

PCCP

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

In situ 2D-extraction of DNA wheels by 3D through-solution transport

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Yusuke Yonamine,^{*a,b} Keitel Cervantes-Salguero,^{*c} Waka Nakanishi,^{*a} Ibuki Kawamata,^c Kosuke Minami,^a Hirokazu Komatsu,^a Satoshi Murata,^{*c} Jonathan P. Hill,^{a,b} and Katsuhiko Ariga^{*a,b}

Controlled transfer of DNA nanowheels from a hydrophilic to a hydrophobic surface was achieved by complexation of the nanowheels with a cationic lipid (2C₁₂N⁺). 2D surface-assisted extraction, '2D-extraction', enabled structure-persistent transfer of DNA wheels, which could not be achieved by simple drop-casting.

DNA nanostructures can be designed by programming of the DNA sequences that consist of Watson-Crick base pairs.¹ One, two, and three-dimensional (1, 2, and 3D) structures can be fabricated to produce DNA origami, tiles, and their assemblies.^{2,3} The techniques for design and fabrication of DNA origami and tiles are collectively known as DNA nanotechnology,^{4,5} which permits the fabrication of nanowires,^{6,7} sensors,^{8,9} semiconductors¹⁰ from DNA components. The observation and analyses of DNA origami are usually performed by using AFM and, since DNA is hydrophilic, DNA origami is semi-statically immobilized on a hydrophilic substrate due to strong ionic interactions with divalent cations.¹¹ Dynamic motion of lipid conjugated DNA origami on hydrophobic surfaces has recently been reported¹²⁻¹⁶ and suggests the application of DNA origami for dynamic positioning of molecular devices and machines.¹⁷ In addition to chemical bonding, complexation with lipids is an alternative method for lipophilization of DNA.^{18,19} However, simple mixing of self-assembled DNA tiles with a cationic lipid results in complex aggregation not suitable for the purpose of designing DNA nanostructures.²⁰ We speculated that this problem partially originates from the self-assembly properties of

cationic lipids and the destabilization of assembled DNA structures on 2D surfaces.

In this work, a wheel-structured DNA tile assembly²⁰ was lipophilized with its wheel structure persisting and obtained in isolated form by addition of cationic lipid on a hydrophilic 2D surface (Fig. 1). During complexation with cationic lipid, the DNA wheel underwent transfer from hydrophilic to hydrophobic regions of a patterned 2D substrate. This transfer between hydrophilic and hydrophobic environments could not be achieved where lipids are covalently bonded¹²⁻¹⁶, and can be considered as a model of a DNA actuator that responds to hydrophobic chemical stimuli. Furthermore, this in situ extraction method is suitable for structure-persistent lipophilization of DNA nanostructures. Since lipophilization of biomaterials is known to improve processability for easier handling and reduced costs,¹⁷ this could be a useful method for fabrication of materials using DNA nanostructures.

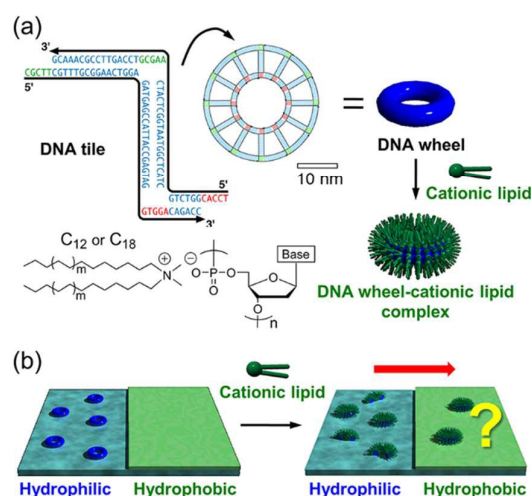


Fig. 1. Schematic view of complexation of DNA wheel and cationic lipid. (a) Formation of DNA wheel from the tile and complexation with lipid. (b) Transfer of the DNA wheel to a hydrophobic surface induced by lipid-complexation.

