CHEMISTRY
A European Journal

A Journal of
ChemPubSoc Europe

Accepted Article

Title: A pH-responsive DNAsome from the self-assembly of DNA-phenyleneethynylene hybrid amphiphile

Authors: Reji Varghese, Shine K Albert, Murali Golla, Hari Veera Prasad Thelu, and Nithiyanandan Krishnan

To be cited as: *Chem. Eur. J.* 10.1002/chem.201701446

Link to VoR: http://dx.doi.org/10.1002/chem.201701446

Supported by
ACES

WILEY-VCH

10.1002/chem.201701446

WILEY-VCH

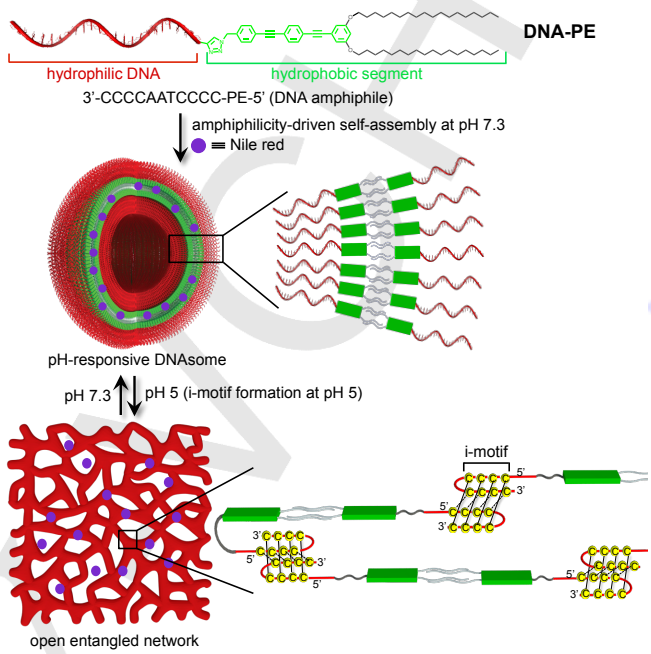
A pH-responsive DNAsome from the self-assembly of DNA-phenyleneethynylene hybrid amphiphile

Shine K. Albert, Murali Golla, Hari Veera Prasad Thelu, Nithiyanandan Krishnan and Reji Varghese*

Abstract: A pH-responsive DNAsome derived from the amphiphilicity-driven self-assembly of DNA amphiphile containing C-rich DNA sequence is reported. The acidification of DNAsome induces a structural change of C-rich DNA from random coil to an i-motif structure that triggers the disassembly of DNAsome and its subsequent morphological transformation into an open entangled network. The encapsulation of a hydrophobic guest into the membrane of DNAsome and its pH-triggered release upon acidification of DNAsome is also demonstrated.

Stimuli-responsive DNA nanostructures have found potential applications in several fields including medicine[1] and nanotechnology.[2,3] Among the various DNA nanostructures, DNAsomes (vesicular nanostructures made of DNA) is particularly attractive due to their remarkable ability to act as nanocarrier in drug delivery applications.[4] The salient structural features of DNAsomes include DNA based corona, hydrophilic internal cavity and hydrophobic membrane. Hence, DNAsomes are capable of encapsulating both hydrophilic and hydrophobic drugs in the internal cavity and membrane, respectively. More importantly, DNA shell of DNAsomes offers the unique opportunity for targeted drug delivery either by incorporating specific cell targeting ligands onto their surface through DNA hybridization[5] or by replacing the random DNA sequence with DNA aptamer for a specific target.[6] Another desirable property that needs to be maintained in DNAsomes for drug delivery applications is the stimuli-responsive nature, which allow them to respond to different stimuli in a predictable manner. Hence the design and synthesis of stimuli-responsive DNAsomes are extremely important for drug delivery applications.

