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Cooperative DNA Compaction by Ternary Supramolecular Complex with Cucurbituril/Cyclodextrin Pair

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DNA condensation plays a vital role in regulating cell life. Herein, we report a supramolecular complex with synergistic and specific DNA-condensing ability, which is achieved by the cucurbituril-induced conformational change and pK_a shift in aqueous solution. The complexation with cucurbit[6]uril can drive the side chain of 1,6-diaminohexane out of β-cyclodextrin's cavity to increase the molecular rigidity and meanwhile, the supramolecular pK_a shift from 10.81 to 12.15 can ensure the amount of positive charges, which facilitate the close contact with DNA. By benefiting from β-cyclodextrin's hydrophobic cavity, the binary cucurbituril–cyclodextrin complex can be further decorated with anthryl adamantane. Furthermore, it is demonstrated that the resultant ternary assembly originating from the integration of cucurbituril–cyclodextrin macrocyclic pair with the protonated ammonium chain and π-conjugated anthryl adamantane can efficiently bind to the DNA backbones, thus resulting in the DNA morphological transition from loose clews to compact nanoparticles. Thus, this supramolecular complex may have powerful potential as compacting agent for nucleic acids in non-viral gene delivery.

Introduction

The rapid degradation and clearance of naked oligonucleotides by serum nucleases in the bloodstream is considered as the major hurdle in truly potent medicines and wider clinical use.[1] To overcome this important barrier, the condensation of DNA in a controlled manner becomes one of the key steps in the gene therapy and it is highly imperative to establish effective delivery strategies that can compact and protect nucleic acids from undesirable inactivation process.[2] To date, researchers have reached a consensus that cationic compounds (e.g., linear and branched polyethylenimines) and π-aromatic rings (e.g., anthracene and pyrene) are among the most potent categories in the DNA condensation, because the former can make electrostatically driven neutralization with phosphate backbones of DNA, whereas the latter can interact with DNA by penetrating into its grooves.[3] Despite that various self-assembled functional nanoarchitectures, such as cationic liposomes, micelles, dendrimers, and surfactants, have been created and utilized as artificial vectors with high complexity,[4,5] there is an increasingly strong demand on the precise and effective gene therapeutic methods using readily available recognition motifs, and the design and synthesis of DNA complexating agents with high efficiency and well-defined structures still remain challenging.[6]

Supramolecular complexes and assemblies, constructed from multiple noncovalent interactions, always far exceed the performance of individual components and have been widely utilized in the construction of advanced gene/drug delivery systems and multistimuli-responsive nanomaterials. For instance, cyclodextrins (CDs), a class of torus-shaped cyclic oligosaccharides, can be functionalized to entrap various neutral and negatively charged substrates in their inherent cavity, which is considered as an ideal scaffold to solubilize hydrophobic drugs and other bioactive molecules in aqueous solution.[7] In comparison, cucurbiturils (CBs), a class of pumpkin-shaped cyclic compounds made of glycoluril monomers, tend to encapsulate inorganic/organic cations mainly through the ion–dipole interactions working at the carbonyl groups in CBs.[8] In particular, it is noteworthy that the complexation with CBs can stabilize the active form of drug molecules through a positive complexation-induced pK_a shift, thus leading to the enhancement of drug's bioavailability under physiological conditions.[9] Thus, possessing mutually complementary molecular-binding characteristics, one can believe that the CD- and CB-based hybrid supramolecular architectures will be developed into a new approach to fabricate biocompatitable nanostructures with fascinating biological functions.

Herein, we report a ternary supramolecular complex originating from the selective self-assembling process involving β-cyclodextrin (β-CD) and cucurbit[6]uril (CB[6]), in which both the cationic and aromatic groups are simultaneously integrated through a noncovalent conjunction. Besides the π-conjugated anthracene, it is also found that the DNA conformational transition process can be triggered by CB[6]; that is, CB[6] can induce a dramatic conformational change in hexane-1,6-diamine-modified β-CD (**1**), in which the ammonium side chain was completely impelled from the β-CD's cavity to increase the mo-

