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Smart Lipids for Programmable Nanomaterials

Matthew P. Thompson[‡], Miao-Ping Chien[‡], Ti-Hsuan Ku, Anthony M. Rush, and Nathan C. Gianneschi^{*}

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093

Abstract

Novel, responsive liposomes are introduced, assembled from DNA-programmed lipids allowing sequence selective manipulation of nanoscale morphology. Short, single stranded DNA sequences form polar head groups conjugated to hydrophobic tails. The morphology of the resulting lipid aggregates depends on sterics and electronics in the polar head groups and therefore, is dependent on the DNA hybridization state. The programmability, specificity and reversibility of the switchable system are demonstrated via dynamic light scattering, transmission electron microscopy and fluorescence microscopy.

Keywords

DNA; nanomaterials; self-assembly; stimuli-responsive; liposomes; lipids

Biological compartments rely on their ability to change morphology in response to patterns of specific stimuli to facilitate many of the processes necessary for life.¹ Mimicking and understanding this type of behavior is of interest in the development of synthetic nanostructures for a diverse range of materials applications.² To this end, a significant body of research exists describing efforts to trigger and manipulate the morphology of discrete assemblies of amphiphiles³ utilizing stimuli such as pH,⁴ temperature,⁵ small molecules⁶ or ions,⁷ enzymes⁸ and light.^{9–10} These triggering mechanisms work primarily by changing and tuning the amphiphilic properties of the building blocks *in situ*.¹¹ However, methods enabling truly programmable soft, discrete nanoscale morphology are rare, requiring a method for encoding materials with information.¹² Herein, we present an approach to such materials inspired by the success of lipid-anchored oligonucleotides as programmable tools for studying and mimicking natural lipid assemblies and their interactions including vesicle fusion processes.^{2j–13}

Nucleic acids are increasingly finding a role as construction and informational elements in chemical systems and in materials because they are unique in their ability to store and transfer encoded information with high fidelity.¹⁴ Here, DNA is utilized to instruct the assembly of three-dimensional aggregates of amphiphiles (Figure 1). The selective DNA-encoded, stimuli-induced shifting of size and morphology of the aggregates is demonstrated via fluorescence microscopy, transmission electron microscopy (TEM) and dynamic light scattering (DLS). Together these studies show that short oligonucleotide conjugated lipids¹³ (DNA-programmed lipids) spontaneously assemble into unilamellar vesicular liposomes,

^{*}ngianneschi@ucsd.edu.

[‡]These authors contributed equally to this paper

Supporting Information Available. Experimental details including synthetic strategies, DNA sequences and controls via DLS, TEM and fluorescence microscopy. This material is available free of charge via the Internet at <http://pubs.acs.org>.

and undergo reversible, *in situ* vesicle-to-micelle phase transitions in response to specific DNA signals. This represents a simple approach to phase switchable soft materials and a tool for building well-defined, programmable supramolecular assemblies.

The DNA-programmed lipids consist of two 18-carbon alkyl hydrophobic tails, linked covalently to the 5'-termini of 9-mer single stranded DNA (ssDNA) oligonucleotides that perform as hydrophilic head groups. The short DNA sequence is necessary to allow vesicle formation, while providing the required binding energy to allow duplex formation at room temperature with complementary DNA sequences (see Supporting Information for particle and duplex stability measurements). The design strategy is based on well-established rules that govern the assembly of amphiphiles in solution¹¹ and it was reasoned that by manipulating the size, shape and charge of the polar head group of each surfactant molecule via DNA hybridization and displacement cycles, one would be able to guide a material through a series of reversible, programmed phase transitions. These systems are designed such that phase transitions are controlled by selective changes in surfactant structure that shift the equilibrium free energy minimum. Therefore, DNA hybridization generates “new” surfactants that are in equilibrium between monomer and vesicle (ssDNA polar head groups), or monomer and micelle (duplex DNA polar head groups). The isothermal phase transitions are enabled by mixing the DNA-lipid assemblies sequentially with several short ssDNA strands, as illustrated in Figure 1.

