UV measurements.

($\Delta S°$) at pH 8.0, 7.0, and 5.0. Upon lowering the pH from 8.0 to 5.0, the T_m was found to increase by ∼29 °C with the $\Delta G°$ reduced by ∼2.2 kcal·mol^{-1}. The more negative $\Delta H°$ at pH 5.0 suggests there are more stabilizing interactions. As revealed from the ^{31}P and ^{1}H NMR spectral features, the MDB became unstable again at pH below 5.0 and fully unfolded at pH 2.0 (Figure S1).

Then, we studied the solution structural features of the CCTG MDB at pH 5.0 in order to elucidate the underlying reasons for the drastic gain in stability. In this MDB, C2 was found to be protonated, resulting in a C2$^+$·C6 mispair in the minor groove. Surprisingly, we also found that C1 was protonated, forming a C1$^+$−G4 Hoogsteen base pair (Figure 2A). The two type II loops in this MDB are evidenced by the unusually downfield C2$^+$ and C6 ^{31}P signals and the unusually upfield T3 and T7 ^{31}P/H7 signals (Figure 2B), which are consistent with the chemical shifts of the corresponding residues in the MDB at pH 7.0.[3] The NOEs of G8 H8−C1$^+$ H5 and G8 H1′/H2′/H2″-C1$^+$ H6 support the presence of a 3′-5′ terminal stacking (Figure 2C), further consolidating the formation of the CCTG MDB. The C1$^+$−G4 Hoogsteen base pair is supported by the strong NOE between C1$^+$ H3 and G4 H8 (Figure 2D). In addition, the downfield chemical shifts of C1$^+$ H3/H41/H42 (16.9/10.2/9.0 ppm) (Figure 2D) also agree with those of the protonated cytosine residues in the reported C$^+$−G Hoogsteen base pairs.[14,15] No protonation of C5 was found and C5−G8 formed a Watson−Crick base pair, as evidenced by the NOEs of G8 H1−C5 H41/H42 and the chemical shifts of C5 H41/H42 (8.5/7.3 ppm) and G8 H1 (13.0 ppm) (Figure 2E). The signal intensity of G4 H1 was also found to be weaker than that of G8 H1 (Figure S2), agreeing with the formation of the C1$^+$−G4 Hoogsteen and C5−G8 Watson−Crick base pairs (Figure 2D,E). There is a protonation of C1 but not C5 probably because the favorable interaction between the positively charged cytosine and the negative π electron cloud of the stacked base[16] from the terminal C1$^+$ and G8 is more crucial toward the MDB formation than that from the nonterminal G4 and C5$^+$.

In this MDB, the protonation of C2 is evidenced by the downfield amino proton signals of C2$^+$ H41 at 9.8 ppm and H42 at 9.3 ppm (Figure 2F), which agree with the corresponding proton chemical shifts of protonated cytosine residues.[17] The other minor groove residue C6 was not protonated, as supported by the chemical shifts of C6 H41 at 8.5 ppm and H42 at 6.9 ppm (Figure 2F). Because both C2$^+$ and C6 sit in the minor groove and their glycosidic bonds are aligned in nearly opposite directions,[5] this provides a unique opportunity for the formation of the C2$^+$·C6 mispair with the C2$^+$ O2−C6 H41, C2$^+$ H3−C6 N3, and C2$^+$ H41−C6 O2 hydrogen bonds (Figure 2F). Such a three hydrogen bond C$^+$·C mispair has also been observed in the highly symmetrical DNA i-motif structures formed by four equivalent strands,[17−19] but wherein protonation was found in alternate cytosine residues and thus the populations of C$^+$·C and C·C$^+$ were equal. To rationalize why the protonation occurred at C2 but