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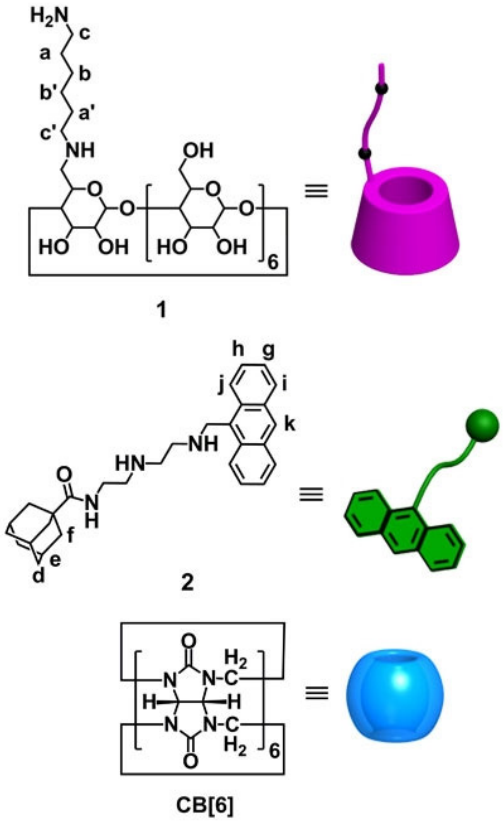
lecular rigidity in the whole supramolecular complex. Second, the positive complexation-induced pK_a shift can guarantee a high charge amount through the $N^{+}\cdots O^{\delta-}$ ion – dipole interconnection with the carbonyl groups in CB[6], thus making the resultant ternary complex more resistant to external pH changes. Consequently, through the supramolecular cooperativity, the resulting ternary assembly gives a specific DNA-condensing capability as compared with the individual anthryl adamantane and hexane-1,6-diamine-modified β-CD, which seems to be a promising system for controlled gene therapy. [10] The molecular structures of hexane-1,6-diamine-modified β-CD (**1**), anthryl adamantane (**2**), and CB[6] were shown in Scheme 1.



Scheme 1. Molecular structures of hexane-1,6-diamine-modified β-CD **1**, anthryl adamantane **2**, CB[6], and the proton designations of **1** and **2.**

Results and Discussion

The selective self-assembling process in **1**·**2**·CB[6] supramolecular complex was preliminarily investigated by ^{1}H NMR titration experiments. Upon addition of equimolar amount of CB[6], the signals assigned to aliphatic protons in **1** (H_a and H_b) exhibited a sizable upfield shift, indicating the propensity of the hydrophobic cavity of CB[6] to encapsulate the protonated diaminohexane through ion–dipole interaction (Figure 1a and 1b). Comparatively, the adamantyl protons in **2** gave a moderate complexation-induced downfield shift in the presence of **1**, un-

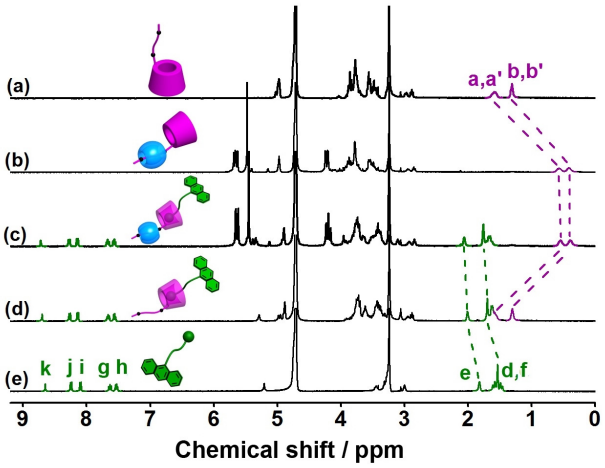


Figure 1. ^{1}H NMR spectra of (a) **1**, (b) **1**·CB[6] complex, (c) **1**·**2**·CB[6] supramolecular complex, (d) **1**·**2** complex, and (e) **2** in D_2O at 25 °C, respectively (400 MHz, [**1**] = [**2**] = [CB[6]] = 2.0 mM).

equivocally corroborating that β-CD preferentially encapsulated the adamantyl moiety (Figure 1d and 1e). Moreover, the resonance peaks of **1**·**2**·CB[6] assembly combined both β-CD- and CB[6]-binding characteristics and it seems that the molecular recognition of adamantane and diaminohexane with two different types of macrocycles could not affect each other (Figure 1c). It is also found that no obvious spectral change was observed in the aromatic region of **2** (H_{g-k}) with and without macrocycles, suggesting that the 9-substituted anthracene was always exposed to the aqueous environment and this structural feature may facilitate the binding with DNA grooves.[11] Furthermore, the thermodynamic parameters were quantitatively examined by isothermal titration calorimetry (ITC), giving the binding constants (K_S) of 9.60 × 10^3 and 7.33 × 10^5 M^{-1} for **1**·**2** and **1**·CB[6] complexes, respectively (Figures 2, S1–S2, and Table S1 in the Supporting Information).