^a World Premier International (WPI) Research Centre for Materials Nanoarchitectonics (MANA), National Institute for Materials Science (NIMS) 1-1 Namiki, Tsukuba, Ibaraki, 305-0044, Japan. E-mail: YONAMINE.Yusuke@nims.go.jp, NAKANISHI.Waka@nims.go.jp, ARIGA.Katsuhiko@nims.go.jp

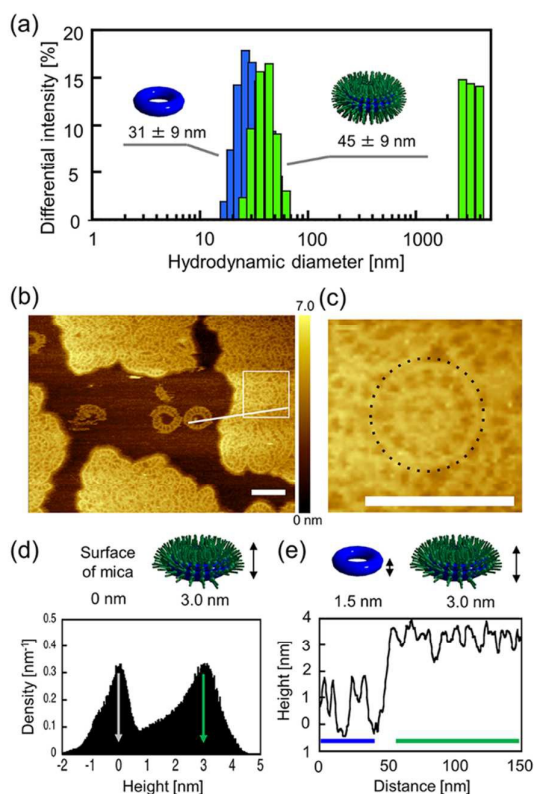
^b CREST, JST, Sanbancho, Chiyoda-ku, Tokyo, 102-0075, Japan

^c Department of Bioengineering and Robotics, Tohoku University, 6-6-1 Aobayama, Sendai, 980-8579, Japan. E-mail: cervantes@molbot.mech.tohoku.ac.jp, murata@molbot.mech.tohoku.ac.jp

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

The wheel-structured DNA tile self-assembly²⁰ was used here since it is available in larger quantities than DNA origami composed of longer nucleic acid sequences, and is suitable for use in analyses of their assembly properties. DNA wheel was prepared by the reported method (Supporting Information (SI), Fig. S1, ESI†).²⁰ The DNA wheel-cationic lipid complex was dissolved in an organic solvent (CHCl_3) and dimensions and optical properties were analyzed using dynamic light scattering (DLS) (Fig. 2a), UV absorption, and circular dichroism (CD) spectroscopy (Fig. S2, ESI†). Diameters of the DNA wheels were increased from 31 ± 9 nm to 45 ± 9 nm following complexation with the cationic lipid, dioctadecyldimethylammonium bromide ($2\text{C}_{18}\text{N}^+$), as indicated by DLS data (Fig. 2a). These data were obtained from the DNA wheel-cationic lipid complex in solution illustrate that the complex can exist in a monodisperse form in solution ($10 \mu\text{M}$ of DNA wheel). Since simple drop casting or spin-coating onto mica of the DNA-cationic lipid mixture resulted in AFM images in which the wheel structure cannot be recognized due to aggregation of the complex (Fig. S3, ESI†),²¹ we adopted an approach of in situ complexation of immobilized DNA wheels

Distributions of height in b. (e) Height profile at the white line in b.



on mica to avoid such aggregation.

