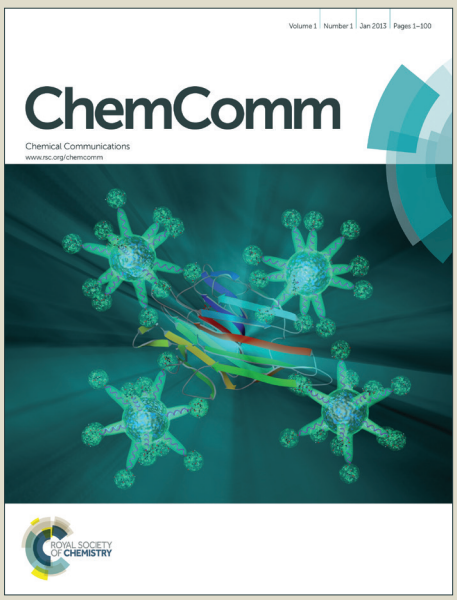
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Chem Comm

RSCPublishing

COMMUNICATION

i-motifs are more stable than G-quadruplexes in a hydrated ionic liquid†

Cite this: DOI: 10.1039/x0xx00000x

Hisae Tateishi-Karimata [a] Miki Nakano, [a] Smritimoy Pramanik [a] Shigenori Tanaka[b] and Naoki Sugimoto[a,c]*

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Thermodynamic analyses and molecular dynamics calculations demonstrated that i-motifs in a hydrated ionic liquid of choline dihydrogen phosphate (choline dhp) were more stable than G-quadruplexes due to choline ions binding to loop regions in the i-motifs. Interestingly, the i-motifs formed even at physiological pH in the choline dhp-containing solution.

Nucleic acids are useful in medical applications and in nanodevices and nanostructures due to their conformational polymorphism.[1-3] A DNA duplex can undergo structural transitions to non-canonical DNA structures in response to environmental stimuli such as pH, ions and cosolutes.[4-6] Two well characterized non-canonical structures are the G-quadruplex and the i-motif. The G-quadruplex is formed by G-rich sequences and the i-motif is adopted under certain conditions by C-rich sequences of DNA.[6] Researchers have taken advantage of these transitions from duplex to these non-canonical structures to develop DNA-based materials such as sensors, logic devices, circuits and drugs.[7-11] For example, G-quadruplexes have been used for delivery of drugs that bind specifically to these structures.[12] Several small molecules have been shown to stabilize G-quadruplex DNA or both G-quadruplex and i-motif DNA.[13, 14] Qu and coworkers reported that single wall carbon nanotubes (SWNTs) can drive i-motif formation without effects on G-quadruplex stability[15] and developed a DNA nanomachine driven by the interaction between SWNTs and an i-motif.[15] Hurley and coworkers showed a small-molecule ligand was able to bind to a C-rich region of the *BCL2* gene and control transcription by affecting i-motif formation.[16] Despite these efforts, selective control of G-quadruplex and i-motif formation is a bottleneck in progress of DNA nanotechnology. Changing cosolute or cosolvent conditions is one of the most convenient methods for controlling the thermodynamic and kinetic properties of DNA structures.[17] Ionic liquids (ILs) have been used as solvents in the field of nanotechnology and are a good media for development of DNA materials because they provide favourable environments for a wide range of chemical reactions and are environmentally friendly solvents.[18, 19] There have been attempts to use the IL as a drug delivery tool,[20, 21] and ILs are attractive for use as vehicles in chemotherapy and gene therapy.[22] A representative IL, choline dihydrogen phosphate (choline dhp), dissolved in a small amount of water (~20 wt%) ensures long-term stability of biomolecules like DNA and proteins.[23, 24] Certain ions are known to preferentially stabilize certain base pairs,[25] and we have recently shown that the A-T base pair is more stable than the G-C base pair in choline dhp due to specific interactions between choline and bases.[26-28] We hypothesized that the presence of the hydrated IL could impact stability of C-C^+ base pairs in i-motif tetraplexes. The choline (2-hydroxy-*N*,*N*,*N*-trimethylethanaminium) ions and their derivatives are in abundant supply in cells and impact metabolism and DNA methylation.[29-31] As non-canonical DNA structures regulate biological reactions such as transcription and translation,[32, 33] these ions may impact cellular processes involving DNA and RNA structural transitions.