Meanwhile, ^{1}H NMR titration experiments were employed to investigate the molecular binding behaviors in **1**·CB[6] and **1**·**2** complexes. The K_S value in **1**·CB[6] complex was calculated as 5.2 × 10^3 M^{-1} by analyzing the sequential changes in chemical shift ($\Delta\delta$) of adamantyl proton H_e in **2** at varying concentrations of **1** using a nonlinear least-squares curve-fitting method (Figure S3 in the Supporting Information). This result is comparable to the one obtained by ITC experiments. Meanwhile, because of the slow exchange equilibrium in the **1**·CB[6] complexation, free and bound species could be clearly distinguished in the NMR timescale. Therefore, the binding constant could be estimated by single-point method from the integral ratio of complexed and uncomplexed protons of **1** in the presence of CB[6], and the lower limit for the K_S value in **1**·CB[6] complex could be calculated as 5.3 × 10^4 M^{-1}, which is also basically accordance with the ITC results (Figures S4–S5 in the Supporting Information). Obviously, these large K_S values obtained from ITC and ^{1}H NMR experiments would facilitate the

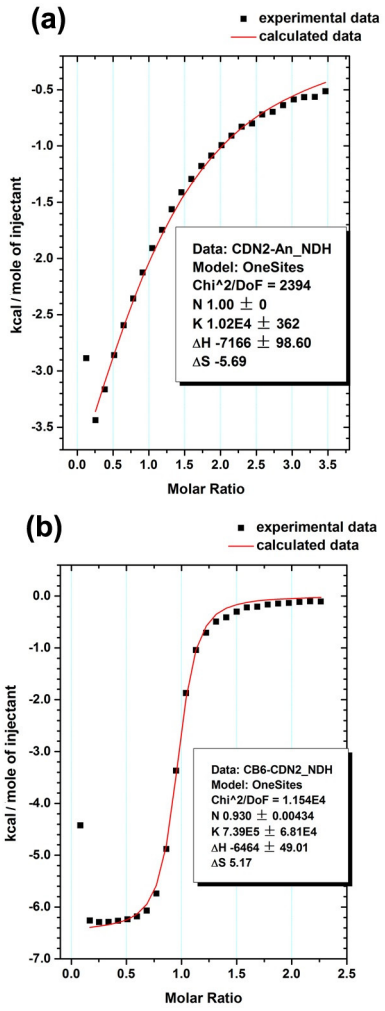


Figure 2. "Net" heat effects of complexation of (a) **1**·**2** and (b) **1**·CB[6] for each injection, obtained by subtracting the dilution heat from the reaction heat, which was fitted by computer simulation with the "one set of binding sites" model.