Amphiphilicity-driven self-assembly is an efficient bottom-up approach for the crafting of stimuli-responsive soft molecular assemblies that has been extensively explored in supramolecular chemistry.[7] Very recently, amphiphiles derived from DNA (DNA amphiphiles) have emerged as a promising candidate for the design of soft DNA nanostructures.[8] In an interesting report, Liu et al. have shown pH-induced morphology switching between spherical micelle and cylindrical micelle in the self-assembly of a DNA amphiphile containing i-motif forming DNA as the hydrophilic segment.[9] Motivated from this report, we envisioned that the replacement of random DNA sequence of DNAsome forming amphiphile reported by us recently[10] with an i-motif forming DNA could lead to a pH-responsive DNAsome. Keeping this in mind, we have designed a DNA-phenyleneethynylene (PE) based amphiphile (**DNA-PE**). The DNA sequence used in our design is 5'-PE-CCCCTAACCCC-3', which can undergo reversible structural change between i-motif



Scheme 1. Chemical structure of **DNA-PE** and its amphiphilicity-driven self-assembly into pH-responsive DNAsome. The encapsulation of Nile red into DNAsome and its release upon i-motif formation with acidification is also shown.

structure through intermolecular cytosine–protonated cytosine ($C:CH^+$) base-pairing at low pH (acidic) and a random coil single stranded DNA (ssDNA) at high pH (neutral or basic).[9] This secondary structural changes of DNA with the change in pH could result in the formation of a pH-responsive DNAsome. Herein, we demonstrate the amphiphilicity-driven self-assembly of **DNA-PE** into DNAsomes and its reversible pH-induced morphological switching between DNAsome and entangled nanostructure (Scheme 1). The potential of pH-responsive DNAsome as a nanocarrier for drug delivery applications is also demonstrated with the encapsulation and pH-triggered release of Nile red, a hydrophobic dye, as a representative example.

Synthesis of **DNA-PE** was achieved through copper(I)-catalyzed azide-Alkyne cycloaddition (CuAAC) following reported procedures[11] and the details are provided in the Supporting Information (SI). Self-assembly of **DNA-PE** was initially studied at pH 7.3 by annealing **DNA-PE** (Tris-HCl buffer, 50 mM) at 90 °C for 5 minutes followed by slow cooling to room temperature. Optical properties of **DNA-PE** aggregates are provided in SI (Figure S29). Denaturing polyacrylamide gel electrophoresis (PAGE) analysis shows no migration of **DNA-PE** on PAGE gel (Figure 1a). The retardation of migration of **DNA-PE** on PAGE gel can be attributed to the spontaneous formation of **DNA-PE** nanostructures having dense display of DNA on its surface, as observed in similar systems.[12] DLS analysis of **DNA-PE** at 20 °C shows unimodal distribution of spherical particles with sizes ranging from 44 nm to 460 nm (σ = 0.189) with an average diameter of 142 nm (Figure 1b). Since the

[a] S. K. Albert, M. Golla, H. V. P. Thelu, N. Krishnan, Dr. R. Varghese
School of Chemistry, Indian Institute of Science Education and Research-Thiruvananthapuram (IISER-TVM)
CET campus, Trivandrum-695016 (India)
E-mail: reji@iisertvm.ac.in

Supporting information for this article is given via a link at the end of the document.

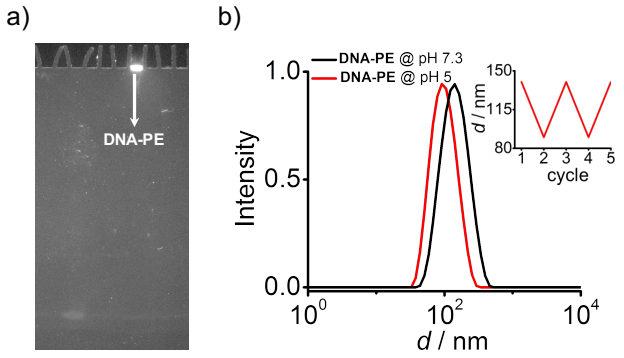


Figure 1. a) Denaturing PAGE (20%) of **DNA-PE**. b) Size distribution graphs from the DLS analyses of **DNA-PE** at pH 7.3 and 5, and the inset shows the pH induced cycling of random coil ss-DNA and i-motif structures monitored using the changes in the average diameter (d) of the nanostructures (c = 1 μM, Tris-HCl buffer, 50 mM).