Fig. 2. Structures of bare non-lipophilized DNA wheel and the lipid-DNA wheel complex. (a) Size histograms measured by DLS for non-lipid-DNA wheel in TAE/ Mg^{2+} buffer (blue) and lipid-DNA wheel complex in CHCl_3 (green). (b) AFM image of lipid-DNA wheel complex on mica surface. (c) Magnification of the position enclosed by a white box in b. A single DNA wheel is indicated by the dotted line. Scale bars in b and c: 50 nm. (d)

Formation of the wheel structures, which include a fine spoke-like framework at their interiors, was confirmed by high speed AFM both prior to (Fig. S4, ESI†) and following addition of the cationic lipid (didodecyldimethylammonium bromide; $2\text{C}_{12}\text{N}^+$) (Fig. 2b, 2c). In this work, a cationic lipid containing shorter alkyl substituents was used in place of $2\text{C}_{18}\text{N}^+$ to simplify the procedure for dissolution of the lipid. (See ESI†). After addition of the cationic lipid, the height perpendicular to the surface of the lipid-modified DNA wheel was 3.0 nm indicating an increase by complexation with lipid of 1.5 nm (Fig. 2d, 2e) obtained by subtracting the corresponding height of the non-complexed DNA (1.5 nm, Fig. S4, ESI†). The height gained is in agreement with the extended length of a $2\text{C}_{12}\text{N}^+$ molecule.²² The fact that the increased height of the DNA wheel-cationic lipid complex is the same as that of the cationic lipid is consistent with the formation of an interdigitated bilayer.²³ Although the fine structure of DNA wheel could be observed, the form of the DNA wheel was distorted after complexation with lipid and the tile assemblies often appeared with a defect (open) structure, which may be caused by aggregation of the cationic lipid and destabilization of the assembly of the DNA wheel (Fig. S5, ESI†).

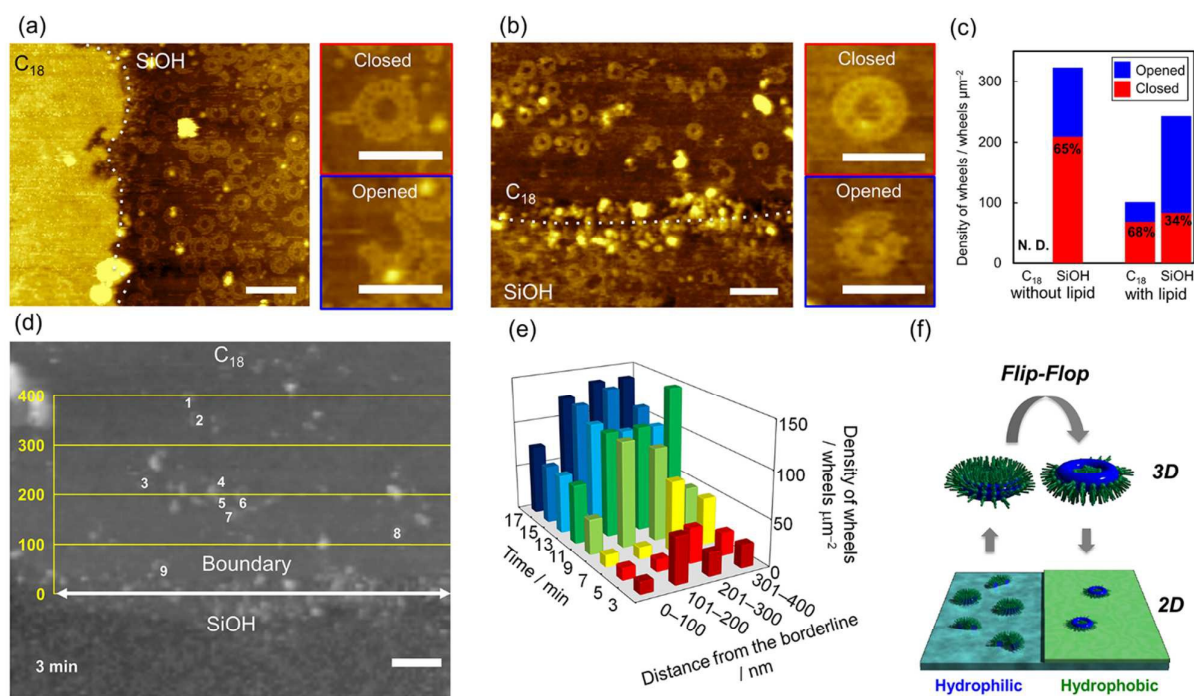
To demonstrate our hypothesis that transfer of DNA wheels to a hydrophobic area immediately after the lipid complexation confers persistence on the DNA wheel structure avoiding the aggregation, we investigated localization of non-lipid-complexed DNA-wheels and their complexes with cationic lipid on a patterned 2D substrate possessing both hydrophilic and hydrophobic regions.²⁴ The patterned 2D substrate was prepared by treatment with an alkyl (C_{18}) silane while untreated regions remained hydrophilic (SiOH) (Fig. S6, ESI†). When DNA wheel alone was dispersed on the patterned 2D substrate, wheels preferred to adsorb on hydrophilic regions (Fig. 3a, Fig. S7, ESI†). When cationic lipid was applied to the patterned 2D substrate, complexation of DNA wheels occurred and the resulting DNA wheel-cationic lipid complex was transferred to hydrophobic regions (Fig. 3b). This lipid-assisted transfer process is similar to solvent-solvent extraction using immiscible mixtures of hydrophobic/hydrophilic (organic) solvents. Based on the transfer by lipid, here we propose '2D-extraction' of DNA wheels using surfaces as an analogy of 3D-extraction using organic solvent. Despite DNA wheels being tightly immobilized on the hydrophilic surface through interactions with divalent magnesium ions,¹¹ lipid assisted 2D extraction resulted in transfer of up to 30% of the DNA wheel population from hydrophilic to hydrophobic regions.

