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Supramolecular nanoparticles based on β-CD modified hyaluronic acid for DNA encapsulation and controlled release†

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Supramolecular nanoparticles composed of doubly positively charged adamantane (ADA2+) and β-CD modified hyaluronic acid (HACD) were constructed. When the ester group in ADA2+ was hydrolyzed to a carboxyl group, the quaternary ammonium chain with a positive charge in ADA2+ converted to a "zwitterionic" structure, and the controlled binding and release of pDNA was realized.

The construction of targeted drug/gene nanocarrier systems through non-covalent interactions has become an emerging strategy for researchers in the chemical and biological/medicinal materials fields.[1–4] The convenient chemical synthesis of building blocks, controllable assembly, large capacity for loading of drugs/genes, and controllable release of cargo are possible advantages of nano-supramolecular delivery systems.[5–11] Among a series of supramolecular interactions, the binding behavior between adamantane and β-cyclodextrin has often been selected due to the large association constant and high reliability of the interactions.[12–15] Recently, several targeted drug/gene delivery systems based on adamantane–β-cyclodextrin interactions were reported by our group and other scientific researchers.[16–19] Zhang *et al.*[20] have reported a targeted siRNA delivery nanoparticle constructed by supramolecular interactions, and the siRNA binding behavior with an adamantane–bis(diamine) conjugate was enhanced by a cucurbit[6]uril complexation-induced pK_a shift. Moreover, Zhao's group[21] have reported a novel mesoporous silica nanoparticle functionalized with β-cyclodextrin by cleavable disulfide bonds, which could hold anti-cancer drugs inside the nanoparticle. PEG-folate modified adamantane was also introduced onto the surface of the silica nanoparticle to improve the biocompatibility and targeting capabilities through supramolecular interactions. Finally, controllable drug release was realized by cleavage of disulfide bonds by glutathione *in vitro*.

On the other hand, one problem in targeted gene delivery is the timely release of the loaded pDNA/siRNA, which might be the determinant for the further promotion of gene transfection efficiency.[22–24] A number of literature studies pay attention to the effective condensation and loading of nucleic acids, however, the stimulus-response release of the nucleic acids when they arrive at the targeting zone should be also considered.[25,26] To solve this problem, Jiang's[27–32] group reported a molecular structure with a connected carboxylic ester group and a positively charged quaternary ammonium group together, and this structure could bind negatively charged biomolecules, such as nuclei acids, proteins, phospholipids, *etc.* When the ester group was hydrolyzed to a negative charged carboxyl, a positively charged group and a negatively charged group simultaneously appeared on one chain, which was deemed to be a "zwitterionic" molecule and was considered as an ultralow-fouling material, and the packaged biomolecules were effectively released.

In this work, we synthesized a doubly positively charged guest, **ADA2+**, bearing one adamantyl group on one side and two quaternary ammonium chains on the other side, and then fabricated β-CD modified hyaluronic acid (**HACD**) which was reported by our group previously. When **ADA2+** and **HACD** were mixed together, the nanoparticle named **ADA2+@HACD**, which had a negatively charged HA shell and a positively charged quaternary ammonium chain core, was constructed through the supramolecular interactions between the adamantane group in **ADA2+** and the β-CD group in **HACD** (Scheme 1). The positive core of the **ADA2+@HACD** nanoparticles could bind negatively charged plasmid DNA (pDNA), which then could be released when the ester group on the quaternary ammonium chain of **ADA2+** was hydrolyzed to form a zwitterionic group, and controllable pDNA binding and release by the **ADA2+@HACD** nanoparticles could be realized.

HACD was synthesized according to our previous work.[33,34] The molecular weight of HA that we employed in this work was 290 kDa, and the degree of substitution on **HACD** was calculated as one β-CD unit grafted on every seven HA repeating sugar

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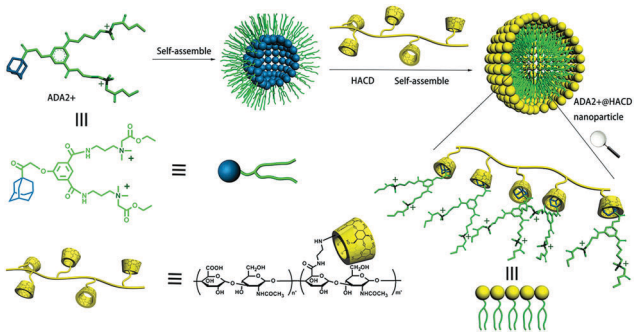