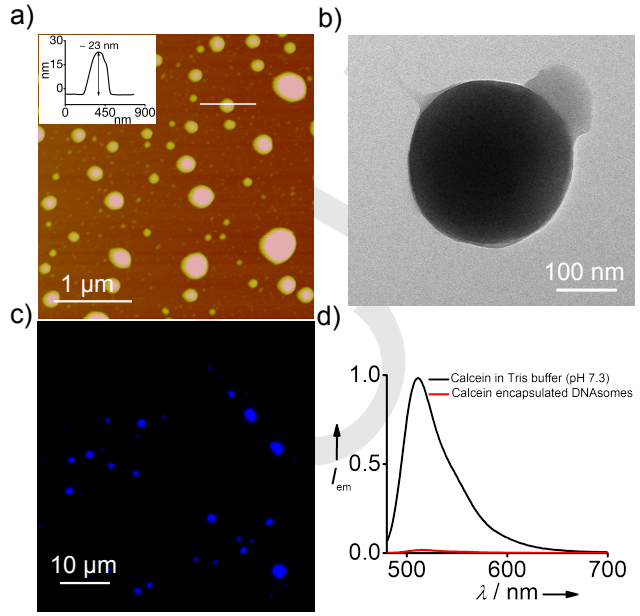


Figure 2. a) T-AFM (height), b) TEM and c) fluorescence microscopic images of **DNA-PE** DNAsome. d) Fluorescence spectra of Calcein encapsulated in **DNA-PE** DNAsome and Calcein in DNAsome-free solution (c = 1 μM, Tris-HCl buffer, λ_{ex} = 470 nm).

diameter of the smallest particle (44 nm) is significantly larger than the calculated bilayer distance of **DNA-PE** (~16 nm), it can be inferred that the spherical aggregates are vesicular (DNAsomes) and not micellar in nature.[13] As expected, zeta potential measurement shows −39.5 mV for **DNA-PE** aggregates, indicating the negatively charged surfaces of the DNAsome (Figure S30).

Tapping-mode atomic force microscopic (T-AFM) analyses of **DNA-PE** on mica surface reveal the formation of spherical particles with size ranging 50 nm to 800 nm (Figure 2a). The average diameter of the spheres of **DNA-PE** assemblies, which were estimated from the fitted histograms of the size distribution curve, is 250 nm. In this case also, it can be seen that the diameter of the smallest particle (~50 nm) is considerably larger than the calculated bilayer distance of **DNA-PE**, confirming the formation of DNAsome for **DNA-PE**. In addition, the section analysis of DNAsomes reveals an average height of ~15 nm, which is considerably smaller when compared with the respective average diameter of the DNAsomes. This clearly indicates that the DNAsomes are “soft” in nature and hence are flattened on the mica surface, as observed for similar systems.[14] Furthermore, transmission electron microscopic (Figure 2b) and fluorescence microscopic analyses (Figure 2c) show the formation of DNAsomes. We have also carried out dye encapsulation experiments to further confirm the DNAsome structure for the **DNA-PE** aggregates using Calcein. Calcein is a fluorescent hydrophilic dye, and hence would be encapsulated in the hydrophilic interior of the DNAsome. Significant quenching is observed for the emission intensity of Calcein encapsulated inside the **DNA-PE** DNAsome when compared with the emission of an absorption matched solution of Calcein in a DNAsome-free solution (λ_{ex} = 470 nm, Figure 2d). This quenching of emission can be attributed to the self-quenching of the dye emission due to the high encapsulation of Calcein inside the hydrophilic cavity of DNAsome. These results unequivocally confirm that **DNA-PE** self-assembles into DNAsomes through van der Walls interaction of the alkyl chains and π-stacking of PE, which constitute the membrane of DNAsome, whereas hydrophilic C-rich DNA is exposed to the polar medium on either side of the membrane as shown in Scheme 1.