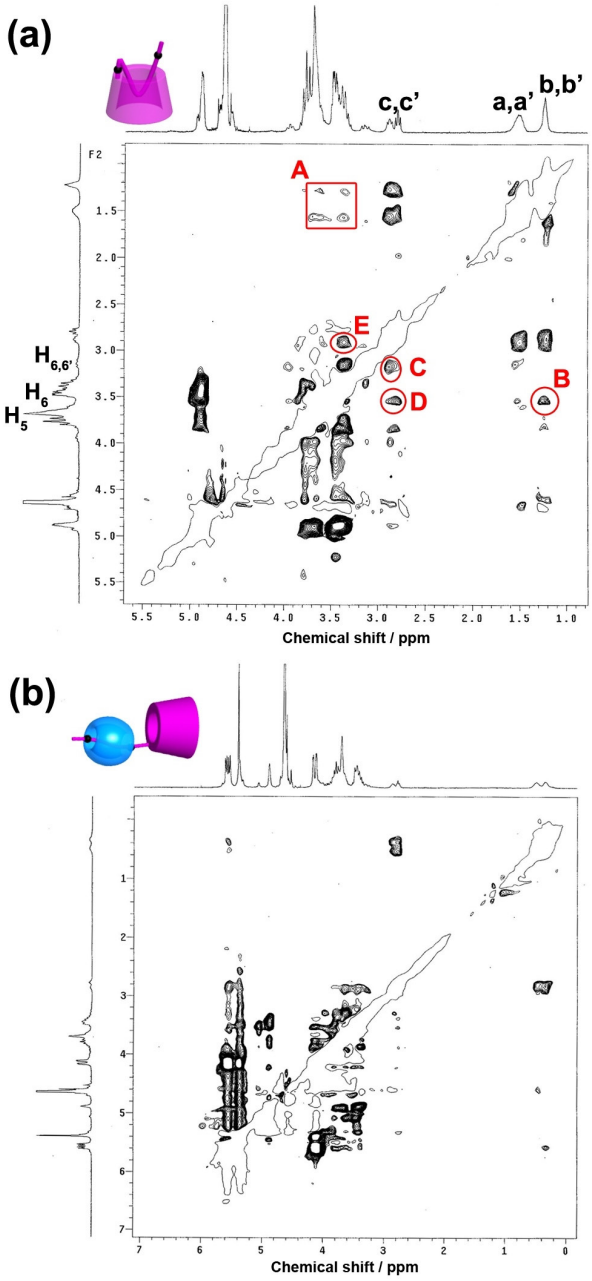


Figure 3. ROESY spectrum of (a) compound **1** with mixing time of 0.240 s ([**1**] = 2.0 mM); (b) **1**·CB[6] complex with mixing time of 0.220 s ([**1**] = [CB[6]] = 2.0 mM).

eventual formation of stable ternary supramolecular complex **1**·**2**·CB[6] for efficient DNA compaction.

The conformational analyses further imply that all the alkyl protons in **1** (H_{a-c}) possessed the strong correlations with H_{5-6} of β-CD's cavity (cross peaks B–E in Figure 3a). Meanwhile, H_{a-b} in **1** gave moderate correlations with β-CD's interior protons (cross peaks A in Figure 3a). Based on this information, we deduce that the diaminohexane moiety in **1** was shallowly accommodated in the β-CD cavity from the narrow side to form a self-inclusion complex. Meanwhile, the introduction of CB[6] can pull the diaminohexane in **1** out of the β-CD's cavity, as no correlation peak was found in alkyl protons with β-CD (Figure 3b). After validating these NMR spectral behaviors, the pK_a values of free **1** and **1**·CB[6] complex were calculated as 10.06 and 12.15, respectively, through a plot of chemical shifts versus pH values. Moreover, a similar pK_a value of 10.81 was obtained after adding 1-adamnatnol to expel the self-included diaminohexane group (Figure S6 in the Supporting Information). These pK_a results jointly demonstrate that the complexation with CB[6] can not only induce the conformational change of **1** but also ensure the positive charge distribution of the resulting complex through the macrocycle-enhanced pK_a shift.[8,12] This supramolecular synergistic effect was further realized by the DNA condensation in gel retardation assay as described below.

Next, agarose gel electrophoresis assay was carried out to examine the different DNA compaction abilities of **1**, **2**, CB[6], and their corresponding complexes. Considering the dynamic

equilibrium and noncovalent complexation of β-CD with adamantane, an excess amount of **1** was used to achieve a high percentage of supramolecular complex in solution. Therefore, the concentrations of **1**,·**2**, and CB[6] were used as 0.49, 0.12, and 0.49 mM, respectively, corresponding to more than 70% inclusion efficiency in **1**·**2** complex and alomost 100% inclusion efficiency in **1**·CB[6] complex. This indicates that the **1**·**2** and **1**·CB[6] complexes were the dominant species in solution. The amount of calf thymus DNA was used as 10 ng/μL. As can be seen in Figure 4, no obvious condensation effect was observed

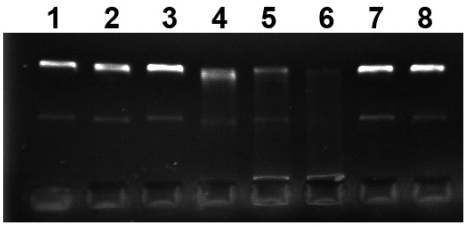


Figure 4. Agarose gel electrophoresis assay of pBR322 DNA condensation induced by **1** (Lane 2), CB[6] (Lane 3), **1**·**2** complex (Lane 4), **1**·CB[6] complex (Lane 5), **1**·**2**·CB[6] assembly (Lane 6), **2** (Lane 7), and **2**·β-CD complex (Lane 8), respectively ([DNA] = 10 ng/μL, [**1**] = [β-CD] = [CB[6]] = 0.49 mM, and [**2**] = 0.12 mM). Lane 1 is the blank control.