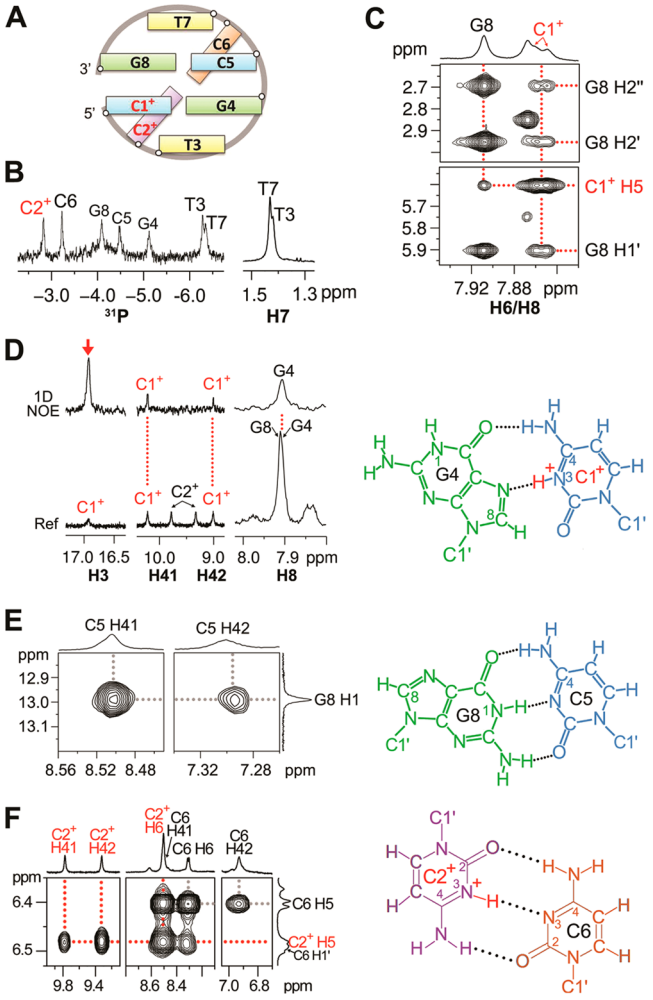


Figure 2. (A) A schematic of the CCTG MDB with C1$^+$ and C2$^+$ at pH 5.0. (B) 1D NMR spectra show the unusually downfield ^{31}P signals of C2$^+$ and C6, and the unusually upfield ^{31}P/H7 signals of T3 and T7. (C) The G8−C1$^+$ NOEs support the 3′-5′ terminal stacking. (D) The C1$^+$−G4 Hoogsteen base pair is supported by the 1D NOE between C1$^+$ H3 and G4 H8 upon selectively irradiating C1$^+$ H3 (red arrow). (E) The C5−G8 Watson−Crick base pair is supported by the G8 H1−C5 H41/H42 NOEs. (F) The downfield chemical shifts of C2$^+$ H41/H42 suggest there is a protonation of C2. The spectra were acquired at 0 °C.

not C6, we built an MDB model containing a C2$^+$·C6 mispair, C1$^+$−G4 Hoogsteen, and C5−G8 Watson−Crick base pairs (Figure S3). In this model, C6 O2 is close to G8 amino H22 and thus capable of forming a hydrogen bond. If C6 instead of C2 were protonated, the electronegativity of C6 O2 would decrease,[20] thus weakening the C6 O2-G8 H22 hydrogen bond and destabilizing the MDB. As C1$^+$−G4 adopts the Hoogsteen pairing geometry, C2$^+$ O2 is far from G4 amino. Therefore, the protonation of C2 does not destabilize the MDB.

DOI: 10.1021/acs.jpclett.7b01666
J. Phys. Chem. Lett. 2017, 8, 3478−3481

The above structural analysis reveals the formation of the $C2^+$·C6 mispair and $C1^+$–G4 Hoogsteen base pair in the CCTG MDB upon lowering the pH from 7.0 to 5.0. It has been shown that a transition from a C–G Watson–Crick to C^+–G Hoogsteen base pair in a CCCG type II loop caused almost no change in the loop stability.[21] In addition, due to the predominant ion-dipole interaction between the positively charged cytosine and the negative dipole moment of the neutral cytosine, the calculated gas-phase three hydrogen bond C^+·C mispair was found to be much more stable than a two hydrogen bond C·C mispair.[22] Therefore, the drastic gain in the MDB stability at pH 5.0 should be predominantly contributed by the $C2^+$·C6 mispair. To verify this, we designed two additional sequences, (i) 5′-CTTG CCTG-3′ and (ii) 5′-CCTG CTTG-3′, by substituting C2 and C6 with T2 and T6, respectively, so that the formation of the $C2^+$·C6 mispair was prohibited. At pH 7.0, these two sequences also folded into MDBs (Figure S4) with a T_m of 28 ± 1 and 24.9 ± 0.6 °C, respectively. At pH 5.0, no significant change in their T_m was found (Table S1), revealing the predominant contribution of the three hydrogen bond $C2^+$·C6 mispair to the unusual stability of the CCTG MDB.