DNA-programmed lipids are conveniently synthesized via conjugation of 3,4-di(octadecyloxy)benzoic acid to 5'-amino modified oligonucleotides bound to solid support immediately following DNA synthesis. After conjugation, the sequences were washed, cleaved and deprotected from the support. The resulting ammonium hydroxide solutions were diluted in buffer (Tris, 50 mM, pH 7.4) giving clear solutions, and were dialyzed against the buffer for several days. This preparation yielded uniformly shaped, spherical vesicles approx. 500 nm in diameter as characterized by cryo-TEM, SEM and AFM (Figure 2), DLS (Figure 3), and fluorescence microscopy (Figure 4). The unilamellar bilayer architecture of these vesicle assemblies was confirmed by cryo-TEM (Figure 2) with bilayer thicknesses of 8-9 nm, consistent with an end-on arrangement of surfactant tails as illustrated in the cartoon in Figure 1. SEM and AFM images show flattened structures for the vesicles on surfaces consistent with their hollow morphology (confirmed by cryo-TEM) leading to collapse in the dried state.¹⁵ In solution, zeta-potential measurements show vesicles have increased stability with increasing ionic strength consistent with a charge shielding effect in the polyanionic shell (−13 mV at 5 mM MgCl₂. −19 mV at 10 mM MgCl₂).

To facilitate phase transition to spherical micelles (Figure 3), the vesicles were mixed for several hours with partially complementary single stranded 19-base DNA sequences modified at their 5'-termini with two 18-member ethylene glycol phosphoramidites (**DNA₂** in Figure 1). The system was designed such that **DNA₂** addition to **DNA₁**-lipid assemblies would cause an increase in steric and electronic repulsion in the polar head group. DNA hybridization was confirmed by melting temperature studies, consistent with the expected stability of the duplex in the micelle shell. Therefore, the newly formed double stranded DNA-lipid is better accommodated within the micelle phase and accordingly, there is a shift in structure of the observed aggregates from vesicles to 20-25 nm micelles (critical micelle concentration = 300 nM). On these grounds we reasoned the process should be reversible as directed by predictable DNA duplex formation. Therefore, **DNA₃**, a 19-base ssDNA strand perfectly complementary to **DNA₂**, was designed to reverse the phase transition by invasion into the 9-base pair duplex **DNA₁-DNA₂**, releasing the more thermodynamically stable 19-base pair duplex, **DNA₂-DNA₃**. In this manner the vesicles could be first “destroyed”

yielding spherical micelles and could be subsequently “repaired” in a cyclical fashion, either isothermally, or thermally.

The DNA-sequence selective, reversible assembly process could be visualized using a two-color dye experiment by fluorescence microscopy and by DLS (Figure 4). DNA-lipids covalently labeled at the 3'-terminus with fluorescein (**DNA₁-Fluorescein**) or with rhodamine (**DNA₄-Rhodamine**) were allowed to assemble separately into “green” or “red” vesicles respectively, and were then mixed together. These solutions were then treated with two different ssDNA strands designed to specifically hybridize with one or the other dye-labeled vesicles. Upon addition of **DNA₅**, a polyethylene glycol modified ssDNA strand partially complementary to the rhodamine-labeled **DNA₄-lipid**, only green fluorescence was observed, with the new 20 nm “red” spheres sized below the optical resolution limit and giving low intensity, diffuse background fluorescence (Figure 4B). The opposite process was observed when only the fluorescein-labeled vesicles were shifted to small spherical micelles via addition of **DNA₂** (Figure 4D). When both labeled vesicles are shifted to micelles, little fluorescence at either wavelength is observed (Figure 4E). The reversibility is illustrated via the addition of strands (**DNA₃** and **DNA₆**) capable of invading into the duplex present in the shell of the small spherical micelles to regenerate the initial surfactant molecule and shift the equilibrium back to the vesicular phase (as shown in Figure 1 for **DNA₃** addition to spheres containing the **DNA₁-DNA₂** duplex). Following this reversion to vesicles, the formation of mixed dye assemblies is observed as orange/yellow structures in merged fluorescence microscopy images (Figures 4C and 4F). Essentially, this is a DNA-directed phase separation process giving mixed dye assemblies for matched surfactants and single color systems for unmatched surfactants.