Scheme 1 The synthetic route for the **ADA2+@HACD** nanoparticles.

units on average. This was determined according to the ^{1}H NMR spectra of **HACD**, and meets the requirements that at least six successive repeating units of HA are needed as one targeting section for achieving multiple HA–CD44 interactions in tumor-targeting bioconjugates for drug/gene delivery.[35] On the other hand, the guest molecule **ADA2+** bearing one adamantyl group and two quaternary ammonium chains was synthesized through a five-step synthetic route (Fig. S1, ESI†) with a total yield of 71%. The characterization of the intermediate products (compound **1**–**4**) and **ADA2+** is presented in Fig. S2–S16 (ESI†). As shown in Scheme 1, the adamantyl group in **ADA2+** could be included in the cavity of β-CD of **HACD** to form **ADA2+@HACD** nanoparticles, and the double quaternary ammonium chain could electrostatically interact with the anionic phosphate backbones of pDNA. The bound pDNA could be released *via* the hydrolysis of the ester group on the quaternary ammonium chain in **ADA2+** by forming a zwitterionic structure.

We employed β-CD as a reference host and then used a ^{1}H NMR titration method to investigate the supramolecular interactions between the adamantane part of **ADA2+** and the β-CD moiety in **HACD**. As shown in Fig. 1a, with the increase of the concentration of β-CD from 0–9 mM, the proton signals of **ADA2+** (1 mM) shifted downfield from δ = 2.01 ppm to δ = 2.22 ppm, accompanied by shape changes, which indicated the inclusion of the adamantane part of **ADA2+** into the cavity of β-CD. Furthermore, by analyzing the nonlinear least-squares fit of the titration data (Fig. 1b), the binding constant (K_S) between **ADA2+** and β-CD was calculated as (6.1 ± 0.8) × 10^3 M^{-1}. Moreover, the stoichiometry between **ADA2+** and β-CD was determined as 1 : 1 according to the Job plot in which the maximum appeared at a molar fraction of 0.5 (Fig. S17, ESI†).

Taking advantage of the strong supramolecular interactions between adamantane and β-CD, the supramolecular nanoparticle **ADA2+@HACD** was obtained by simply mixing aqueous solutions of **ADA2+** and **HACD** together. The morphology, size, and surface charge of the **ADA2+@HACD** nanoparticles were characterized by atomic force microscopy (AFM), high-resolution transmission electron microscopy (HR-TEM), dynamic light scattering (DLS), and ζ potential experiments. As shown in Fig. S18a (ESI†), the guest molecule **ADA2+** could form spherical self-assemblies whose diameter was measured as *ca.* 600 nm, and the height of the loose and collapsed assembly was obtained as 2.7 nm. However, in Fig. 2a, after complexation with HACD, spherical