The pH-dependent stability of the CCTG MDB brings about the opportunity of constructing a simple and efficient pH-controlled molecular switch, which can further be applied in areas such as drug delivery[23] and DNA nanostructure assembly.[24] At present, the reported pH-controlled DNA molecular switches involve the use of triplexes[24−26] and i-motifs.[23,27] These systems are based on toehold activation strategy, which requires an invading strand to completely displace the other strand, and their kinetics are usually on a time scale of minutes.[26,27] As a proof-of-concept study for the applicability of the CCTG MDB as a pH-controlled molecular switch, we first prepared a complementary duplex system composed of 5′-CCTG CCTG-3′/5′-CAGG CAGG-3′ at pH 7.0. Using the mfold Web server,[28] the T_m of this duplex was predicted to be ∼35 °C, which is ∼13 °C higher than that of the CCTG MDB at pH 7.0 but ∼11 °C lower than that of the CCTG MDB at pH 5.0, revealing the CCTG CCTG strand can possibly dissociate from the CAGG CAGG strand to form an MDB at pH 5.0 (Figure 3A). To investigate this possibility, we lowered the pH to 5.0 and immediately acquired the 1D ^{1}H NMR spectrum at 25 °C. The system was found to completely convert to the MDB state, as supported by the appearance of the unusually upfield T3 and T7 H7 signals (Figure 3B,C). Then, we raised the pH back to 7.0 and the system was found to reverse back to the original duplex state (Figure 3D). To further confirm these structural conversions, we also acquired the imino spectra at 0 °C (Figure S5). Compared to the other reported pH-controlled DNA molecular switches,[23−27] this MDB-duplex system employs simply two short strands of 8-nt oligomers, shows instant and complete structural conversions, and does not require an invading strand to function.

In short, we have demonstrated that upon adjusting the pH to 5.0, the thermodynamic stability of the CCTG MDB can be drastically improved, predominantly due to the formation of a three hydrogen bond C^+·C mispair in the minor groove. The 8-nt CCTG MDB sequence, when combined with its complementary CAGG CAGG strand, forms a simple and efficient pH-controlled molecular switch. We anticipate that this tiny DNA system will have intriguing applications in biomedical research,[29] as several diseases such as cancer are accompanied by pH dysregulation.[30]

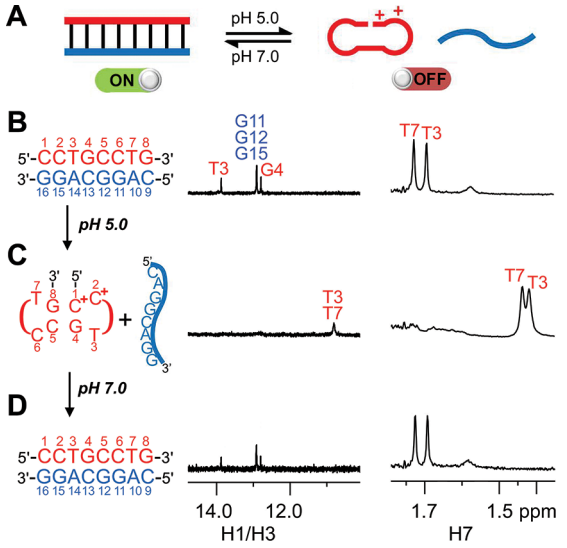


Figure 3. (A) Structural conversions of the CCTG CCTG strand (red) at pH 7.0 and 5.0 form the basis of a pH-controlled molecular switch. The imino and methyl NMR signals support the formation of (B) a duplex at pH 7.0, (C) an MDB at pH 5.0, and (D) the duplex again upon readjusting the pH back to 7.0. The spectra were acquired at 25 °C.