in the case of individual compounds or binary inclusion complexes, with the exception of **1**·CB[6] complex that could retard the movement of DNA in the gel well to some extent, mainly due to CB[6]-induced conformational change and pK_a shift in aqueous solution (Lane 5). More gratifyingly, it is found that **1**·**2** complex could achieve a complete DNA compaction with the assistance of CB[6] (Lane 6). In our case, it is noted that the minimum concentration of **1**·**2**·CB[6] supramolecular complex for the DNA compaction was 30.8 μM, which was much lower than the ones in other control groups under the same experimental conditions (Figures S7–S10 in the Supporting Information). In addition, it is found that the compact DNA could be further released upon addition of an excess amount of 1-adamantanol (40 equiv) as the exogenous competitor and decompacting agent, and this result further demonstrates the importance of noncovalent cooperation between adamantane in **2** and CD's cavity in **1** (Figure S11 in the Supporting Information). Therefore, we can conclude that the condensing ability of discrete anthracene and unbound aliphatic amine is so weak that it cannot efficiently induce DNA conformational transition, and the complexation of CB[6] with diaminohexane plays an indispensable role in tuning up the DNA conformation.

The DNA morphology before and after treating by **1**·**2**·CB[6] assembly was further investigated by atomic force microscopy (AFM). The stretched single DNA molecules originally existed as loose clews (Figure 5a), but turned to the condensed globular nanoparticles in the presence of compacting agent **1**·**2**·CB[6] assembly (Figure 5b). This morphological change clearly indicates the good DNA condensation ability of **1**·**2**·CB[6] supramolecular complex, which is jointly ascribable to the synergistic

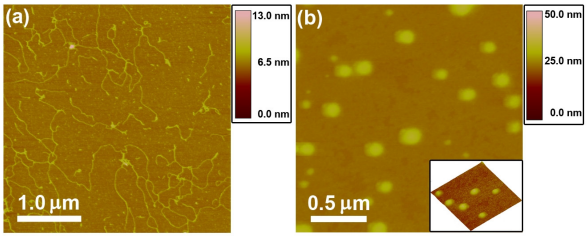


Figure 5. AFM images of (a) naked calf thymus DNA and (b) its condensation induced by **1**·**2**·CB[6] supramolecular complex. Inset: topographic image of a specific area.

effect of CB[6]-activated ammonium chain on **1** and the anthryl ring on **2**.

To explore the possible DNA binding mode and mechanism, viscosity and circular dichroism spectroscopic measurements were carried out. It is well-established that the relative viscosity of calf thymus-DNA does not show any significant changes, but increases dramatically when small molecules interact with DNA via intercalation. Meanwhile, a slight or no perturbation in DNA's circular dichroism signals should be found when small molecules interact with DNA through groove and/or electrostatic interaction, while intercalation binding always increases the intensities of both the negative band at 245 nm and the positive band at 277 nm.[13] As discerned from Figures S13 and 6, in our case, no obvious change was found in the

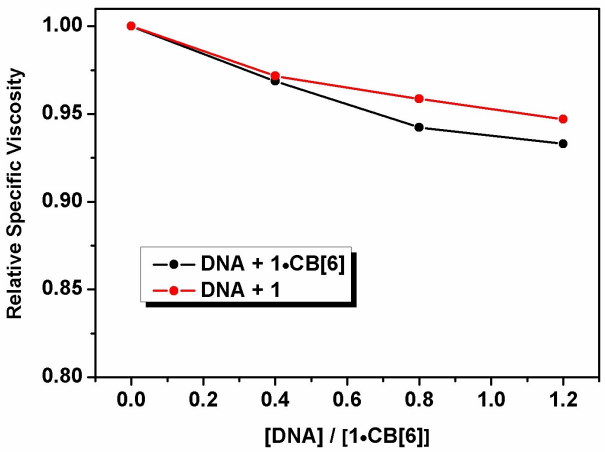


Figure 6. Effect of increasing the concentrations of **1** and **1**·CB[6] complex on the relative specific viscosities of ct-DNA in TAE buffer at 25 °C ([**1**] = [CB[6] and [DNA] = 0.11 mM).