In order to investigate the pH-responsiveness of DNAsome self-assembly of DNAsomes was studied at pH 5 (c = 1 μM, Tris-HCl buffer). Circular dichroism (CD) studies of DNAsome (pH 7.3) show a CD signal with positive Cotton effect at 280 nm and a negative Cotton effect at 250 nm, which is a characteristic CD signal of random C-rich ssDNA present on the surface of DNAsomes.[9] Interestingly, a red-shift of CD signal to 290 nm with an increase in CD signal intensity is observed upon acidifying the DNAsome to pH 5 (Figure 3a). The CD signal observed at pH 5 is a characteristic spectral feature for the formation of i-motif structure through C-CH^+ hydrogen bonding as shown in Scheme 1. More interestingly, pH-induced structural transformation between the random ssDNA and i-motif structures is completely reversible for several cycles as evident from the CD studies (Figure 3a inset). At pH 5, the absorption spectrum of DNAsome shows a decrease in absorbance corresponds to DNA region with a red-shift to 272 nm, further supporting the i-motif formation at pH 5 (Figure S29).[9] DLS analysis of DNAsome at pH 5 shows a decrease in the average size of the aggregates to 91 nm (σ = 0.13, Figure 1b). Interestingly, DLS analyses also reveal that the switching of size distribution upon acidification is reversible for several cycles, in agreement with the CD studies (Figure 1b, inset). These experiments obviously indicate that the DNAsomes are undergoing reversible morphological transformation with the change in pH. AFM and scanning electron microscopic (SEM) analyses of DNAsomes at pH 5 show the formation of

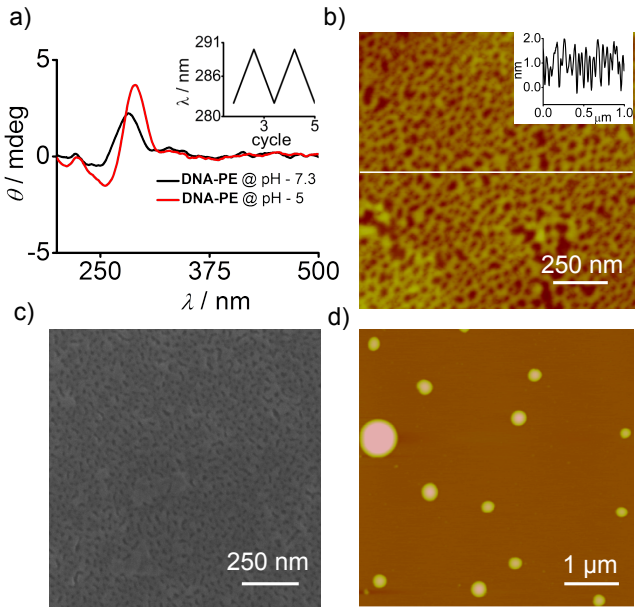


Figure 3. a) CD spectral changes of **DNA-PE** with the change in pH and the inset shows the cycling of random coil ssDNA and i-motif structures monitored using the CD spectral changes. b) AFM and c) SEM images of entangled **DNA-PE** network at pH 5. d) AFM image of reformed DNAsome with the increase of pH to 7.3 (c = 1 µM, Tris-HCl buffer).

highly entangled network that are extended over several micrometers (Figure 3b, c). Zoom-in AFM images reveals that the network is made of thin fibers that are grown in 3D to form the network. Section analysis of the fibrous structures shows that fiber has a width of ~35 nm (after reducing the tip broadening factor)[15] and a height of ~2 nm (Figure 3b inset). More interestingly, the morphological transition of DNAsome to the entangled network is fully reversible. DNAsomes are reformed upon increasing the pH of the solution of intertwined structure to pH 7.3 as evident from the AFM analysis (Figure 3d). Based on these results, it is proposed that **DNA-PE** undergoes amphiphilicity-driven self-assembly into DNAsome at pH 7.3. Acidification of DNAsome to pH 5 induces a structural transformation of random-coil C-rich ssDNA on the surface of DNAsome into i-motif structure through intermolecular C-CH$^+$ base pairing. Since there is no directionality for the i-motif formation it leads to highly entangled 3D networks with the bilayer packing of the amphiphile intact as schematically shown in Scheme 1.