EXPERIMENTAL METHODS

See the Supporting Information for a detailed description of the experimental methods.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.7b01666.

Experimental methods; additional experimental data (Table S1 and Figures S1–S10) (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: lams@cuhk.edu.hk.

ORCID

Sik Lok Lam: 0000-0001-5368-706X

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Mr. Chi Chiu Chan for making the nitrogen purge system on our UV absorption spectrophotometer. The work described in this paper was supported by General Research Fund (CUHK14302114 and CUHK14302915) and Special Equipment Grant (SEG/CUHK09) from the Research Grants Council of the Hong Kong Special Administrative Region, and Direct Grants (3132671 and 3132672) from the Faculty of Science of The Chinese University of Hong Kong.

REFERENCES

(1) Wells, R. D. Non-B DNA Conformations, Mutagenesis and Disease. *Trends Biochem. Sci.* **2007**, *32*, 271–278.

(2) Choi, J.; Majima, T. Conformational Changes of Non-B DNA. *Chem. Soc. Rev.* **2011**, *40*, 5893–5909.

(3) Guo, P.; Lam, S. L. New Insights into the Genetic Instability in CCTG Repeats. *FEBS Lett.* **2015**, *589*, 3058–3063.

DOI: 10.1021/acs.jpclett.7b01666
J. Phys. Chem. Lett. 2017, 8, 3478–3481

(4) Guo, P.; Lam, S. L. Unusual Structures of TTTA Repeats in IcaC Gene of Staphylococcus Aureus. *FEBS Lett.* **2015**, *589*, 1296−1300.
(5) Guo, P.; Lam, S. L. Minidumbbell: A New Form of Native DNA Structure. *J. Am. Chem. Soc.* **2016**, *138*, 12534−12540.
(6) Guo, P.; Lam, S. L. The Competing Mini-Dumbbell Mechanism: New Insights into CCTG Repeat Expansion. *Signal Transduct. Target. Ther.* **2016**, *1*, 16028.
(7) Guo, P.; Lam, S. L. Unusual Structures of CCTG Repeats and Their Participation in Repeat Expansion. *Biomol. Concepts* **2016**, *7*, 331−340.
(8) Zhang, D. Y.; Seelig, G. Dynamic DNA Nanotechnology Using Strand-Displacement Reactions. *Nat. Chem.* **2011**, *3*, 103−113.
(9) Rinkel, L. J.; Tinoco, I., Jr. A Proton NMR Study of a DNA Dumbbell Structure with Hairpin Loops of Only Two Nucleotides: d(CACGTGTGTGCGTGCA). *Nucleic Acids Res.* **1991**, *19*, 3695−3700.
(10) Ippel, J. H.; Lanzotti, V.; Galeone, A.; Mayol, L.; Van den Boogaart, J. E.; Pikkemaat, J. A.; Altonaf, C. Thermodynamics of Melting of the Circular Dumbbell d < pCGC-TT-GCG-TT>. *Biopolymers* **1995**, *36*, 701−710.
(11) Lam, S. L.; Wu, F.; Yang, H.; Chi, L. M. The Origin of Genetic Instability in CCTG Repeats. *Nucleic Acids Res.* **2011**, *39*, 6260−6268.
(12) SantaLucia, J., Jr.; Hicks, D. The Thermodynamics of DNA Structural Motifs. *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 415−440.
(13) Boulard, Y.; Cognet, J. A.; Fazakerley, G. V. Solution Structure as a Function of pH of Two Central Mismatches, C. T and C. C, in the 29 to 39 K-Ras Gene Sequence, by Nuclear Magnetic Resonance and Molecular Dynamics. *J. Mol. Biol.* **1997**, *268*, 331−347.
(14) Asensio, J. L.; Lane, A. N.; Dhesi, J.; Bergqvist, S.; Brown, T. The Contribution of Cytosine Protonation to the Stability of Parallel DNA Triple Helices. *J. Mol. Biol.* **1998**, *275*, 811−822.
