Controllable DNA condensation through cucurbit[6]uril in 2D pseudopolyrotaxanes†

Chen-Feng Ke,[a] Sen Hou,[b] Heng-Yi Zhang,[a] Yu Liu,*[a] Kun Yang[b] and Xi-Zeng Feng*[b]

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2D pseudopolyrotaxanes containing β-cyclodextrins and cucurbit[6]urils can induce DNA condensation, and the number of cucurbit[6]urils threaded onto the side chains of β-cyclodextrins plays important roles in this process.

Gene therapy can be defined as the treatment of human disease by transferring the genetic material into specific cells of patients.[1] The first step is packing of large DNA plasmids by synthetic vectors or viral vectors. Contrary to the latter, synthetic vectors can provide opportunities for improved of safety, greater flexibility and more facile manufacturing, so these vectors such as cationic lipids,[2] gemini surfactants,[3] cationic polymers[4] have received much attention in recent years.[5] In this respect, cationic polymers are specially designed for gene delivery owing to their possessing DNA binding moieties.[6,7] Recently, cyclodextrins (CDs) bearing cationic moieties were used as vectors for gene delivery and solubilizing agents in FDA-approved pharmaceutical formulations because CDs are biocompatible materials.[8] Pseudopolyrotaxanes which contain many CDs on the linear polymer chain were investigated for gene transfection due to their advantage of good solubility,[9] improved cell viability,[10] pH controllable ability,[11] good DNA binding ability and high gene transfection efficiency.[12] As the good binding does not correlate directly with gene-delivery efficiency, controlling the binding of vectors with DNA to a suitable intensity can be both advantageous for DNA protection and transfection.[13] It was reported that threading cucurbit[6]uril (CB[6]) on spermidine and spermine affects their ability to adjust the activity of DNA enzyme.[14] Thus, CB[6]s might be suitable reagents for modulation of DNA condensation. Herein, pseudopolyrotaxane **1** bearing 6-[(6-aminohexyl)amino]-6-deoxy-β-CD dichloride was selected as the predecessor. By adding different amount of CB[6]s, a serials of 2D pseudopolyrotaxanes (PPRs) were formed to detect their ability to induce DNA condensation.‡

Pseudopolyrotaxane **1** was synthesized through PPG4000 and 6-[(6-aminohexyl)amino]-6-deoxy-β-CD dichloride. Then, (0, 20, 40, 70, 100)% CB[6]s (calculated to 6-[(6-aminohexyl)amino]-6-deoxy-β-CD) were added to the aqueous solutions of **1** to form these novel 2D PPRs, as shown in Fig. 1 (see ESI†). PPRs bearing (0, 20, 40, 70, 100)% CB[6]s are named as PPR-0, PPR-20, PPR-40, PPR-70 and PPR-100, respectively. In general, PPRs were prepared by threading the rings onto the main chain or side chains of polymers. In our recent work,[15] 2D PPRs were prepared *via* pseudorotaxanes threading onto the PPG chain. In contrast, the present 2D PPRs were firstly prepared by threading modified CDs on the polymer backbone, and then CB[6]s were threaded on the arms of modified CDs. Because of the strong binding ability between hexane-1,6-diamine and CB[6] ($K = 4.49 \times 10^8$ M^{-1}) in aqueous solution,[16] the complexation in our experiment conditions is essentially quantitative. Consequentially, we can control arbitrarily the degree of substitution of CB[6]s in these obtained 2D PPRs through this method.

CB[6]s threading on side chains can easily be confirmed by ^1H NMR spectra. As can be seen from Fig. 2, when 40% CB[6]s were added to the aqueous solutions of **1**, the resonance signals of $H_{b/b'}$ and $H_{c/c'}$ in the side chain of β-CD exhibit two sets of different peaks, *i.e.*, the free side chains and the included ones. The resonance signals of included moieties shift upfield about 1 ppm from 1.2–1.5 ppm to 0.3–0.5 ppm. Upon further addition of 100% CB[6], the free $H_{b/b'}$ and $H_{c/c'}$ signals almost disappear. These observations indicate that all CB[6] molecules are threaded on the side chains of β-CDs.