In this study, we evaluated the effect of an IL on i-motif and G-quadruplex stability. We first investigated stability of DNA structures in solutions containing hydrated choline dhp by evaluation of ultraviolet (UV) melting curves. A concentration of 4 M (80 wt%) choline dhp was selected, because solutions with high concentrations of choline dhp (around 4 M) have unique properties such as decreased water activity, lower dielectric constant and altered ion networks relative to aqueous buffer. We also evaluated the stability in a solution containing 100 mM NaCl, 50 mM Tris-HCl (pH 7.0) and 1 mM Na_2EDTA as this solution is often used as a mimic of physiological conditions. Human telomere sequences of

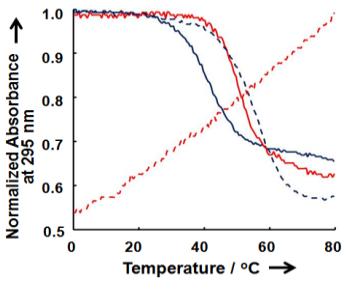


Fig. 1 (a) Normalized UV melting curves at 295 nm for ODN1 (blue) and ODN2 (red) (a) in physiological (100 mM NaCl, pH 7.0) (dashed line) and choline dhp solution (solid line). DNA strand concentration was 2 μM. The experiments were performed in a solution containing 4 M choline dhp and 1 mM Na_2EDTA without pH control because we wanted to investigate DNA stability in a pure hydrated liquid. The original melting curve of ODN2 in physiological condition was used due to unclear melting transition.

$dTAG_3(TTAG_3)_3$ [ODN1] and $d(C_3TAA)_3C_3TA$ [ODN2] were selected (Table S1) for analysis, because these sequences fold into typical antiparallel G-quadruplex or i-motif structures in 100 mM NaCl solution.[5] Moreover, it was confirmed by CD spectral analysis at different temperatures that the ODN1 and ODN2 showed two-state transitions (Fig. S1). Although ODN1 in both the physiological conditions and choline dhp and ODN2 in the choline dhp showed the typical G-quadruplex and i-motif spectra, respectively, ODN2 in the physiological condition showed dissociated i-motif spectra (See supporting information).

The melting temperature (T_ms) of 2 μM ODN1 in the physiological condition was 55.5 °C (Fig. 1). ODN2 did not show a clear melting transition, indicating that ODN2 was not structured under these conditions corresponding to CD analysis (Fig. S1). The T_m values of ODN1 and ODN2 in choline dhp-containing solution were 41.5 and 49.9 °C, respectively (Fig.1). As a result, ODN2 in choline dhp was markedly stabilized relative to that in the physiological condition, whereas ODN1 was slightly destabilized. Moreover, to eliminate the effects of ionic strength and solution pH on the i-motif, the UV melting curves in solutions containing 50 mM Tris (pH 7.0), 1 mM Na_2EDTA with 4 M NaCl or 4 M choline dhp were also estimated (Fig. S2). Although the T_m value for ODN2 in choline dhp (pH 7.0) was decreased relative to that in choline dhp with uncontrolled pH, stable i-motif formation was observed. In contrast, an i-motif did not form in 4 M NaCl. To quantify how choline dhp altered the stabilities of DNAs, we determined thermodynamic parameters for ODN1 and ODN2 (Table 1). The ΔG°_{25} values (free energy changes for DNA structure formation at 25 °C) of ODN1 in physiological and choline dhp solution were −4.7 and −2.2 kcal mol^{-1}, respectively. The lower stability of ODN1 in choline dhp solution was due to unfavourable enthalpy relative to that in the physiological condition. Choline ions preferentially bind to G bases in single strands.[26] Similarly, the choline ions could destabilize the G-quadruplex formation by preferential binding to the single strands. Moreover, the ΔG°_{25} value of ODN2 in the 4 M choline dhp was −5.2 kcal mol^{-1}; the higher stability relative to ODN1 in choline dhp was due to a favourable enthalpic contribution. A favourable enthalpic contribution to DNA duplex formation in choline dhp was previously observed.[26] Choline ions may bind specifically to the i-motif structure and stabilize it.