One of the potential applications of pH-responsive DNAsomes is to act as a nanocarrier in drug delivery application, particularly in cancer therapy. Drugs could easily be loaded either into the hydrophobic membrane (hydrophobic drugs) or hydrophilic cavity (hydrophilic drugs) of the DNAsome during the self-assembly, and by taking the advantage of acidic pH of cancerous cell, the loaded drugs can be released upon entry into the cancerous cell. As a proof-of-concept, we demonstrate the encapsulation of Nile red, a hydrophobic dye, into the membrane of DNAsome, and its release upon acidification. Fluorescence changes of Nile red were monitored in order to

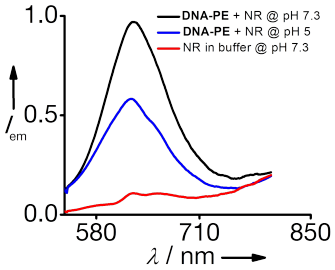


Figure 4. Fluorescence spectra of Nile red encapsulated in **DNA-PE** DNAsome at pH 7.3 (black trace), DNAsome-free solution at pH 7.3 (red trace) and at pH 5 (blue trace). (c = 1 µM, Tris-HCl buffer, λ_{ex} = 514 nm).

study the encapsulation and release of the dye (λ_{ex} = 514 nm). Initially, Nile red was encapsulated into the membrane of DNAsome by annealing **DNA-PE** in the presence of Nile red. Nile red is nearly non-emissive in buffer (Tris-HCl buffer) at pH 7.3 (c = 2 µM). The emission intensity of Nile red encapsulated in the membrane of the DNAsome was compared with the emission intensity of Nile red alone in buffer at pH 7.3 (Figure 4). A significant fluorescence enhancement is observed for Nile red emission after its encapsulation into the DNAsome membrane, which clearly indicates that the dye is encapsulated into the hydrophobic membrane of DNAsome. Interestingly, a decrease in the fluorescence intensity is observed upon acidification of DNAsome to pH 5. On the other hand, control experiments of effect of pH on the fluorescence of Nile red show a slight increase in fluorescence intensity with the change in pH from 7.3 to 5 (Figure S38). Hence the observed decrease in fluorescence intensity of Nile red encapsulated inside the DNAsome with the change in pH from 7.3 to 5 is indeed due to the disassembly and transformation of DNAsome into an open entangled network and the subsequent release of Nile red to a more polar medium. However, the reasonable emission of Nile red after its release from the DNAsome implies that the released dye still finds the hydrophobic domains available in the 3D network. These results suggest that the modulation of the hydrophobicity of the amphiphile may allow the efficient release of the encapsulated guest molecules, and could be a potential nanocarrier for drug delivery applications in cancer therapy.

In conclusion, we have demonstrated, for the first time, a pH-responsive DNAsome from the self-assembly of DNA amphiphile. Spectroscopic, light scattering and microscopic studies have shown that **DNA-PE** undergone amphiphilicity-driven self-assembly into nano-to-micro sized DNAsome at pH 7.3. DNAsomes consists of a hydrophobic membrane constituting of self-assembled alkyl chains tethered PE, and a shell made of hydrophilic C-rich DNA. The C-rich DNA segment undergoes a structural change from random coil ssDNA to an i-motif structure upon acidification that triggered the opening of DNAsomes and its subsequent morphological transformation into entangled 3D network structure in a reversible manner. We have also shown the potential of our system in the encapsulation of a hydrophobic guest into the membrane of DNAsome and its release upon acidification of DNAsome. We hope the unique structural features of DNAsome and its pH-responsiveness may find potential applications in drug delivery, particularly in cancer

therapy. Our results are also expected to motivate other researchers to design new stimuli-responsive DNA nanostructure to address the challenges in drug delivery.

Acknowledgements

Financial support from DBT (BT/PR7030/NNT/28/63/2012) is gratefully acknowledged. S. K. A. thanks CSIR, and H. V. P. T. and N. K. thank UGC for Research Fellowships. We thank MGU for ESI-MS analyses. The help of Rafeeque P. P. with the schematic drawing is acknowledged.