circular dichroism spectra of DNA with excess amount of **1**·CB[6] complex, and the specific viscosity of DNA was slightly declined in the presence of **1** and **1**·CB[6] complex. These results are indicative of a nonintercalative mode in the DNA compaction with **1**·CB[6] complex and provide evidence on their groove binding nature.[13b,14] Furthermore, it was also observed that DNA could be rapidly flocculated at the relatively higher

concentrations of **1**·CB[6] and **1**·**2**·CB[6] systems, here again corroborating the good DNA condensation ability of CB[6]-involved complexes (Figure S14 in the Supporting Information). Moreover, the energy-minimized structures of DNA with **1**·CB[6] complex and **1**·**2**·CB[6] assembly suggest that the CB[6]-protected ammonium group and the pendant anthracene mediated by β-CD can strongly interact with DNA backbone;[15] that is, the anthryl group in **2** may prefer the binding in the minor DNA groove, whilst the molecular size and spatial arrangement of **1**·CB[6] complex are beneficial for the adjacent major DNA groove binding (Figure S15 in the Supporting Information). Actually, anthracene and its analogues may exhibit intercalation and groove binding modes toward DNA, both of which have been previously reported.[13b,16] In our case, as discerned from the decreased viscosity and the unchanged circular dichroism intensity, the anthryl group in **1**·**2**·CB[6] complex was prone to the groove binding with DNA backbone. It is reasonable, because the bulky CB and CD moieties around the π-conjugated anthracene in **1**·**2**·CB[6] complex can seriously impede the anthryl group from the close contact with DNA's nucleobases.[13b,17]

Overall, the different DNA condensation abilities of the resulting complexes may be explained as follows. The efficient interaction may not occur when mixing DNA with **1** alone, mainly due to the undesirable formation of self-inclusion complex. This situation is slightly changed upon complexation of **1** with **2**, but the flexible amino group and isolated anthryl graft are not very favorable to compact DNA. In comparison, despite of the shielding effect on ammonium sites, the extensive charge distribution and the steric stabilization through CB[6] complexation may be jointly attributed to the enhanced DNA binding ability of **1**·CB[6] complex. That is, the association with CB[6] can greatly delocalize the intensive positive charges of ammonium side chain through the $N^{+}\cdots O^{\delta-}$ ion – dipole interconnection with the carbonyl groups in CB[6], which is frequently observed in the CB-involved crystalline complexes.[18] Moreover, the introduction of CB[6] can not only ensure the cationic amount through the host-assisted pK_a shift, but also impel the ammonium side chain out of the CD's cavity to increase the molecular rigidity of the protonated 1,6-diaminohexane moiety. These binding features in **1**·CB[6] complex can eventually facilitate the electrostatic and hydrogen-bonding contact with DNA phosphate backbone.[19] As a result, the ternary **1**·**2**·CB[6] supramolecular complex with combined advantages of anthracene and cucurbituril emerged into the limelight, thus showing the best DNA compaction ability among all the examined complexes.

Conclusions

In conclusion, benefiting from both the ion-dipole interaction between hexane-1,6-diamine and CB[6] and the hydrophobic interaction between the adamantyl group and the β-CD cavity, we constructed a ternary assembly as a supramolecularly combinatorial agent for the synergistic and specific DNA condensation. In our case, the **1**·**2**·CB[6] supramolecular complex gives a specific DNA-condensing ability and CB[6] as a promoter can directly trigger and govern the DNA condensation process. Microscopic measurements and electrophoretic assay jointly demonstrate that the naked DNA can be efficiently condensed into uniform spherical nanoparticles driven by the groove binding forces. The supramolecular pK_a and conformational regulation and the effective DNA compaction in this work further stress the power of synergistic effect arising from different macrocyclic receptors and may display a new principle of an allosterically controlled DNA-condensing agent. Considering that the noncovalent linkage is intrinsically dynamic and reversible, it is anticipated that this supramolecular triad with dual DNA binding sites can be utilized as a smart candidate in the controlled capture and the release of nucleic acid and other biomacromolecules.