ODN1 with 3' quencher (ODN3) and ODN2 with 5' fluorophore (ODN4) were designed to compare the stability of i-motif and duplex structures (Fig. 2a and Table S1). When ODN3 and ODN4

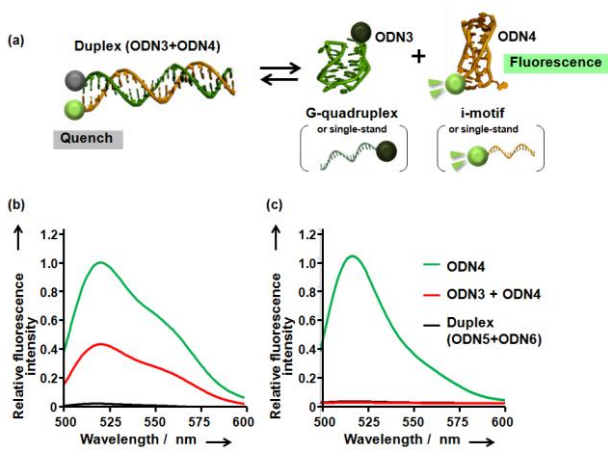


Fig. 2 (a) Schematic illustration of ODN3 and ODN4 structures. Emission spectra recorded at 25 °C for 1 μM ODN4 (green), 1 μM ODN3 + 1 μM ODN4 (red), or 1 μM ODN5 + 1 μM ODN6 (black) in (b) choline dhp (without pH control) and (c) physiological (100 mM NaCl, pH 7.0) solutions.

Table 1. Thermodynamic parameters for i-motif and G-quadruplex formation measured in NaCl or choline dhp

	ΔH° (kcal mol^{-1})	$T\Delta S^\circ$ (kcal mol^{-1})	ΔG°_{25} (kcal mol^{-1})	T_m[a] (°C)
100 m M NaCl (pH 7.0) [b]				
ODN1	−50.8 ± 1.1	−46.1 ± 1.0	−4.7 ± 0.1	55.5
ODN2 [c]	-	-	-	-
4 M Choline dhp (without pH control)[d]				
ODN1	−42.9 ± 5.9	−40.7 ± 1.3	−2.2 ± 0.6	41.5
ODN2	−68.0 ± 2.1	−62.8 ± 0.6	−5.2 ±0.2	49.9

[a] T_m values were measured at 2 μM DNA concentration. [b] The experiments were carried out in a buffer containing 100 mM NaCl, 50 mM Tris-HCl (pH 7.0) and 1 mM Na_2EDTA. [c] n.d. indicates that the thermodynamic parameters could not be determined from the data. [d]The experiments were performed in a solution containing 4 M choline dhp and 1 mM Na_2EDTA without pH control. Thermodynamic parameters were evaluated from the average values obtained from curve fitting at different DNA concentrations (2 to 20 μM).

form a duplex, the fluorescence intensity of ODN4 decreased relative to ODN4 in the absence of ODN3 (Fig. 2a). The ratio of i-motif to duplex can be estimated from the fluorescence intensity at 525 nm. Interestingly, in the choline dhp in the presence of ODN3, 43% of ODN4 was in the i-motif structure (Fig. 2b); only duplex was observed in physiological solution (Fig. 2c). Moreover, the i-motif formation in the choline dhp was confirmed by fluorescence melting experiments for i-motif (Fig. S3). To estimate the stability of the ODN3/ODN4 duplex, we carried out fluorescence melting experiments (Fig. S4). The T_m values of the duplex in choline dhp and in physiological solution were 50.8 °C and 68.9 °C, respectively. Although the duplex with G-C base pairs of Watson-Crick are the most stable base pairs in an aqueous solution, the choline dhp stabilized the i-motif structures comparable as duplex with the G-C base pairs.

Because the stability of i-motif structure is affected strongly by pH, the pH dependence of i-motif formation in choline dhp was examined by CD.[15] To eliminate the effect of ionic strength on the i-motif structure, a solution with 4 M NaCl was used. In the NaCl-containing solution, the i-motif structure of ODN2 was formed at pH 5.5 and pH 6.0 as demonstrated by the presence of two i-motif-characteristic CD bands (Fig. S5): a positive band near 288 nm and a negative band near 256 nm. The positive and negative CD bands were shifted to shorter wavelengths at pH 6.3, pH 6.5 and pH 7.0 when compared to the spectrum at pH 5.5 consistent with previous studies.[15, 34] These blue shifts are due to dissociation of the i-motif. In the choline dhp-containing solution, the CD bands characteristic of the i-motif structure were observed at pH 7.0 (Fig. 3a), although the positive CD band was shifted slightly to shorter wavelengths, suggesting that the i-motif structure did not dissociate if the cytosine base was deprotonated[35]; the i-motif was still stable at pH 7 in choline dhp. The transition between the i-motif and unstructured DNA as a function of pH is visible in the plot shown in Fig. 3b. The pH of the transition mid-point in the solution containing 4 M choline dhp was higher than that in 4 M NaCl solution, suggesting that the p*K*a of the N3 position of cytosine was higher in the solution containing 4 M choline dhp. We determined thermodynamic parameters for ODN2 at pH 5.5 and pH 6.0, because this oligonucleotide adopts the i-motif structure in both NaCl and choline dhp solution at these pHs. The i-motif of ODN2 was stabilized by