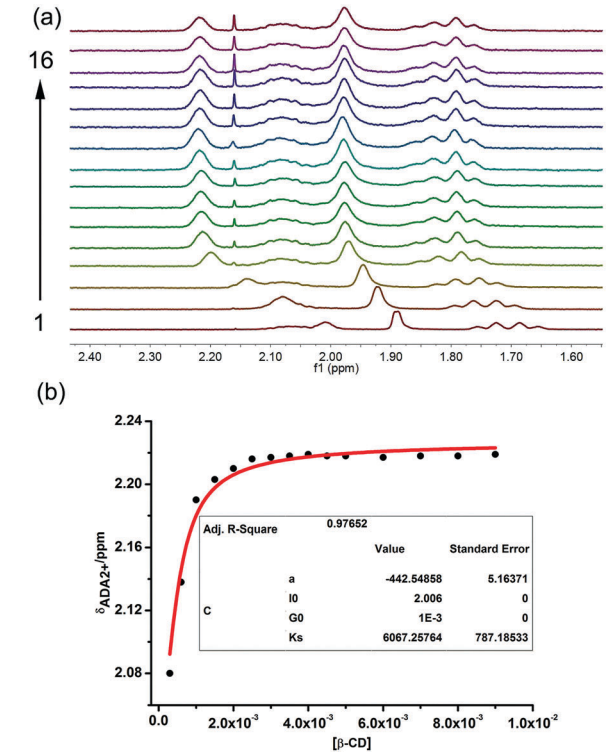


Fig. 1 ^{1}H NMR titration of **ADA2+** with β-CD. (a) ^{1}H NMR spectra of **ADA2+** (1 mM) with addition of 0, 0.3, 0.6, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0 mM β-CD (spectra from 1 to 16) in D_2O at 25 °C. (b) Nonlinear least-squares fit of the chemical shift changes of the **ADA2+** peaks at δ = 2.01 ppm as a function of the concentration of β-CD.

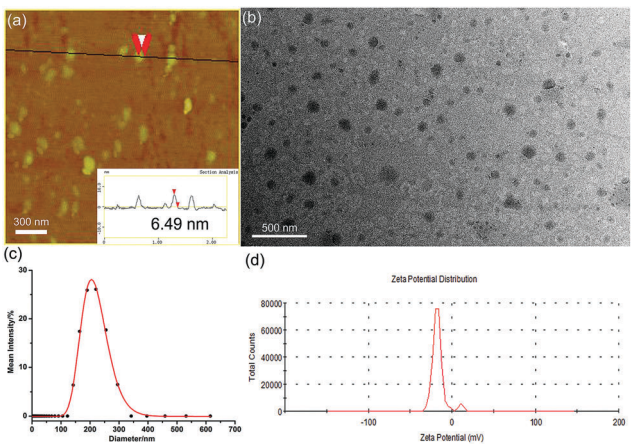


Fig. 2 Typical (a) AFM and (b) HR-TEM images of **ADA2+@HACD** nanoparticles, and (c) DLS and (d) ζ potential experiment results of **ADA2+@HACD** nanoparticles in deionized aqueous solution.

shaped **ADA2+@HACD** nanoparticles were observed with a diameter of around 120 nm, and the height of the collapsed nanoparticle was measured as 6.5 nm, which was roughly equal to the sum of the values of two HA backbones (*ca.* 1.5 nm), two β-CD units (*ca.* 1.6 nm), and two flexible double quaternary ammonium chains with bromide counter anions (3.5 nm). The HR-TEM image (Fig. 2b) also showed the 100–130 nm homogeneous

spherical nanoparticles with homogeneous dispersity. Moreover, as shown in Fig. 2c, the hydrodynamic diameter of the **ADA2+@HACD** nanoparticles was measured by DLS experiments as *ca.* 286 nm, with a narrow distribution, which was much less than that of the self-assemblies of **ADA2+** of *ca.* 616 nm (Fig. S18b, ESI†). Finally, the surface charges of the **ADA2+** self-assemblies and the **ADA2+@HACD** nanoparticles were measured using ζ potential experiments. As shown in Fig. S18c (ESI†), the surface charge of the **ADA2+** self-assemblies was measured as *ca.* +23 mV, implying that the quaternary ammonium chains, with positive charges, pointed toward the outside of the **ADA2+** self-assemblies. After addition of **HACD**, as shown in Fig. 2d, the resulting **ADA2+@HACD** nanoparticles showed a typical negative charge on the surface of *ca.* −17 mV, indicating the coverage of negatively charged HA on the surface of the **ADA2+@HACD** nanoparticles. Subsequently, according to the results we discussed above, we could conclude that the self-assembly of **ADA2+** which had a positively charged hydrophilic quaternary ammonium chain surface, and a hydrophobic adamantane core, could be formed in aqueous solution in an incompact way due to the amphiphilic nature of **ADA2+**. After inclusion of the adamantane part of **ADA2+** into the cavity of β-CD in **HACD**, the **ADA2+@HACD** nanoparticles could be constituted, which had a negatively charged HA shell, and a positively charged quaternary ammonium chain core. The resulting **ADA2+@HACD** nanoparticles could not only recognize the HA receptor positive cancer cells, but also reduced the cytotoxicity of **ADA2+** because of the cytomembrane protection from the cytotoxic positively charged HA shell.