Numbers of pristine assembled 'closed' wheel structures (close) and broken 'opened' structures (open) were observed in the hydrophilic and the hydrophobic regions of the patterned substrate (Fig. S8-S10, ESI†). In the hydrophilic region, the percentage of the closed wheels was reduced from 65% to 34% after lipid-modification (Figure 3c), perhaps due to strain caused by complexation by the cationic lipid. On the

other hand, the percentage of closed wheel in the hydrophobic region was similar (68%) to that of the initially immobilized naked DNA wheel. These results indicate that the lipid modified DNA wheel had been extracted to the hydrophobic region due to complexation with the cationic lipid. During this process wheel structures remain intact, are not aggregated, and are immobilized through interactions between alkyl chains on the substrate.

The population of wheels per unit area (density of DNA wheels) was analyzed as a function of their distances from the hydrophilic/hydrophobic boundary. If the DNA wheel-cationic lipid complexes were transferred across the surface by simple 2D diffusion, their density should decrease according to the distance from the border.²⁵ However, there is no apparent distance dependency (Fig. 3d, Fig. S11, ESI[†]) and, additionally, the time-dependency of the density suddenly increased at 9 min after the addition of the cationic lipid (Fig. 3e, Fig. S12, ESI[†]). These features indicate that the complexes are transferred through solution between hydrophilic and

involving first filling of the hydrophobic region with the aqueous solution, which is then re-extracted to the hydrophobic region. Furthermore, the DNA-lipid complex is suggested to be inverted (flip-flop) during transfer between the hydrophilic and hydrophobic surfaces. This is supported by the height of DNA wheels on the hydrophobic (C₁₈) surface. It was observed to be 1.5 nm following complexation with the lipid, the same as the thickness of the bare DNA wheel suggesting the interdigitation of the surface C₁₈ alkyl chains²⁶ with those of the cationic lipids of the DNA wheel-lipid complex (Fig. S9, S10, ESI[†]). The small diffusion coefficient constant (D) for wheels found in the hydrophobic region, which is 6 orders lower ($D = 0.7 \pm 0.9$ [nm²/sec]) (Fig. S13, Table S1, ESI[†]) than for those of lipid conjugated DNA origami,^{12, 16} can be understood by the large quantity of hydrophobic anchors expected to exist in our case. In addition, solid (rigid) features of the C₁₈ alkyl chains at the hydrophobic surface can also contribute to the small diffusion constants.