DLS data of the switching cycles confirm the reversibility of this process (Figure 4G) with successive additions of DNA input sequences accessing large and small aggregates of spherical or vesicular morphology (see Supporting Information for correlated TEM data). Similarly, raising the temperature of the micelle solution above 30 °C (observed by variable temperature DLS) gave an increase in aggregate size (hydrodynamic diameter, D_h) consistent with melting of the duplex and recovery of ssDNA-lipid.

In conclusion, DNA-programmed lipids are a synthetic tool for building soft, nanoscale materials. Moreover, this approach to surfactant assembly and manipulation of phase represents an example of a programmable, reversible trigger for nanostructure morphology. With this tool in hand, informational, encoded membranes are made possible where DNA-programmed lipids are selectively responsive, in a logical manner to multiple input signals. It is expected that such materials will be valuable in a range of settings including the development of programmable liposomes for smart, triggerable biodiagnostic and drug delivery vehicles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES AND NOTES

- (1). Gennis, RB. Biomembranes. Springer-Verlag; New York: 1989.
- (2). (a) Hartgerink JD, Beniash E, Stupp SI. *Science*. 2001; 294:1684–1688. [PubMed: 11721046] (b) Discher Dennis E, Eisenberg A. *Science*. 2002; 297:967–73. [PubMed: 12169723] (c) Zhang Q, Ariga K, Okabe A, Aida T. *J. Am. Chem. Soc.* 2004; 126:988–989. [PubMed: 14746444] (d) Gong Y, Luo Y, Bong D. *J. Am. Chem. Soc.* 2006; 128:14430–14431. [PubMed: 17090005] (e) Vriezema DM, Garcia PML, Oltra NS, Natzakis NS, Kuiper SM, Nolte RJM, Rowan AE, van Hest JCM. *Angew. Chem., Int. Ed.* 2007; 46:7378–7382. (f) Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. *Nat. Nanotechnol.* 2007; 2:751–760. [PubMed: 18654426] (g) Lee S-M, Dettmer CM, O'Halloran TV, Nguyen ST. *J. Am. Chem. Soc.* 2007; 129:15096–15097. [PubMed: 17999499] (h) Smart T, Lomas H, Massignani M, Flores-Merino MV, Perez LR, Battaglia G. *Nano Today*. 2008; 3:38–46. (i) Chen C-L, Zhang P, Rosi NL. *J. Am. Chem. Soc.* 2008; 130:13555–13557. [PubMed: 18800838] (j) Chandrawati R, Stadler B, Postma A, Connal LA, Chong S-F, Zelikin AN, Caruso F. *Biomaterials*. 2009; 30:5988–5998. [PubMed: 19683341] (k) Chan Y-HM, Lengerich B, Boxer SG. *Proc. Natl. Acad. Sci.* 2009; 106:979–984. [PubMed: 19164559]
- (3). Wang Y, Xu H, Zhang X. *Adv. Mat.* 2009; 21:1–16.
- (4). (a) Butun V, Billingham NC, Armes SP. *J. Am. Chem. Soc.* 1998; 120:11818–11819. (b) Li Y, Du W, Sun G, Wooley KL. *Macromolecules*. 2008; 41:6605–6607. (c) Versluis F, Tomatsu I, Kehr S, Fregonese C, Tepper AWJW, Stuart MCA, Ravoo BJ, Koning RI, Kros A. *J. Am. Chem. Soc.* 2009; 131:13186–13187. [PubMed: 19711889]
- (5). Sundararaman A, Stephan T, Grubbs RB. *J. Am. Chem. Soc.* 2008; 130:12264–12265. [PubMed: 18722446]
- (6). Ishihara Y, Bazzi HS, Toader V, Godin F, Sleiman HF. *Chem. Eur. J.* 2007; 13:4560–4570.
- (7). Zhang L, Yu K, Eisenberg A. *Science*. 1996; 272:1777–1779. [PubMed: 8662482]
- (8). (a) Kim J-H, Lee S, Park K, Nam HY, Jang SY, Youn I, Kim K, Jeon H, Park R-W, Kim I-S, Choi K, Kwon IC. *Angew. Chem., Int. Ed.* 2007; 46:5779–5782. (b) Amir RJ, Zhong S, Pochan DJ, Hawker CJ. *J. Am. Chem. Soc.* 2009; 131:13949–13951. [PubMed: 19739628] (c) Azagarsamy MA, Sokkalingam P, Thayumanavan S. *J. Am. Chem. Soc.* 2009; 131:14184–14185. [PubMed: 19757790]
- (9). Zou J, Tao F, Jiang M. *Langmuir*. 2007; 23:12791–12794. [PubMed: 18004898]
- (10). Roy D, Cambre JN, Sumerlin BS. *Chem. Commun.* 2009:2106–2108.
- (11). (a) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*. 2nd ed.. John Wiley & Sons, Inc; New York: 1980. (b) Israelachvilli JN, Mitchell DJ, Ninham BW. *J. Chem. Soc., Faraday Trans. 2.* 1976; 72:1525–68. (c) Jain S, Bates FS. *Science*. 2003; 300:460–464. [PubMed: 12702869] (d) Nagarajan R. *Langmuir*. 2002; 18:31–38.
- (12). (a) Alemdaroglu FE, Herrmann A. *Org. Biomol. Chem.* 2007; 5:1311. [PubMed: 17464398] (b) Alemdaroglu FE, Alemdaroglu NC, Langguth P, Herrmann A. *Macromol. Rapid Commun.* 2008; 29:326–329. (c) Chien M-P, Rush AP, Thompson MP, Gianneschi NC. *Angew. Chem. Int. Ed.* 2010 In Press.
- (13). (a) Boutorin AS, Gus'kova LV, Ivanova EM, Kobetz ND, Zarytova VF, Ryte AS, Yurchenko LV, Vlassov VV. *FEBS Lett.* 1989; 254:129–132. [PubMed: 2776879] (b) Shea RG, Marsters JC, Bischofberger N. *Nucleic Acids Res.* 1990; 18:3777–3783. [PubMed: 2165251] (c) MacKellar C, Graham D, Will DW, Burgess S, Brown T. *Nucleic Acids Res.* 1992; 20:3411–3417. [PubMed: 1630912] (d) Krieg AM, Tonkinson J, Matson S, Zhao Q, Saxon M, Zhang LM, Bhanja U, Yakubov L, Stein CA. *Proc. Natl. Acad. Sci.* 1993; 90:1048–1052. [PubMed: 8430072] (e) Patolsky F, Lichtenstein A, Willner I. *J. Am. Chem. Soc.* 2000; 122:418–419. (f) Pfeiffer I, Hook F. *J. Am. Chem. Soc.* 2004; 126:10224–10225. [PubMed: 15315417] (g) Yoshina-Ishii C, Miller GP, Kraft ML, Kool ET, Boxer SG. *J. Am. Chem. Soc.* 2005; 127:1356–1357. [PubMed: 15686351] (h) Kurz A, Bunge A, Windek A-K, Rost M, Flasche W, Arbuzova A, Strobbach D, Muller S, Liebscher J, Huster D, Herrmann A. *Angew. Chem. Int. Ed.* 2006; 45:4440–4444. (i) Stengel G, Zahn R, Hook F. *J. Am. Chem. Soc.* 2007; 129:9584–9585. [PubMed: 17629277] (j) Gissot A, Di Primo C, Bestel I, Giannone G, Chapuis H, Barthelemy P. *Chem. Commun.* 2008:5550–5552. (k) Jakobsen U, Simonsen AD, Vogel S. *J. Am. Chem. Soc.*