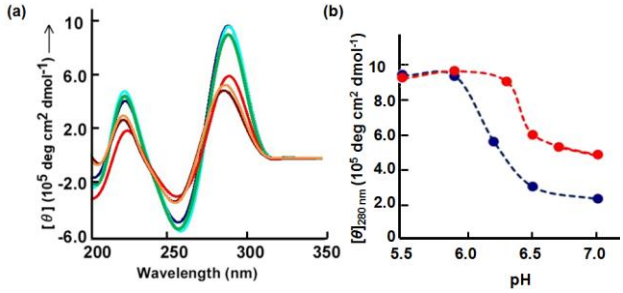


Fig. 3 (a) CD spectra of 20 μM ODN2 in a buffered solution containing 4 M choline dhp at pH 5.5 (black), pH 6.0 (light blue), pH 6.3 (green), pH 6.5 (red), pH 6.7 (orange), or pH 7.0 (brown) at 25 °C. (b) The plot of pH vs. molar ellipticity at 288 nm of ODN2 in a solution containing 4 M NaCl (blue) at pH 5.5, 6.0, 6.2, 6.5, or 7.0 or 4 M choline dhp (red) at pH 5.5, 6.0, 6.3, 6.5, 6.7, or 7.0 at 25 °C. DNA strand concentration was 20 μM. The pH was controlled using 50 mM MES (pH 5.0-6.5) or 50 mM Tris (pH 7.0).

choline dhp relative to that in NaCl (Table S2), although the pH of solution is affect the stability of i-motif (Table S2). The stabilization of i-motif in choline dhp was also due to a favourable enthalpic contribution. A previous report showed that SWNTs stabilize the human telomeric i-motif at acidic and neutral pHs through hydrophobic and electrostatic interactions.[36] Because the i-motif structure was stabilized via a favourable enthalpic contribution at various pHs, choline ions may also bind to the i-motif structure.

To understand how choline ions interact with G-quadruplex and i-motif structures, we carried out molecular dynamic (MD) simulations of the DNA structures with choline and sodium ions (see Supporting Information). Structures were constructed from coordinates obtained from the Protein Data Bank (see Supporting Information). To ensure that i-motif structures were maintained during the simulations, N3 atoms of cytosines were protonated. After 20-ns simulations, we counted the number of cations (N_+) and waters (N_w) within 3.5 Å of DNA atoms: 30.4 choline ions bound to $ODN1_{MD}$ and 27.5 bound to $ODN2_{MD}$ and 15.2 and 15.1 sodium ions bound to $ODN1_{MD}$ and $ODN2_{MD}$, respectively (Table S3). To investigate quantitatively how ion binding affected the stability of DNA structures, we calculated the energy changes of cation binding to DNA structures (ΔG_+) using the Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) module included in Amber12. Despite differences in the DNA structures, the values of N_+ and ΔG_+ for $ODN1_{MD}$ and $ODN2_{MD}$ with choline and sodium ions were not significantly different (Table S3).

We next analysed the positions of choline ions from 5000 snapshots taken from the trajectories during 19–20 ns intervals of the MD simulations. It is reported previously that choline ions are buried inside minor groove in A-T rich duplex or triplex and enhance the stability of DNA structures.[27, 28, 37, 38] Choline ions were not localized in the grooves of $ODN2_{MD}$ but rather shielded the negatively charged backbone. There was no clear preference for binding to a particular a base in $ODN1_{MD}$ (Fig. 4a), but in $ODN2_{MD}$ choline ions bound preferentially to bases in loops (Fig. 4b, orange bars). To explore this loop preference, we evaluated UV melting of the intermolecular i-motif formed by dC_3TAAC_3 [ODN7], which lacks one loop region relative to ODN2. Choline dhp stabilized the ODN7 structure relative to that in aqueous solution, but the degree of stabilization was smaller than that for ODN2 (Fig. S6). A choline ion has three methyl groups that result in a local hydrophobic environment. When the choline ions bind to loop regions, the ions might stack on the $C\text{-}C^+$ base pair inducing a p*K*a shift by locally