As can been seen from Fig. 3, 2D PPRs bearing different number of CB[6]s show different condensation abilities with pEGFP-C2 plasmid DNA in their agarose gel electrophoresis assay. It needs to be pointed out that this DNA exists as a mixture of circular supercoiled DNA (*form I*) and relaxed circular DNA (*form II*) arising from single-strand cleavage. Comparing with the lowest concentration needed for DNA to remain in the well, we

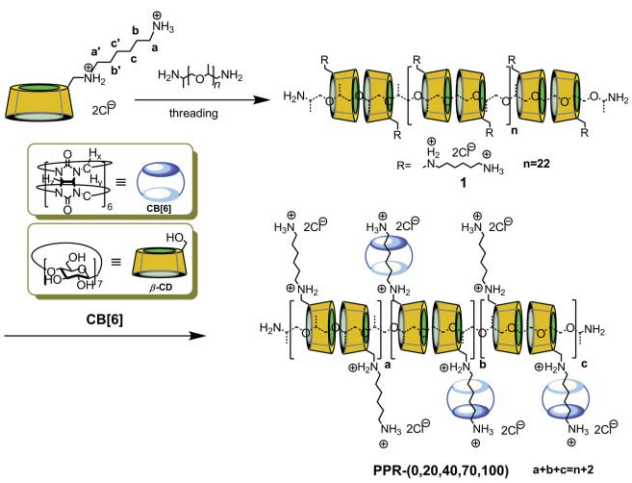

Fig. 1 Preparation of 2D PPRs (PPR-0, 20, 40, 70, 100).

[a]*Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin, 300071, P. R. China. E-mail: yuliu@nankai.edu.cn; Fax: +8622 2350 3625; Tel: +8622 2350 3625*
[b]*College of Life Science, Nankai University, Tianjin, 300071, P. R China. E-mail: xzfeng@nankai.edu.cn; Fax: +8622 23507022; Tel: +8622 23507022*
† Electronic supplementary information (ESI) available: Detailed synthesis of PPRs, and reference experiments. See DOI: 10.1039/b704279e

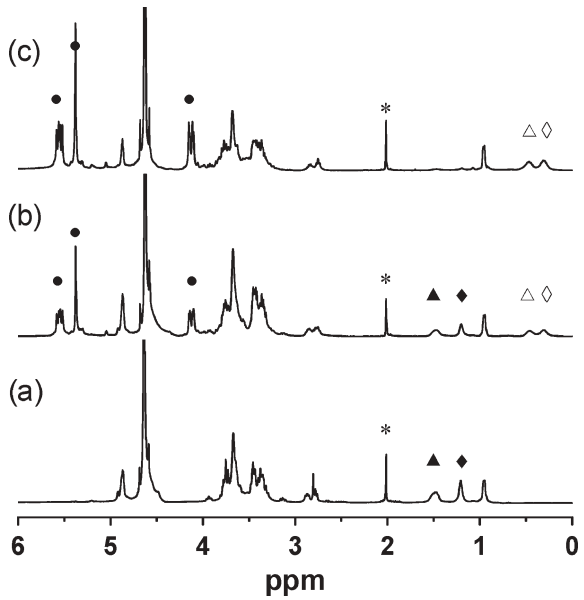

Fig. 2 ^{1}H NMR spectra PPRs. (a) PPR-0, (b) PPR-40, (c) PPR-100. Symbols ● and * indicate CB[6] and acetone resonances, respectively, and the latter was chosen as references in D_2O. Symbols (▲/△) and (◆/◇) indicate Hb/b′ and Hc/c′ side chains of β-CDs outside/inside CB[6]s′ cavities, respectively.

found that PPR-70 has the most efficiency to retard DNA in the gel well. This phenomenon is very strange that the retardation of DNA by PPRs neither increased nor decreased linearly with the percentage of CB[6]s. In a control experiment, the compound 6-[(6-aminohexyl)amino]-6-deoxy-β-CD did not show obvious condensation effect on DNA in agarose gel electrophoresis.

To get further insight into the DNA structural changes in the presence of PPRs, we employed atomic force microscope (AFM). In order to give a notable difference, we respectively chose DNA/PPR-0 and DNA/PPR-70 with the same concentration and molar ratio as shown in lane 7 in Fig. 3(a) and (d). In the absence of PPRs, free plasmid DNAs with about 4730 bp deposited in their plectonemic form with several supercoils which cause the double helix to cross itself a number of times (Fig. 4(a)). Upon interaction with PPR-0, DNA was induced to form small nanoparticles (about 120 nm diameter, Fig. 4(b)). When PPR-70 was added, larger nanoparticles with about 600 nm diameter were observed (Fig. 4(c)). In the case of PPR-100, the diameter of the nanoparticles decreased (see ESI†). That is to say, the percentage of CB[6] threading on the side chain does not increase linearly with the size of DNA complex particles. Similar phenomena were reported that the DNA condensation abilities did not grow linearly with the generation of cationic dendrimers.[17]