Furthermore, the formation of **ADA2+@HACD** nanoparticles could be also recognized by the naked eye. As shown in Fig. S19 (ESI†), **ADA2+**, **HACD**, and **ADA2+@HACD** nanoparticles existed as steady and transparent aqueous solutions. Among them, **ADA2+** exhibited the strongest Tyndall effect due to the largest size (*ca.* 600 nm) of the self-assembly in aqueous solution. **ADA2+@HACD** nanoparticles showed much more of an obvious Tyndall effect than **HACD**, which indicated the formation of nanoscale particles with modest sizes (*ca.* 130 nm).

Due to the quaternary ammonium chains of **ADA2+** with positive charge in aqueous solution, **ADA2+** was considered to bind DNA through electrostatic interactions. Subsequently, the pDNA binding behaviors of **ADA2+** and **ADA2+@HACD** nanoparticles were examined by electrophoresis experiments by analyzing the electrophoretic mobility at different N/P ratios (the charge ratio of the cationic quaternary ammonium units in **ADA2+** to the anionic phosphate units in pDNA). As shown in Fig. 3a, the pDNA was completely retarded by **ADA2+** at N/P $\geq$ 30. After adding **HACD**, the negatively charged HA chain did not impede the pDNA binding behavior of **ADA2+**, and the formed **ADA2+@HACD** nanoparticles exhibited similar pDNA binding capabilities as **ADA2+**, thus the minimum N/P ratio for complete pDNA retardation was also observed as 30 (Fig. 3b). This phenomenon might be attributed to the encapsulation of pDNA into the **ADA2+@HACD** nanoparticles, which had a negatively charged HA shell, and a positively charged quaternary ammonium chains core, and the HA chains had no interfering electrostatic interaction with **ADA2+**.

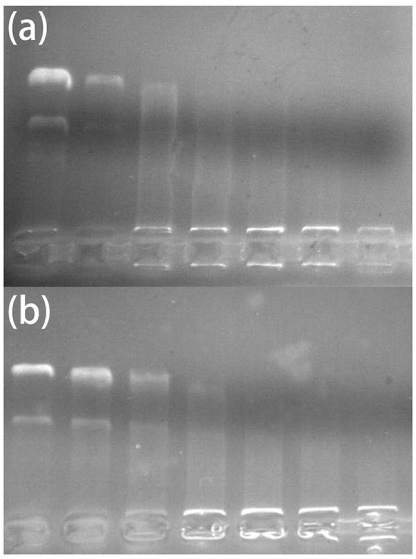


Fig. 3 Electrophoresis experiments of (a) **ADA2+** and (b) **ADA2+@HACD** nanoparticles with pDNA at different N/P ratios (N/P = 0, 5, 10, 20, 30, 40, 60 from left to right).

On the other hand, the controllable pDNA release by hydrolysis of ester groups in **ADA2+** was performed by electrophoresis experiments of pDNA–**ADA2+** and pDNA–**ADA2+@HACD** adducts before and after hydrolysis with aqueous NaOH solution. As shown in Fig. 4, at N/P = 30, **ADA2+** and **ADA2+@HACD** nanoparticles showed satisfactory pDNA retardation effects (line 1 and line 3, respectively), due to the electrostatic interaction between the positively charged quaternary ammonium in **ADA2+** and the negatively charged phosphate of the pDNA. After hydrolysis with NaOH aqueous solution, the neutral ester group in **ADA2+** changed to a negatively charged carboxyl group, and a zwitterionic **ADA2+′** appeared which had a positive charge and a negative charge on one chain. This zwitterionic species was highly resistant