Keywords: self-assembly • amphiphiles • DNA nanostructures • vesicles • responsive systems

[1] a) A. S. Walsh, H. Yin, C. M. Erben, M. J. A. Wood, A. J. Turberfield, *ACS Nano* **2011**, *7*, 5427–5432; b) Q. Jiang, C. Song, J. Nangreave, X. Liu, L. Lin, D. Qiu, Z.-G. Wang, G. Zou, X. Liang, H. Yan, B. Ding, *J. Am. Chem. Soc.* **2012**, *134*, 13396–13403; c) S. Modi, C. Nizak, S. Surana, S. Halder, Y. Krishnan, *Nature Nanotech.* **2013**, *8*, 459–467.

[2] a) N. C. Seeman, *Nature* **2013**, *421*, 427–431; b) M. R. Jones, N. C. Seeman, C. A. Mirkin, *Science* **2015**, *347*, 1260901–11.

[3] a) U. Feldkamp, C. M. Niemeyer, *Angew. Chem.* **2006**, *118,* 1888–1910; *Angew. Chem. Int. Ed*. **2006**, *45*, 1856–1876; b) J. Bath, A. J. Turberfield, *Nature Nanotech*. **2007**, *2*, 275–284; c) E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M. M. Golas, B. Sander, H. Stark, C. L. P. Oliveira, J. S. Pedersen, V. Birkedal, F. Besenbacher, K. V. Gothelf, J. Kjems, *Nature* **2009**, *459*, 73–76; d) M. Liu, J. Fu, C. Hejesen, Y. Yang, N. W. Woodbury, K. Gothelf, Y. Liu, H. Yan, *Nature Commun*. **2013**, *4*, 2127; e) C. Song, Z.-G. Wang, B. Ding, *Small* **2013**, *9*, 2382–2392; f) C.-H. Lu, I. Willner, *Angew. Chem.* **2015**, *127*, 12380–12405; *Angew. Chem. Int. Ed*. **2015**, *54*, 12212–12235.

[4] c) M. P. Thompson, M.-P. Chien, T.-H. Ku, A. M. Rush, N. C. Gianneschi, *Nano Lett.* **2010**, *10*, 2690–2693; b) Y. H. Roh, J. B. Lee, P. Kiatwuthinon, M. R. Hartman, J. J. Cha, S. H. Um, D. A. Muller, D. Luo, *Small* **2011**, *7*, 74–78; c) Y. Dong, Y. Sun, L. Wang, D. Wang, T. Zhou, Z. Yang, Z. Chen, Q. Wang, Q. Fan, D. Liu, *Angew. Chem.* **2014**, *126*, 2645–2648; *Angew. Chem. Int. Ed.* **2014**, *53*, 2607–2610.

[5] F. E. Alemdaroglu, N. C. Alemdaroglu, P. Langguth, A. Herrmann, *Adv. Mater*. **2008**, *20*, 899–902.

[6] S. S. Oh, B. F. Lee, F. A. Leibfarth, M. Eisenstein, M. J. Robb, N. A. Lynd, C. J. Hawker, H. T. Soh, *J. Am. Chem. Soc*. **2014**, *136*, 15010–15015.

[7] a) K. Jalani, M. Kumar, S. J. George, *Chem. Commun.* **2013**, *49*, 5174–5176; b) C. Rest, M. J. Mayoral, K. Fucke, J. Schellheimer, V. Stepanenko, G. Fernandez, *Angew. Chem.* **2014**, *126*, 716–722; *Angew. Chem. Int. Ed.* **2014**, *53*, 700–705; c) Y. Taniguchi, T. Takishita, T. Kawai, T. Nakashima, *Angew. Chem*. **2016**, *128*, 2123–2126; *Angew. Chem. Int. Ed.* **2016**, *55*, 2083–2086.