hydrophobic regions (i.e. by a 3D rather than 2D process),

Fig. 3. Migration of DNA wheels from hydrophilic (SiOH) to hydrophobic (C₁₈) surface induced by lipid complexation. (a) AFM images of DNA wheels on the patterned hydrophilic/hydrophobic surface before and (b) after complexation with lipid. The figures at the right side of a and b are magnifications of the wheels on hydrophilic or hydrophobic surfaces, respectively. Scale bars: 100 nm and 50 nm for the original and the magnified image, respectively. Image in b was taken 22 min after the addition of lipid. (c) Density of closed and opened wheels on the hydrophilic/hydrophobic surfaces before and after complexation with the lipid. Numbers indicate the ratio of closed wheel. N.D. = Not detected. (d) An AFM image of DNA wheel migration to the hydrophobic region at 3 min after addition of the cationic lipid (2C₁₂N⁺). Lines indicate the distance from the hydrophilic/hydrophobic boundary. Sum of opened and closed wheels were counted and are indicated. Only opened wheels with over 50% of the structure remaining were counted. Scale bars: 100 nm. a, b, and d: Rate = 50 sec/frame (e) Time-dependent

density of wheels as a function of the distance from the hydrophilic/hydrophobic boundary from d. (f) Schematic view of lipid-assisted 2D-3D-2D transfer of DNA wheel-lipid complex.

Conclusions

In summary, we have successfully achieved in situ lipophilization of DNA wheels with preservation of the self-assembled wheel structure by addition of cationic lipid ($2C_{12}N^+$) and 2D-extraction using hydrophilic/hydrophobic patterned 2D surfaces. This 2D-extraction enables non-distance-limited and pinpoint transfer of DNA nanostructures. Detailed analysis of AFM images obtained during transfer gave physical insight into lipid-lipid and lipid-DNA interactions, which are important for the flip-flop transfer. By using this technique, DNA machines and DNA devices can be fabricated in hydrophilic regions with subsequent transfer to hydrophobic regions, where functions involving the bare non-lipophilized DNA nanostructures exposed at the surface can be anticipated. Furthermore, alignment of DNA tiles can be designed and constructed with architectural precision using a patterned substrate, i.e. DNA nanoarchitectonics²⁷: by combination with nanolithography techniques, DNA tiles can be formed in nanometric patterns to form complex nanostructures.

Experimental Section

General methods, materials, experimental details, additional data are shown in the Supporting Information.

Acknowledgements

This work was partly supported by World Premier International Research Center Initiative (WPI Initiative), MEXT, Japan, the Core Research for Evolutional Science and Technology (CREST) program of Japan Science and Technology Agency (JST), Japan, and JSPS KAKENHI Grant Number 26790003, 26104541, 24104005, 15K12136. Preparation of the patterned substrate was supported by the NIMS Nanofabrication Platform in Nanotechnology Platform Project sponsored by MEXT. We thank Dr. K. Kawakami (NIMS) for helpful discussion.