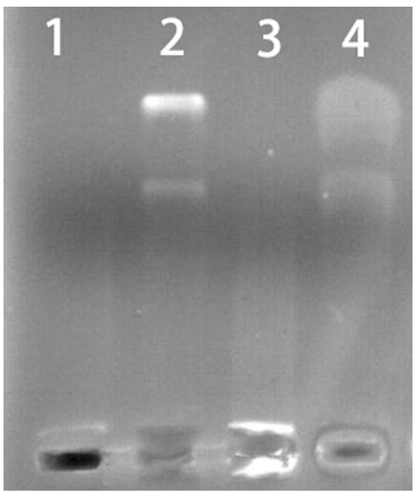
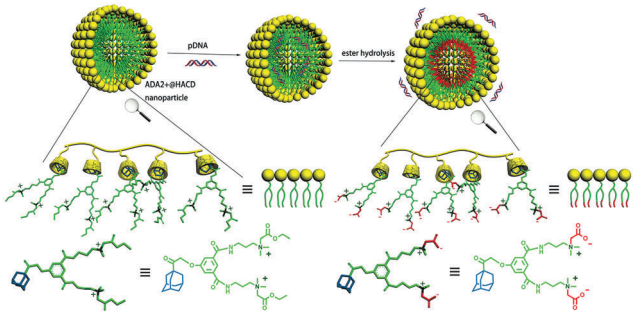


Fig. 4 Electrophoresis experiments of **ADA2+** with pDNA before (lane 1) and after (lane 2) esterolysis at N/P = 30, and electrophoresis experiments of **ADA2+@HACD** nanoparticles with pDNA before (lane 3) and after (lane 4) esterolysis at N/P = 30.



Scheme 2 The process of pDNA binding and release of the **ADA2+@HACD** nanoparticles.

to bio-molecular absorption, and pDNA release from **ADA2+′** and **ADA2+′@HACD** were observed (line 2 and line 4 in Fig. 4). The process of pDNA binding and release is exhibited in Scheme 2.

In conclusion, a targeted pDNA encapsulation system of **ADA2+@HACD** nanoparticles was successfully constructed by **ADA2+** and **HACD** through supramolecular interactions. The nanoparticles were well-characterized, and had negatively charged HA shells and positively charged quaternary ammonium chain cores. The nanoparticles could bind pDNA effectively. Moreover, after hydrolysis of the ester group in **ADA2+**, the quaternary ammonium chain with a positive charge was converted to a zwitterionic group, and the encapsulated pDNA could be released. The controllable binding and release of pDNA by this approach might give important insights into methods of gene transfection of target cells. Finally, the construction of **ADA2+@HACD** nanoparticles provides an attractive strategy and platform for targeted delivery, and controllable packaging and release of pharmaceutical nucleic acids. Continual optimization of the quaternary ammonium chains in **ADA2+** is the goal of endeavor for our group.

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Conflicts of interest

The authors declare no competing financial interest.