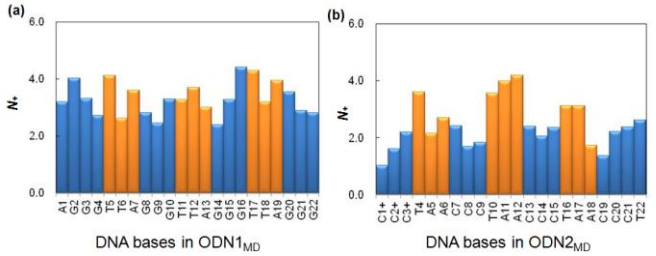


Fig. 4 Comparison of the number of choline ions located within 3.5 Å of DNA base atoms (N_+) in (a) $ODN1_{MD}$ and (b) $ODN2_{MD}$. DNA bases in loop regions are highlighted by orange.

decreasing the dielectric constant as seen inside proteins.[39] Alternatively, binding of choline ions may alter solvation, dehydrating loop regions and stabilizing the i-motif. Thus, choline ion binding to loop regions may stabilize i-motifs relative to aqueous solution.

The extent of DNA structure formation depends on the relative stabilities of the single-stranded state and the structured state. It is difficult to investigate the ion effect on single-stranded DNA experimentally. Thus, we carried out MD simulations of single-stranded G-rich (ssODN1) and C-rich (ssODN2) oligonucleotides (Table S1). Because solvation is critical to stability, the number of waters (N_w) located within 3.5 Å of DNA atoms were estimated. ssODN1 and ssODN2 in the presence of choline ions bound 189 and 211 waters, respectively. The values of ΔG_+ for ssODN1 and ssODN2 with choline ions were −85.6 and −73.1 kcal mol^{-1}, respectively (Table S3). In contrast, the N_w values of ssODN1 and ssODN2 in the presence of sodium ions were the same. Thus the destabilization of structures formed by G-rich DNAs in the presence of choline ions is likely due to choline ion binding preferentially to single-stranded rather than structured DNA via solvation changes.

DNA structure formation is accompanied by the formation of a hydrogen-bonded network of water surrounding the nucleic acid surface, and the formation of the water network is highly sensitive to the water activity of the solution.[40, 41] For example, DNA structures such as i-motifs and G-quadruplexes, which dehydrate during structure formation, should stabilize at lower water activity solutions.[34, 40, 42] Therefore, we measured the water activity of the solutions (Table S4). At the same concentration of NaCl and choline dhp, water activity in the solution containing the choline dhp was lower than that of NaCl solution. For example, the water activitiy of a 50 mM MES (pH 6.0), 1 mM Na_2EDTA and 4 M NaCl solution and that of a 4 M choline dhp solution were 0.868 and 0.861, respectively. Ionic liquids decrease water activity owing to an interaction network of cations and anions.[43] Because a decline in water activity is favorable for i-motif and G-quadruplex formation accompanied by release of water, the water activity change was not the main factor changing DNA stability in choline dhp.

Control of DNA structure formation is necessary for applications of DNA in sensors, logic devices and circuits and as drugs. The data reported here suggest DNA structure switches can be controlled by use of hydrated ILs as solvent. It is noteworthy that the i-motif was also stabilized in a choline chloride solution that was not a hydrated IL: the T_m value of 2 μM ODN2 in the presence of choline chloride was 12.4 °C higher than that in the presence of sodium chloride (Table S2 and Fig. S7). Thus, our results may also be relevant to the environment in inside cells where DNA is found because choline ions or their derivatives are abundant in cells.[31, 44] The biological role of the telomeric repeat-containing RNAs that are transcription

products of the C-rich telomeric DNA strand is unclear.[45, 46] Phosphatidyl choline found in the nuclear membrane has a choline head group, and in preliminary experiments the i-motif was stabilized at a liposome surface, whereas the G-quadruplex was destabilized (data not shown). The structure of telomeric DNA and that of the transcription product, localized at the nuclear membrane surface, is likely affected by the presence of the choline group. Additionally, the incidence of cardiovascular disease and of arteriosclerosis disease is correlated with the concentration of choline ions and their derivatives.[31, 47] Because transcription and translation are inhibited by G-quadruplex and i-motif structures,[17a] choline ions or their derivatives may control these biological reactions. There are many C-rich sites in the human genome including several in the *E2F1* gene, which is implicated in growth of cancer cells.[48]

Conclusions

Our data show that the i-motif structure is stabilized in the presence of choline ions; these ions are present in cells and likely play an important role in biological reactions involving nucleic acids.