- 2008; 130:10462–10463. [PubMed: 18642914] (l) Godeau G, Staedel C, Barthelemy P. J. Med. Chem. 2008; 51:4374–4376. [PubMed: 18605715] (i) Muller UF, Bartel DP. RNA. 2008; 14:552–562. [PubMed: 18230767] (m) Loew M, Kang J, Dahne L, Hendus-Altenburger R, Kaczmarek O, Liebscher J, Huster D, Ludwig K, Bottcher C, Herrmann A, Arbuzova A. Small. 2009; 5:320–323. [PubMed: 19123173] (n) Chung M, Lowe RD, Chan Y-HM, Ganesan PV, Boxer SG. J. Struct. Biol. 2009; 168:190–199. [PubMed: 19560541] (o) Chan, Wu, Y.; Sefah, K.; Liu, H.; Wang, R.; Tan, W. Proc. Natl. Acad. Sci. 2010; 107:5–10. [PubMed: 20080797]
- (14). (a) Seeman NC. J. Theor. Biol. 1982; 99:237–247. [PubMed: 6188926] (b) Adleman LM. Science. 1994; 266:1021–4. [PubMed: 7973651] (c) Winfree E, Liu FR, Wenzier LA, Seeman NC. Nature. 1998; 394:539–544. [PubMed: 9707114] (d) Storhoff JJ, Mirkin CA. Chem. Rev. 1999; 99:1849–1862. [PubMed: 11849013] (e) Yurke B, Turberfield AJ, Mills AP Jr. Simmel FC, Neumann JL. Nature. 2000; 406:605–608. [PubMed: 10949296] (f) Stojanovic MN, Stefanovic D. Nat. Biotechnol. 2003; 21:1069–1074. [PubMed: 12923549] (g) Frezza BM, Cockroft SL, Ghadiri MR. J. Am. Chem. Soc. 2007; 129:14875–14879. [PubMed: 17994734] (h) Seeman NC. Mol. Biotechnol. 2007; 37:246–257. [PubMed: 17952671] (i) Venkataraman S, Dirks Robert M, Rothmund Paul WK, Winfree E, Pierce Niles A. Nat Nanotechnol. 2007; 2:490–494. [PubMed: 18654346] (j) Aldaye FA, Palmer AL, Sleiman HF. Science. 2008; 321:1795. [PubMed: 18818351] (k) He Y, Ye T, Su M, Zhang C, Ribbe AE, Jiang W, Mao C. Nature. 2008; 452:198–201. [PubMed: 18337818] (l) Dietz H, Douglas SM, Shih WM. Science. 2009; 325:725–730. [PubMed: 19661424]
- (15). Moughton AO, O'Reilly RK. Chem. Commun. 2010; 46:1091–1093.