In order to assess the relative binding abilities of DNA with all PPRs, ethidium bromide (EB) displacement assays were carried out (see ESI†).[18] The weakly fluorescent ethidium bromide upon intercalation into DNA exhibits a strong fluorescence, and any competent ligand binding DNA that can displace the intercalated EB, will lead to fluorescence quenching. The obtained results showed that about 80% intercalated EB was displaced after the addition of sufficient PPRs. We define that the lower concentration of sample to quench fluorescence to about 80% of its original intensity is, the stronger interaction with DNA it has. Hence, the

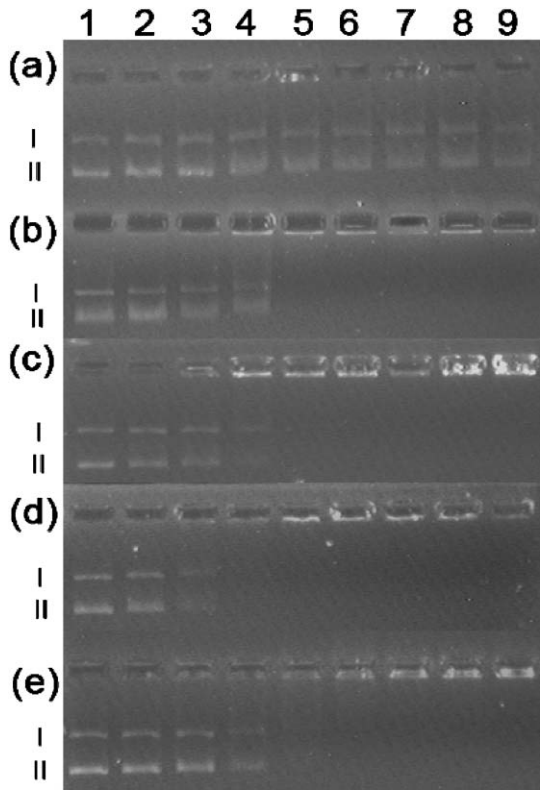

Fig. 3 Agarose gel electrophoresis assay to investigate the DNA condensation induced by (a) PPR-0; (b) PPR-20; (c) PPR-40; (d) PPR-70; (e) PPR-100. Lane 1, DNA alone (5 ng μL^{-1}); lanes 2–9, DNA + PPRs. The molar ratios between PPR-*n* and DNA nucleotide from lane 1 to lane 9 are 0, 1.557, 3.1, 6.197, 9.295, 12.393, 15.492, 18.59 and 31, respectively.

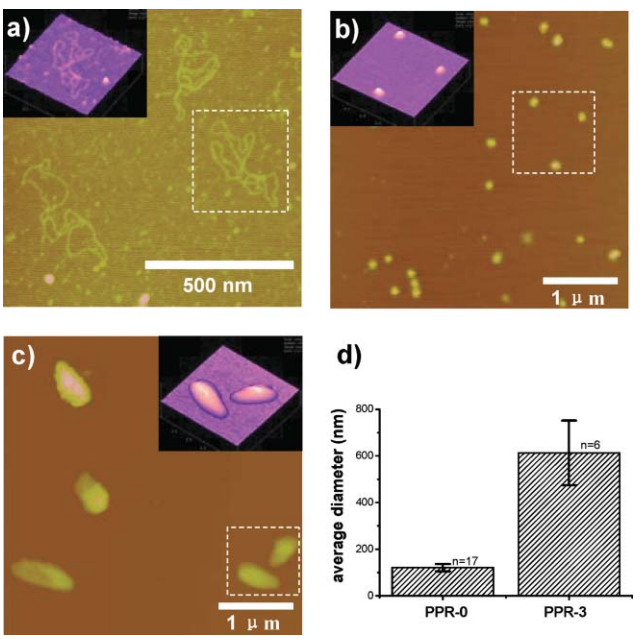

Fig. 4 AFM height images of a plasmid DNA, and its condensates induced by 2D PPRs on mica in tapping mode in the air. (a) An intact plasmid DNA pEGFP-C2, (b) DNA condensate induced by PPR-0, (c) a larger DNA condensates induced by PPR-70, (d) statistic analysis of particle sizes in (b) and (c).

interaction intensities of PPRs with DNA (from strong to weak) are PPR-0, PPR-20, PPR-40, PPR-100, and PPR-70.