Notes and references

1 W. Al-Jamal and K. Kostarelos, *Acc. Chem. Res.*, 2011, **44**, 1094.
2 K. M. Park, D.-W. Lee, B. Sarkar, H. Jung, J. Kim, Y. H. Ko, K. E. Lee, H. Jeon and K. Kim, *Small*, 2010, **6**, 1430.
3 R. Dong, Y. Zhou, X. Huang, X. Zhu, Y. Lu and J. Shen, *Adv. Mater.*, 2015, **27**, 498.
4 X. Ma and Y. Zhao, *Chem. Rev.*, 2015, **115**, 7794.
5 R. Dong, Y. Zhou and X. Zhu, *Acc. Chem. Res.*, 2014, **47**, 2006.
6 Z. Yu, J. Zhang, R. J. Coulston, R. M. Parker, F. Biedermann, X. Liu, O. A. Scherman and C. Abell, *Chem. Sci.*, 2015, **6**, 4929.
7 K. Yang, Y. Pei, J. Wen and Z. Pei, *Chem. Commun.*, 2016, **52**, 9316.
8 S. Hou, J. Choi, M. A. Garcia, Y. Xing, K.-J. Chen, Y.-M. Chen, Z. K. Jiang, T. Ro, L. Wu, D. B. Stout, J. S. Tomlinson, H. Wang, K. Chen, H.-R. Tseng and W.-Y. Lin, *ACS Nano*, 2016, **10**, 1417.
9 M. H. Bakker, C. C. Lee, E. W. Meijer, P. Y. W. Dankers and L. Albertazzi, *ACS Nano*, 2016, **10**, 1845.
10 T. D. Nguyen, Y. Liu, S. Saha, K. C.-F. Leung, J. F. Stoddart and J. I. Zink, *J. Am. Chem. Soc.*, 2007, **129**, 626.
11 L.-L. Tan, H. Li, Y.-C. Qiu, D.-X. Chen, X. Wang, R.-Y. Pan, Y. Wang, S. X.-A. Zhang, B. Wang and Y.-W. Yang, *Chem. Sci.*, 2015, **6**, 1640.
12 Q.-D. Hu, G.-P. Tang and P. K. Chu, *Acc. Chem. Res.*, 2014, **47**, 2017.
13 C. Y. Ang, S. Y. Tan, X. Wang, Q. Zhang, M. Khan, L. Bai, S. T. Selvan, X. Ma, L. Zhu, K. T. Nguyen, N. S. Tan and Y. Zhao, *J. Mater. Chem. B*, 2014, **2**, 1879.
14 C. Stoffelen and J. Huskens, *Small*, 2016, **12**, 96.
15 R. Mejia-Ariza, L. Grana-Suárez, W. Verboom and J. Huskens, *J. Mater. Chem. B*, 2017, **5**, 36.
16 N. Nayak and K. R. Gopidas, *J. Mater. Chem. B*, 2015, **3**, 3425.
17 L. Xiong, Q. Luo, Y. Wang, X. Li, Z. Shen and W. Zhu, *Chem. Commun.*, 2015, **51**, 14644.
18 J. E. Mealy, C. B. Rodell and J. A. Burdick, *J. Mater. Chem. B*, 2015, **3**, 8010.
19 H. Chen, H. Jia, H. P. Tham, Q. Qu, P. Xing, J. Zhao, S. Z. F. Phua, G. Chen and Y. Zhao, *ACS Appl. Mater. Interfaces*, 2017, **9**, 23536.
20 Y.-M. Zhang, Y. Yang, Y.-H. Zhang and Y. Liu, *Sci. Rep.*, 2016, **6**, 28848.
21 Q. Zhang, F. Liu, K. T. Nguyen, X. Ma, X. Wang, B. Xing and Y. Zhao, *Adv. Funct. Mater.*, 2012, **22**, 5144.
22 N. Kamaly, B. Yameen, J. Wu and O. C. Farokhzad, *Chem. Rev.*, 2016, **116**, 2602.
23 W. Chen, F. Meng, R. Cheng, C. Deng, J. Feijen and Z. Zhong, *J. Controlled Release*, 2014, **190**, 398.
24 S. M. Sarett, C. E. Nelson and C. L. Duvall, *J. Controlled Release*, 2015, **218**, 94.
25 U. Lächelt and E. Wagner, *Chem. Rev.*, 2015, **115**, 11043.
26 J. M. Ren, T. G. McKenzie, Q. Fu, E. H. H. Wong, J. Xu, Z. An, S. Shanmugam, T. P. Davis, C. Boyer and G. G. Qiao, *Chem. Rev.*, 2016, **116**, 6743.
27 L. Zhang, Z. Cao, T. Bai, L. Carr, J.-R. Ella-Menye, C. Irvin, B. D. Ratner and S. Jiang, *Nat. Biotechnol.*, 2012, **31**, 533.
28 A. J. Keefe and S. Jiang, *Nat. Chem.*, 2012, **4**, 59.
29 S. Jiang and Z. Cao, *Adv. Mater.*, 2010, **22**, 920.
30 L. Zhang, A. Sinclair, Z. Cao, J.-R. Ella-Menye, X. Xu, L. R. Carr, S. H. Pun and S. Jiang, *Small*, 2013, **9**, 3439.
31 Z. Cao, Q. Yu, H. Xue, G. Cheng and S. Jiang, *Angew. Chem., Int. Ed.*, 2010, **49**, 3771.
32 H. S. Sundaram, J.-R. Ella-Menye, N. D. Brault, Q. Shao and S. Jiang, *Chem. Sci.*, 2014, **5**, 200.
33 Y.-H. Zhang, Y.-M. Zhang, Y. Yang, L.-X. Chen and Y. Liu, *Chem. Commun.*, 2016, **52**, 6087.
34 Y. Yang, Y.-M. Zhang, Y. Chen, J.-T. Chen and Y. Liu, *J. Med. Chem.*, 2013, **56**, 9725.
35 S. Jaracz, J. Chen, L. V. Kuznetsova and I. Ojima, *Bioorg. Med. Chem.*, 2005, **13**, 5043.