Notes and references

- J. D. Watson and F. H. Crick, *Nature*, 1953, **171**, 737-738.
- P. W. K. Rothemund, *Nature*, 2006, **440**, 297-302.
- B. Sacca and C. M. Niemeyer, *Angew. Chem. Int. Ed.*, 2012, **51**, 58-66.
- A. V. Pinheiro, D. R. Han, W. M. Shih and H. Yan, *Nat. Nanotechnol.*, 2011, **6**, 763-772.
- F. Zhang, J. Nangreave, Y. Liu and H. Yan, *J. Am. Chem. Soc.*, 2014, **136**, 11198-11211.
- Y. Z. Ma, H. N. Zheng, C. E. Wang, Q. Yan, J. Chao, C. H. Fan and S. J. Xiao, *J. Am. Chem. Soc.*, 2013, **135**, 2959-2962.
- E. P. Gates, J. K. Jensen, J. N. Harb and A. T. Woolley, *RSC Adv.*, 2015, **5**, 8134-8141.
- A. Kuzuya, Y. Sakai, T. Yamazaki, Y. Xu and M. Komiyama, *Nat. Commun.*, 2011, **2**.
- D. Koirala, P. Shrestha, T. Emura, K. Hidaka, S. Mandal, M. Endo, H. Sugiyama and H. B. Mao, *Angew. Chem. Int. Ed.*, 2014, **53**, 8137-8141.
- Z. T. Deng, A. Samanta, J. Nangreave, H. Yan and Y. Liu, *J. Am. Chem. Soc.*, 2012, **134**, 17424-17427.
- J. Vesenska, M. Guthold, C. L. Tang, D. Keller, E. Delaine and C. Bustamante, *Ultramicroscopy*, 1992, **42-44 (Pt B)**, 1243-1249.
- K. Borjesson, E. P. Lundberg, J. G. Woller, B. Norden and B. Albinsson, *Angew. Chem. Int. Ed.*, 2011, **50**, 8312-8315.
- M. Langecker, V. Arnaut, T. G. Martin, J. List, S. Renner, M. Mayer, H. Dietz and F. C. Simmel, *Science*, 2012, **338**, 932-936.
- J. R. Burns, K. Gopfrich, J. W. Wood, V. V. Thacker, E. Stulz, U. F. Keyser and S. Howorka, *Angew. Chem. Int. Ed.*, 2013, **52**, 12069-12072.
- Y. Suzuki, M. Endo, Y. Yang and H. Sugiyama, *J. Am. Chem. Soc.*, 2014, **136**, 1714-1717.
- A. Czogalla, D. J. Kauert, R. Seidel, P. Schwillie and E. P. Petrov, *Nano Lett.*, 2015, **15**, 649-655.
- M. Langecker, V. Arnaut, J. List and F. C. Simmel, *Acc. Chem. Res.*, 2014, **47**, 1807-1815.
- K. Tanaka and Y. Okahata, *J. Am. Chem. Soc.*, 1996, **118**, 10679-10683.
- Y. Yonamine, T. Kawasaki and Y. Okahata, *Chem. Lett.*, 2010, **39**, 84-85.
- S. Hamada and S. Murata, *Angew. Chem. Int. Ed.*, 2009, **48**, 6820-6823.
- A. S. Finch, C. M. Anton, C. M. Jacob, T. J. Proctor and D. N. Stratis-Cullum, *Nanomaterials*, 2012, **2**, 275-285.
- K. Okuyama, Y. Soboi, K. Hirabayashi, A. Harada, A. Kumano, T. Kaziyama, M. Takayanagi and T. Kunitake, *Chem. Lett.*, 1984, 2117-2120.
- J. H. K. Rozenfeld, E. L. Duarte, T. R. Oliveira, C. Loney, J. M. Ruyschaert and M. T. Lamy, *Langmuir*, 2013, **29**, 11102-11108.
- N. Herzer, S. Hoepfner and U. S. Schubert, *Chem. Commun.*, 2010, **46**, 5634-5652.
- R. Malik, D. Burch, M. Bazant and G. Ceder, *Nano Lett.*, 2010, **10**, 4123-4127.
- A. Dhotel, H. Li, L. Fernandez-Ballester, L. Delbreilh, B. Youssef, X. C. Zeng and L. Tan, *J Phys Chem C*, 2011, **115**, 10351-10356.
- S. Howorka, *Langmuir*, 2013, **29**, 7344-7353.