Experimental Section

General Methods and characterizations were attached in the Supporting information.

Acknowledgements

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Keywords: Cyclodextrin · Cucurbituril · Noncovalent cooperativity · Supramolecular pKa shift · DNA condensation

[1] a) H. M. Aliabadi, B. Landry, C. Sun, T. Tang, H. Uludağ, *Biomaterials* **2012**, *33*, 2546–2569; b) M. A. Mintzer, E. E. Simanek *Chem. Rev.* **2009**, *109*, 259 – 302.

[2] a) N. V. Berezhnoy, N. Korolev, L. Nordenskiöld, *Adv Colloid Interface Sci.* **2014**, *205*, 221–229; b) V. Vijayanathan, E. Agostinelli, T. Thomas, T. J. Thomas, *Amino Acids* **2014**, *46*, 499–509; c) R. de Vries, *Biochimie* **2010**, 92, 1715–1721; d) S. Jiang, Z. Cao, *Adv. Mater.* **2010**, *22*, 920–932; e) P. Saccardo, A. Villaverde, N. González-Montalbán, *Biotechnol. Adv.* **2009**. *27*, 432–438; f) C. M. Jewell, D. M. Lynn, *Adv. Drug Delivery Rev.* **2008**, *60*, 979–999; g) A. A. Zinchenko, N. Chen, *J. Phys.: Condens. Matter* **2006**, *18*, R453 – R480; h) I. M. Porter, G. A. Khoudoli, J. R. Swedlow, *Curr. Biol.* **2004**, *14*, R554–R556; i) Y. Burak, G. Ariel, D. Andelman, *Curr. Opin. Colloid Interface Sci.* **2004**, *9*, 53 – 58; j) R. Dias, M. Rosa, A. C. Pais, M. Miguel, B. Lindman, *J. Chin. Chem. Soc.* **2004**, *51*, 447 – 469; k) V. A. Bloomfield, *Biopolymers* **1997**, *44*, 269 – 282;l) V. A. Bloomfield, *Curr. Opin. Struct. Biol.* **1996**, *6*, 334–341.

[3] a) G.-Y. Li, R.-L. Guan, L.-N. Ji, H. Chao, Coord. *Chem. Rev.* **2014**, *28*, 100–113; b) A. González-Pérez, *Curr. Top. Med. Chem.* **2014**, *14*, 766–773; c) P. Carrivain, A. Cournac, C. Lavelle, A. Lesne, J. Mozziconacci, F. Paillusson, L. Signon, J.-M. Victor, M. Barbi, *Soft Matter* **2012**, *8*, 9285–9301; d) A. Estévez-Torres, D. Baigl, *Soft Matter* **2011**, *7*, 6746–6756.

[4] a) G. Navarro, J. Pan, V. P. Torchilin, *Mol. Pharmaceutics* **2015**, *12*, 301 – 313; b) T. Zhou, A. Llizo , C. Wang, G. Xu, Y. Yang, *Nanoscale* **2013**, *5*, 8288–8306; c) M.-L. Ainalema, T. Nylander, *Soft Matter* **2011**, *7*, 4577 – 4594.

[5] a) M. Yu, S.-Z. Zu, Y. Chen, Y.-P. Liu, B.-H. Han, Y. Liu, *Chem. Eur. J.* **2010**, *16*, 1168–1174; b) Y. Liu, Z.-L. Yu, Y.-M. Zhang, D.-S. Guo, Y.-P. Liu, *J. Am. Chem. Soc.* **2008**, *130*, 10431–10439;

[6] a) X. Ma, Y. Zhao, *Chem. Rev.* **2015**, *115*, 7794–7839; b) C. Ortiz Mellet, J. M. García Fernández, J. M. Benito, *Chem. Soc. Rev.* **2011**, *40*, 1586–1608; c) Y. Chen, Y. Liu, *Chem. Soc. Rev.* **2010**, *39*, 495–505.

[7] Y. Chen, Y. Liu, *Adv. Mater.* **2015**, *27*, 5403–5409

[8] a) C. Marquez, R. R. Hudgins, W. M. Nau *J. Am. Chem. Soc.* **2004**, *126*, 5806–5816.;b) J. W. Lee, S. Samal, N. Selvapalam, H.-J. Kim, K. Kim. *Acc. Chem. Res.* **2003**, 36, 621–630.