It is known that agarose gel electrophoresis experiment actually reports on size-charge ratio. Thus, more highly positively charged complexes do indeed remain close to the well, but also larger and less charged complexes stay near the well as they are less able to migrate into the gel. From AFM images, we can find out that PPR-70 interacting with DNA form the biggest particles while PPR-0 form tightly compact ones. On the other hand, EB displacement experiments showed that PPR-70 had the lowest interacting ability with DNA. It is known that EB dissociation from the DNA/EB complex depends on the charge neutralization, which was attributed to a collapse of DNA into packed forms proceeding as a highly cooperative process.[19] When the hexane-diamino moieties of PPRs are included in CB[6]s′ cavities, the charged cations are shielded by CB[6]s, which already had been proved by ^{1}H NMR spectra. As a result, the effective charges of PPRs interacting with DNA should decrease with the increasing of CB[6]s. However, the rigidity of PPRs should increase with the addition of CB[6]s, which could also be an important factor for the interaction of PPRs with DNA. This would lead to an unusual binding interaction taking place in the case of DNA/PPR-70. Combining the results from the EB displacement assays and AFM images, we conclude that the migration of DNA/PPR complexes in agarose gel electrophoresis is mainly dominated by the particles size.

In summary, a series of 2D PPRs bearing CB[6] on their side chains have been synthesized, and these new materials show different abilities on DNA condensation through controlling the number of CB[6]. The results obtained indicate that the effective charges on the side chains of PPRs play a critical role in condensing DNA. Particularly, PPR-70 could form the largest particles with DNA, resulting in the most efficient DNA condensation. The controllable DNA condensation by supramolecular technology could be very helpful in designing gene delivery vectors to gain higher efficiency in the future.

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Notes and references

‡ Plasmid DNA pEGFP-C2 (4.7 kb) was purified from *E. coli* DH5α and was stored in TE buffer (10 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} EDTA, pH 8.0) at −20 °C. The concentration of plasmid DNA was measured through spectrophotometric analysis using a DU-7 Spectrophotometer (Beckman). PPRs were diluted with phosphate-buffered saline (PBS) solution. DNA condensation induced by PPRs was confirmed by agarose gel electrophoresis. Complexes of PPRs and DNA were formed by adding 5 μL PPRs solution into 5 μl DNA solution (5 ng μL^{-1}). The molar ratios between PPRs and DNA nucleotide were 1.557, 3.1, 6.197, 9.295, 12.393, 15.492, 18.59 and 31 separately. Free DNA was applied on the gel as a control. All the samples were kept for 4 h for balance under room temperature, and then loaded into 0.8% agarose gel, which contained ethidium bromide (1 μg mL^{-1}). The DNA binding abilities of PPRs were measured using an ethidium bromide fluorescence quenching assay experiments according to the reported works. This method is rapid and involves the addition of microlitre aliquots of PPRs to a solution of ethidium bromide (1.3 μM) and plasmid DNA ([DNA base pair] = 3.0 μM) in TE buffer (3 mL) with the decrease of fluorescence. For synthesis and characteristic of PPRs and reference experiments see ESI.†