Figure 1.

DNA-programmed lipid assemblies to form spherical lamellar vesicles capable of switching phase to form small spherical micelles in a fully reversible fashion via DNA hybridization (+ DNA₂) and strand invasion (+ DNA₃) cycles.

Figure 2. Characterization of unilamellar vesicle structures formed from the self-assembly of **DNA₁**-lipid in aqueous solution. TOP: Cryogenic-TEM (cryo-TEM) images showing unilamellar bilayer morphology of the vesicles. MIDDLE: SEM of vesicles. BOTTOM: AFM data showing the flattened height profile of the vesicle structures dry, on a mica surface.

Figure 3.

DNA-directed vesicle to micelle phase transition. (A) Monitored by DLS: hydrodynamic diameter (D_h). (B) Monitored by TEM (negative stain). CONDITIONS: Tris (pH 7.4, 50 mM), $MgCl_2$ (50 mM), **DNA₁**-lipid (1 μ M), **DNA₂** (2 μ M), room temperature.

Figure 4.

Specificity and reversibility of DNA-programmed phase shifting. Scale bar = 1 μ m. Two different DNA-lipids were 3'-labeled with two different dyes: **DNA₁**-Fluorescein and **DNA₄**-Rhodamine. All fluorescence images are red/green channel merges. (A) Representative bright field image of labeled DNA-lipid assemblies. (B) **DNA₄**-Rhodamine hybridizes to **DNA₅** leaving observed green fluorescence only. (C) **DNA₆** (complementary to **DNA₅**) causes mixing of the two surfactants to give a yellow colocalized signal from both dyes. (D) **DNA₁**-Fluorescein hybridizes to **DNA₂** to generate micelles leaving observed red fluorescence only. (E) Addition of **DNA₂** and **DNA₅** shifts all structures to micelles leaving little visible fluorescence. (F) Upon addition of **DNA₃** (complementary to **DNA₂**) and **DNA₆** (complementary to **DNA₅**), surfactants reassemble to give yellow colocalization indicative of vesicles containing both dyes. (G) DLS data of phase switching cycles with sequential ssDNA input additions. Solution conditions utilized in DLS experiment and prior to slide preparation: Tris (pH 7.4, 50 mM), $MgCl_2$ (50 mM), DNA-surfactant (1 μ M), ssDNA input sequences (2 μ M), room temperature.