[9] I. Ghosh, W. M. Nau, *Adv Drug Deliv Rev.* **2012**,*64*, 764–783.

[10] a) X.-Y. Hu, T. Xiao, C. Lin, F. Huang, L. Wang, *Acc. Chem. Res.* **2014**, *47*, 2041–2051; b) D.-H. Qu, Q.-C. Wang, Q.-W. Zhang, X. Ma, H. Tian, *Chem. Rev.* **2015**, *115*, 7543–7588; c) N. Song, Y.-W. Yang, *Chem. Soc. Rev.* **2015**, *44*, 3474–3504; d) X. Zhao, Z.-T. Li, *Chem. Commun.* **2010**, *46*, 1601–1616.

[11] T. Ikeda, K. Yoshida, H.-J. Schneider, *J. Am. Chem. Soc.* **1995**, *117*, 1453–1454.

[12] a) N. Barooah, M. Sundararajan, J. Mohanty, and A. C. Bhasikuttan, *J. Phys. Chem. B* **2014**, *118*, 7136 – 7146;b) A. Praetorius, D. M. Bailey, T. Schwarzlose, W. M. Nau, *Org. Lett.* **2008**, *10* , 4089–4092.

[13] a) N. K. Modukuru, K. J. Snow, B. Scott Perrin Jr. , A. Bhambhani, M. Duff, C. V. Kumar, *J. Photochem. Photobiol., A* **2006**, *177*, 43–54; b) Y. Huang, Y. Zhang, J. Zhang, D.-W. Zhang, Q.-S. Lu, J.-L. Liu, S.-Y. Chen, H.-H. Lin, X.-Q. Yu, *Org. Biomol. Chem.* **2009**, *7*, 2278–2285; c) P. Sureshbabu, A. A. J. S. Tjakraatmadja, C. Hanmandlu, K. Elavarasan, N. Kulak, S. Sabiah, *RSC Adv.* **2015**, *5,* 22405–22418; d) C. Pérez-Arnaiz, N. Busto, J. M. Leal, B. García, *J. Phys. Chem. B* **2014**, *118*, 1288 – 1295.

[14] a) R. Adam, P. Bilbao-Ramos, B. Abarca, R. Ballesteros, M. E. González-Rosende, M. A. Dea-Ayuela, F. Estevand, G. Alzuet-Piña, *Org. Biomol. Chem.* **2015**, *13*, 4903–4917; b) K. Duskova, S. Sierra, M.-J. Fernández, L. Gude, A. Lorente, *Bioorg. Med. Chem.* **2012**, *20*, 7112–7118; c) S. Roy, A. K. Patra, S. Dhar, A. R. Chakravarty, *Inorg. Chem.* **2008**, *47*, 5625–5633; d) J. Liu, T. Zhang, T. Lu, L. Qu, H. Zhou, Q. Zhang, L. Ji, *J. Inorg. Biochem.* **2002** , *91*, 269–276.

[15] a) M. Hardouin–Lerouge, P. Hudhomme, M. Sallé, *Chem. Soc. Rev.* **2011**, *40*, 30–43; b) F.-G. Klarner, B. Kahlert, *Acc. Chem. Res.* **2003**, *36*, 919–932.

[16] Y. Liu, L. Yu, Y. Chen, Y.-L. Zhao, H. Yang, *J. Am. Chem. Soc.* **2007**, *129*, 10656–10657.

[17] H.-C. Becker, B. Norden, *J. Am. Chem. Soc.* **1999**, *121*, 11947–11952

[18] a) W.-S. Jeon, K. Moon, S.-H. Park, H. Chun, Y.-H. Ko, J.-Y. Lee, E.-S. Lee, S. Samal, N. Selvapalam, M. V. Rekharsky, V. Sindelar, D. Sobransingh, Y. Inoue, A. E. Kaifer, K. Kim, *J. Am. Chem. Soc.* **2005**, *127*, 12984–12989; b) J. Kim, I.-S. Jung, S.-Y. Kim, E. Lee, J.-K. Kang, S. Sakamoto, K. Yamaguchi, K. Kim, *J. Am. Chem. Soc.* **2000**, *122*, 540–541.

[19] J. DeRouchey, B. Hoover, D. C. Rau, *Biochemistry* **2013**, *52*, 3000 – 3009.

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