1 (*a*) D. W. Pack, A. S. Hoffman, S. Pun and P. S. Stayton, *Nat. Rev. Drug Discovery*, 2005, **4**, 581–593; (*b*) R. C. Mulligan, *Science*, 1993, **260**, 926–932.
2 (*a*) M. Sainlos, M. Hauchecorne, N. Oudrhiri, S. Zertal-Zidani, A. Aissaoui, J.-P. Vigneron, J.-M. Lehn and P. Lehn, *ChemBioChem*, 2005, **6**, 1023–1033.
3 A. J. Kirby, P. Camilleri, J. B. F. N. Engberts, M. C. Feiters, R. J. M. Nolte, O. Söderman, M. Bergsma, P. C. Bell, M. L. Fielden, L. G. Rodríguez Cristina, P. Guédat, A. Kremer, C. McGregor, C. Perrin, G. Ronsin and M. C. P. van Eijk, *Angew. Chem., Int. Ed.*, 2003, **42**, 1448–1457.
4 M. Guillot, S. Eisler, K. Weller, H. P. Merkle, J.-L. Gallani and F. Diederich, *Org. Biomol. Chem.*, 2006, **4**, 766–769.
5 (*a*) N. Duzgunes, C. Tros de Ilarduya, S. Simoes, R. I. Zhdanov, K. Konopka and M. C. Pedroso de Lima, *Curr. Med. Chem.*, 2003, **10**, 1213–1220; (*b*) D. J. Chen, B. S. Majors, A. Zelikin and D. Putnam, *J. Controlled Release*, 2005, **103**, 273–283; (*c*) J. Zabner, *Adv. Drug Delivery Rev.*, 1997, **27**, 17–28.
6 S. H. Pun, N. C. Bellocq, A. Liu, G. Jensen, T. Machemer, E. Quijano, T. Schluep, S. Wen, H. Engler, J. Heidel and M. E. Davis, *Bioconjugate Chem.*, 2004, **15**, 831–840.
7 F. Sansone, M. Dudic, G. Donofrio, C. Rivetti, L. Baldini, A. Casnati, S. Cellai and R. Ungaro, *J. Am. Chem. Soc.*, 2006, **128**, 14528–14536.
8 (*a*) J. Cheng, R. Zeidan, S. Mishra, A. Liu, S. H. Pun, R. P. Kulkarni, G. S. Jensen, N. C. Bellocq and M. E. Davis, *J. Med. Chem.*, 2006, **49**, 6522–6531; (*b*) N. C. Bellocq, D. W. Kang, X. Wang, G. S. Jensen, S. H. Pun, T. Schluep, M. L. Zepeda and M. E. Davis, *Bioconjugate Chem.*, 2004, **15**, 1201–1211; (*c*) S. H. Pun, N. C. Bellocq, A. Liu, G. Jensen, T. Machemer, E. Quijano, T. Schluep, S. Wen, H. Engler, J. Heidel and M. E. Davis, *Bioconjugate Chem.*, 2004, **15**, 831–840; (*d*) N. C. Bellocq, S. H. Pun, G. S. Jensen and M. E. Davis, *Bioconjugate Chem.*, 2003, **14**, 1122–1132; (*e*) S. R. Popielarski, S. Mishra and M. E. Davis, *Bioconjugate Chem.*, 2003, **14**, 672–678.
9 X. Shuai, T. Merdan, F. Unger and T. Kissel, *Bioconjugate Chem.*, 2005, **16**, 322–329.
10 A. Yamashita, H. S. Choi, T. Ooya, N. Yui, H. Akita, K. Kogure and H. Harashima, *ChemBioChem*, 2006, **7**, 297–302.
11 T. Ooya, H. S. Choi, A. Yamashita, N. Yui, Y. Sugaya, A. Kano, A. Maruyama, H. Akita, R. Ito, K. Kogure and H. Harashima, *J. Am. Chem. Soc.*, 2006, **128**, 3852–3853.
12 J. Li, C. Yang, H. Li, X. Wang, S. H. Goh, J. L. Ding, D. Y. Wang and K. W. Leong, *Adv. Mater.*, 2006, **18**, 2969–2974.
13 (*a*) O. Zelphati and F. C. Szoka, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 11493–11498; (*b*) D. V. Schaffer, N. A. Fidelman, N. Dan and D. A. Lauffenburger, *Biotechnol. Bioeng.*, 2000, **67**, 598–606.
14 (*a*) H. Isobe, S. Sato, J. W. Lee, H.-J. Kim, K. Kim and E. Nakamura, *Chem. Commun.*, 2005, 1549–1551; (*b*) Y. B. Lim, T. Kim, J. W. Lee, S. M. Kim, H. J. Kim, K. Kim and J. S. Park, *Bioconjugate Chem.*, 2002, **13**, 1181–1185; (*c*) D. Tuncel, Ö. Özsar, H. B. Tiftik and B. Salih, *Chem. Commun.*, 2007, 1369–1371.
15 Y. Liu, C.-F. Ke, H.-Y. Zhang, W.-J. Wu and J. Shi, *J. Org. Chem.*, 2007, **72**, 280–283.
16 S. Liu, C. Ruspic, P. Mukhopadhyay, S. Chakrabarti, P. Y. Zavalij and L. Isaacs, *J. Am. Chem. Soc.*, 2005, **127**, 15959–15967.
17 (*a*) F. Kihara, H. Arima, T. Tsutsumi, F. Hirayama and K. Uekama, *Bioconjugate Chem.*, 2002, **13**, 1211–1219; (*b*) C. S. Braun, J. A. Vetro, D. A. Tomalia, G. S. Koe, J. G. Koe and C. R. Middaugh, *J. Pharm. Sci.*, 2005, **94**, 423–436.
18 (*a*) A. J. Geall, M. A. W. Eaton, T. Baker, C. Catterall and I. S. Blagbrough, *FEBS Lett.*, 1999, **459**, 337–342; (*b*) B. F. Cain, B. C. Baguley and W. A. Denny, *J. Med. Chem.*, 1978, **21**, 658–668.
19 (*a*) V. A. Izumrudov, M. V. Zhiryakova and A. A. Goulko, *Langmuir*, 2002, **18**, 10348–10356; (*b*) G. S. Manning, *Biopolymers*, 1980, **19**, 37–59.