[8] a) F. E. Alemdaroglu, K. Ding, R. Berger, A. Herrmann, *Angew. Chem. 118*, **2006**, 4313–4317; *Angew. Chem. Int. Ed.* **2006**, *45*, 4206–4210; b) X.-J. Chen, B. L. Sanchez-Gaytan, S. E. N. Hayik, M. Fryd, B. B. Wayland, S.-J. Park, *Small* **2010**, *6*, 2256–2260; c) A. Patwa, A. Gissot, I. Bestel and P. Barthélémy, *Chem. Soc. Rev*. **2011**, *40*, 5844–5854; d) T. G. W. Edwardson, K. M. M. Carneiro, C. K. McLaughlin, C. J. Serpell H. F. Sleiman, *Nature Chem*. **2013**, *5*, 868–875; e) M. Humenik, M. Drechsler, T. Scheibel, *Nano Lett.* **2014**, *14*, 3999−4004; f) S. E. Averick, S. K. Dey, D. Grahacharya, K. Matyjaszewski, S. R. Das, *Angew. Chem.* **2014**, *126*, 2777–2782; *Angew. Chem. Int. Ed*. **2014**, *53* 2739−2744; g) Y. Vyborna, M. Vybornyi, A. V. Rudnev, R. Häner, *Angew. Chem.* **2015**, *127*, 8045–8049; *Angew. Chem. Int. Ed.* **2015**, *54*,7934–7938;

[9] Z. Zhao, L. Wang, Y. Liu, Z. Yang, Y.-M. He, Z. Li, Q.-H. Fan, D. Liu, *Chem. Commun.* **2012**, *48*, 9753–9755.

[10] a) S. K. Albert, H. V. P. Thelu, M. Golla, N. Krishnan, S. Chaudhary, R. Varghese, *Angew. Chem.* **2014**, *126*, 8492–8497; *Angew. Chem. Int. Ed.* **2014**, *53*, 8352–8357; b) S. K. Albert, M. Golla, H. V. P. Thelu, N. Krishnan, P. Deepak, R. Varghese, *Org. Biomol. Chem*. **2016**, *14*, 6960–6969; c) S. K. Albert, H. V. P. Thelu, M. Golla, N. Krishnan, R. Varghese, *Nanoscale* **2017**, DOI: 10.1039/c6nr08370f.

[11] a) J. Gierlich, G. A. Burley, P. M. E. Gramlich, D. M. Hammond, T. Carell, *Org. Lett*. **2006**, *8*, 3639–3642; b) C. Beyer, H.-A. Wagenknecht, *Chem. Commun.* **2010**, *46*, 2230–2231; c) A. H. El-Sagheerab, T. Brown, *Chem. Soc. Rev*. **2010**, *39*, 1388–1405.

[12] T. Chen, C. S. Wu, E. Jimenez, Z. Zhu, J. G. Dajac, M. You, D. Han, X. Zhang, W. Tan, *Angew. Chem.* **2013**, *125*, 2066–2070; *Angew. Chem. Int. Ed.* **2013***, 52,* 2012–2016.

[13] B.-S. Kim, D.-J. Hong, J. Bae, M. Lee, *J. Am. Chem. Soc*. **2005**, *127*, 16333–16337.

[14] M. Yang, W. Wang, F. Yuan, X. Zhang, J. Li, F. Liang, B. He, B. Minch, G. Wegner, *J. Am. Chem. Soc*. **2005**, *127*, 15107–15111.

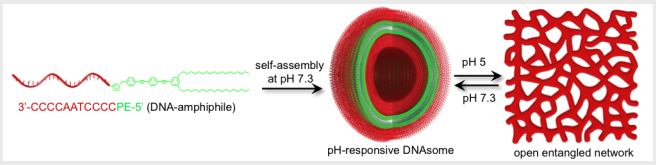
[15] P. Samori, V. Francke, T. Mangel, K. Müllen, J. P. Rabe, *Opt. Mater.* **1998**, *9*, 390–393.

10.1002/chem.201701446

WILEY-VCH

Entry for the Table of Contents (Please choose one layout)

COMMUNICATION



Amphiphilicitty-driven self-assembly of DNA amphiphile containing C-rich DNA as the hydrophilic segment leads to the formation of DNAsome at basic pH (7.3). The acidification of DNAsome induces a structural change of C-rich DNA from random coil to an i-motif structure that triggers the disassembly of DNAsome and its subsequent morphological transformation into an open entangled network in a reversible manner.

*S. K. Albert, M. Golla, H. V. P. Thelu, N. Krishnan, R. Varghese**

Page No. – Page No.

A pH-responsive DNAsome from the self-assembly of DNA-phenyleneethynylene hybrid amphiphile