This work was partly supported by Grants-in-Aid for Scientific Research and MEXT (Ministry of Education, Culture, Sports, Science and Technology)-Supported Program for the Strategic Research Foundation at Private Universities (2014-2019), Japan, the Hirao Taro Foundation of KONAN GAKUEN for Academic Research, and the Hyogo Science and Technology Association. We thank Ms. Yuki Matsumura, and Ms. Yumi Ueda for collecting the UV and CD data.

Notes and references

[a] *Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University,7-1-20 Minatojima-minamachi, Kobe 650-0047, Japan.*
[b] *Graduate School of System Informatics, Department of Computational Science, Kobe University, 1-1, Rokkodai, Nada-ku, Kobe, 657-8501, Japan* [c] *Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, 7-1-20 Minatojima-minamachi, Kobe 650-0047, Japan, Fax: +81-78-303-1495, Tel: +81-78-303-1457, E-mail: sugimoto@konan-u.ac.jp*

† Electronic Supplementary Information (ESI) available

1. D. Liu and S. Balasubramanian, *Angew Chem Int Ed Engl*, 2003, **42**, 5734-5736.
2. J. H. Lee, Z. Wang, J. Liu and Y. Lu, *J Am Chem Soc*, 2008, **130**, 14217-14226.
3. J. Grimes, Y. V. Gerasimova and D. M. Kolpashchikov, *Angew. Chem. Int. Ed.*, 2010, **49**, 8950-8953.
4. D. Miyoshi, H. Karimata and N. Sugimoto, *Angew. Chem. Int. Ed.*, 2005, **44**, 3740-3744.
5. D. Miyoshi, S. Matsumura, S. Nakano and N. Sugimoto, *J. Am. Chem. Soc.*, 2004, **126**, 165-169.
6. A. T. Phan and J. L. Mergny, *Nucleic Acids Res.*, 2002, **30**, 4618-4625.
7. Y. Peng, X. Wang, Y. Xiao, L. Feng, C. Zhao, J. Ren and X. Qu, *J. Am. Chem. Soc.*, 2009, **131**, 13813-13818.
8. Y. Peng, X. Li, J. Ren and X. Qu, *Chem Commun*, 2007, 5176-5178.
9. M. I. D. Miyoshi, N. Sugimoto, *Angew. Chem. Int. Ed.*, 2006, **45**, 7587-7590.
10. D. Miyoshi, M. Inoue and N. Sugimoto, *Angew Chem Int Ed Engl*, 2006, **45**, 7716-7719.
11. T. G. Drummond, M. G. Hill and J. K. Barton, *Nat Biotechnol*, 2003, **21**, 1192-1199.
12. Y. A. Shieh, S. J. Yang, M. F. Wei and M. J. Shieh, *ACS Nano*, 2010, **4**, 1433-1442.
13. A. K. Jain and S. Bhattacharya, *Bioconjug Chem*, 2011, **22**, 2355-2368.
14. Y. Esaki, M. M. Islam, S. Fujii, S. Sato and S. Takenaka, *Chem Commun (Camb)*, 2014, **50**, 5967-5969.
15. X. Li, Y. Peng, J. Ren and X. Qu, *Proc Natl Acad Sci U S A*, 2006, **103**, 19658-19663.
16. H. J. Kang, S. Kendrick, S. M. Hecht and L. H. Hurley, *J Am Chem Soc*, 2014, **136**, 4172-4185.
17. S. Nakano, H. T. Karimata, Y. Kitagawa and N. Sugimoto, *J. Am. Chem. Soc.*, 2009, **131**, 16881-16888.
18. K. R. Seddon, *J. Chem. Technol. Biotechnol.*, 1997, **68**, 351-356.
19. T. Welton, *Chem. Rev.*, 1999, **99**, 2071-2084.
20. L. Zhang, J. Liu, T. Tian, Y. Gao, X. Ji, Z. Li and Y. Luan, *Chemphyschem*, 2013, **14**, 3454-3457.