(15) Nikolova, E. N.; Goh, G. B.; Brooks, C. L., III; Al-Hashimi, H. M. Characterizing the Protonation State of Cytosine in Transient G.C Hoogsteen Base Pairs in Duplex DNA. *J. Am. Chem. Soc.* **2013**, *135*, 6766−6769.
(16) Keppler, M. D.; Fox, K. R. Relative Stability of Triplexes Containing Different Numbers of T.AT and C+.GC Triplets. *Nucleic Acids Res.* **1997**, *25*, 4644−4649.
(17) Leroy, J. L.; Gehring, K.; Kettani, A.; Gueron, M. Acid Multimers of Oligodeoxycytidine Strands: Stoichiometry, Base-Pair Characterization, and Proton Exchange Properties. *Biochemistry* **1993**, *32*, 6019−6031.
(18) Gehring, K.; Leroy, J. L.; Gueron, M. A Tetrameric DNA Structure with Protonated Cytosine.Cytosine Base Pairs. *Nature* **1993**, *363*, 561−565.
(19) Esmaili, N.; Leroy, J. L. I-Motif Solution Structure and Dynamics of the d(AACCCC) and d(CCCCAA) Tetrahymena Telomeric Repeats. *Nucleic Acids Res.* **2005**, *33*, 213−224.
(20) Petrov, A. S.; Lamm, G.; Pack, G. R. The Triplex-Hairpin Transition in Cytosine-Rich DNA. *Biophys. J.* **2004**, *87*, 3954−3973.
(21) van Dongen, M. J.; Wijmenga, S. S.; van der Marel, G. A.; van Boom, J. H.; Hilbers, C. W. The Transition from a Neutral-pH Double Helix to a Low-pH Triple Helix Induces a Conformational Switch in the CCCG Tetraloop Closing a Watson-Crick Stem. *J. Mol. Biol.* **1996**, *263*, 715−729.
(22) Sponer, J.; Leszczynski, J.; Hobza, P. Hydrogen Bonding and Stacking of DNA Bases: A Review of Quantum-Chemical Ab Initio Studies. *J. Biomol. Struct. Dyn.* **1996**, *14*, 117−135.
(23) Liu, J.; Ma, X. W.; Lei, C. N.; Xue, X. D.; Wei, T.; Zhao, J.; Li, S.; Liang, X. J. A Self-Assembled DNA Nanostructure for Targeted and pH-Triggered Drug Delivery to Combat Doxorubicin Resistance. *J. Mater. Chem. B* **2016**, *4*, 3854−3858.
(24) Amodio, A.; Adedeji, A. F.; Castronovo, M.; Franco, E.; Ricci, F. pH-Controlled Assembly of DNA Tiles. *J. Am. Chem. Soc.* **2016**, *138*, 12735−12738.
(25) Amodio, A.; Zhao, B.; Porchetta, A.; Idili, A.; Castronovo, M.; Fan, C.; Ricci, F. Rational Design of pH-Controlled DNA Strand Displacement. *J. Am. Chem. Soc.* **2014**, *136*, 16469−16472.
(26) Liu, Z.; Mao, C. Reporting Transient Molecular Events by DNA Strand Displacement. *Chem. Commun. (Cambridge, U. K.)* **2014**, *50*, 8239−8241.
(27) Tang, W.; Wang, H.; Wang, D.; Zhao, Y.; Li, N.; Liu, F. DNA Tetraplexes-Based Toehold Activation for Controllable DNA Strand Displacement Reactions. *J. Am. Chem. Soc.* **2013**, *135*, 13628−13631.
(28) Zuker, M. Mfold Web Server for Nucleic Acid Folding and Hybridization Prediction. *Nucleic Acids Res.* **2003**, *31*, 3406−3415.
(29) Sellner, S.; Kocabey, S.; Nekolla, K.; Krombach, F.; Liedl, T.; Rehberg, M. DNA Nanotubes as Intracellular Delivery Vehicles in Vivo. *Biomaterials* **2015**, *53*, 453−463.
(30) Webb, B. A.; Chimenti, M.; Jacobson, M. P.; Barber, D. L. Dysregulated pH: A Perfect Storm for Cancer Progression. *Nat. Rev. Cancer* **2011**, *11*, 671−677.

DOI: 10.1021/acs.jpclett.7b01666
J. Phys. Chem. Lett. 2017, 8, 3478−3481