21. L. Viau, C. Tourne-Peteilh, J. M. Devoisselle and A. Vioux, *Chem Commun (Camb)*, 2010, **46**, 228-230.
22. C. Ghatak, V. G. Rao, S. Mandal, S. Ghosh and N. Sarkar, *J Phys Chem B*, 2012, **116**, 3369-3379.
23. I. A. Vijayaraghavan R, Ganesh V, Surianarayanan M, MacFarlane DR., *Angew. Chem. Int. Ed.* , 2010, **49**, 1631-1633.
24. M. D. Fujita K, Forsyth M., *Chem.Commun*, 2005, 4804-4806.
25. E. Stellwagen, Q. Dong and N. C. Stellwagen, *Biochemistry*, 2007, **46**, 2050-2058.
26. H. Tateishi-Karimata and N. Sugimoto, *Angew Chem Int Ed Engl*, 2012, **51**, 1416-1419.
27. M. Nakano, H. Tateishi-Karimata, S. Tanaka and N. Sugimoto, *J Phys Chem B*, 2014, **118**, 379-389.
28. H. Tateishi-Karimata and N. Sugimoto, *Nucleic Acids Res*, 2014, **in press**.
29. E. S. Courtenay, M. W. Capp, C. F. Anderson and M. T. Record, Jr., *Biochemistry*, 2000, **39**, 4455-4471.
30. S. H. Zeisel and K. A. da Costa, *Nutr Rev*, 2009, **67**, 615-623.
31. P. M. Ueland, *J Inherit Metab Dis*, 2011, **34**, 3-15.
32. H. Tateishi-Karimata, N. Isono and N. Sugimoto, *PLoS One*, 2014, **9**, e90580.
33. T. Endoh and N. Sugimoto, *Anal Chem*, 2013, **85**, 11435-11439.
34. A. Rajendran, S. Nakano and N. Sugimoto, *Chem Commun*, 2010, **46**, 1299-1301.
35. H. A. Day, C. Huguin and Z. A. Waller, *Chem Commun (Camb)*, 2013, **49**, 7696-7698.
36. P. Y. Li X, Ren J, Qu X., *Proc Natl Acad Sci U S A.*, 2006, **103**, 19658-196563.
37. H. Tateishi-Karimata, M. Nakano and N. Sugimoto, *Sci Rep*, 2014, **4**, 3593.
38. G. Portella, M. W. Germann, N. V. Hud and M. Orozco, *J Am Chem Soc*, 2014, **136**, 3075-3086.
39. Y. Fukunaga, H. Tamaoki, F. Sakiyama and K. Narita, *J Biochem*, 1982, **92**, 143-153.
40. S. Nakano, D. Miyoshi and N. Sugimoto, *Chem Rev*, 2014, **114**, 2733-2758.
41. C. H. Spink and J. B. Chaires, *Biochemistry*, 1999, **38**, 496-508.
42. D. Miyoshi, H. Karimata and N. Sugimoto, *J Am Chem Soc*, 2006, **128**, 7957-7963.
43. M. Armand, F. Endres, D. R. MacFarlane, H. Ohno and B. Scrosati, *Nat Mater*, 2009, **8**, 621-629.
44. H. F. Lodish, *Molecular cell biology*, 7th edn., W.H. Freeman and Co., New York, 2013.
45. C. M. Azzalin, P. Reichenbach, L. Khoriauli, E. Giulotto and J. Lingner, *Science*, 2007, **318**, 798-801.
46. S. Schoeftner and M. A. Blasco, *Nat Cell Biol*, 2008, **10**, 228-236.
47. Z. Wang, E. Klipfell, B. J. Bennett, R. Koeth, B. S. Levison, B. Dugar, A. E. Feldstein, E. B. Britt, X. Fu, Y. M. Chung, Y. Wu, P. Schauer, J. D. Smith, H. Allayee, W. H. Tang, J. A. DiDonato, A. J. Lusis and S. L. Hazen, *Nature*, 2011, **472**, 57-63.
48. E. Neuman, W. R. Sellers, J. A. McNeil, J. B. Lawrence and W. G. Kaelin, Jr., *Gene*, 1996, **173**, 163-169.

