Organic & Biomolecular Chemistry

Selective binding of nucleosides to gapped DNA duplex revealed by orientation and distance dependence of FRET

Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID	OB-ART-04-2019-000946.R1
Article Type:	Paper
Date Submitted by the Author:	15-May-2019
Complete List of Authors:	Kashida, Hiromu; Nagoya University, Graduate School of Engineering Kokubo, Yuta; Nagoya University, Graduate School of Engineering Makino, Koki; Nagoya University, Graduate School of Engineering Asanuma, Hiroyuki; Nagoya University, Biomolecular Engineering

ARTICLE

Selective binding of nucleosides to gapped DNA duplex revealed by orientation and distance dependence of FRET

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

DOI: 10.1039/x0xx00000x

Hiromu Kashida,*[a] Yuta Kokubo,[a] Koki Makino[a] and Hiroyuki Asanuma*[a]

Herein we used orientation and distance dependence of Förster resonance energy transfer (FRET) to analyze the binding of nucleosides to a gapped DNA duplex. Binding isotherms and information on the structures of the complexes were obtained by monitoring FRET between pyrene and perylene, which were introduced into the DNA through D-threoninol. FRET efficiency significantly changed upon formation of a duplex with a 1-nucleotide gap and a nucleoside. The FRET plot indicated that the complex has a double helical structure similar to a nicked duplex. Cooperative binding of two nucleosides to a duplex with a 2-nucleotide gap was also revealed using FRET. Various drug-nucleic acids interactions could be investigated using this sensitive and facile method.

Introduction

Förster resonance energy transfer (FRET) is a powerful tool for analysis of structures and dynamics of biomolecules.[1-4] FRET is most often used as a molecular ruler to determine the distance between two molecules. Recent studies have revealed that not only distance but also the orientation dependence of FRET provides structural information.[5-12] For example, we have reported that FRET efficiency shows orientation dependence when a donor and an acceptor are introduced into DNA through D-threoninol.[10] Recently, structures and dynamics of nicked and gapped DNA duplexes have been elucidated using FRET.[13]

Herein, we used FRET to analyse the binding of nucleosides into a gap in a DNA duplex (Fig. 1). Binding of nucleosides or nucleotides with DNA and RNA are of interest since these complexes are key intermediates in non-enzymatic nucleic acid synthesis and enzymatic gap-filling reactions.[14-18] We hypothesized that the structure of a gapped duplex is altered after nucleoside binding. This structural change should alter dye orientation so that the binding can be detected by monitoring FRET efficiency. This structural change is expected to be too small to detect using only the distance dependence of FRET. Previously, complex formation was indirectly analysed by the analysis of the inhibitory effect of free nucleotides on primer extension,[19] and binding constants were directly determined from NMR and isothermal titration calorimetry (ITC).[19-22] NMR and ITC require large amounts of sample, which makes systematic analyses difficult. In contrast, binding isotherms and structural information can be directly and easily obtained through orientation-dependent FRET.

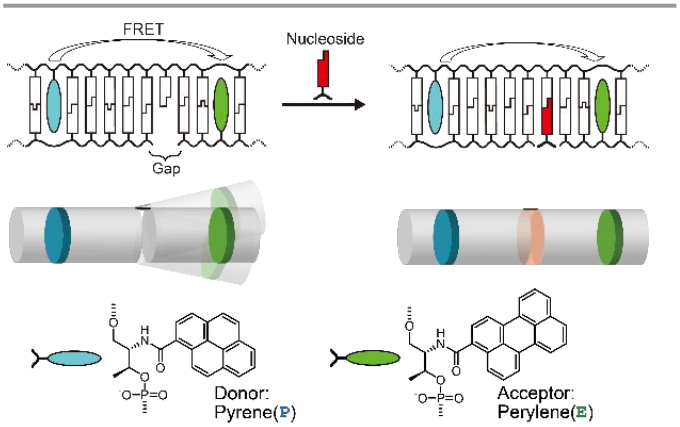


Fig. 1 Illustration of the strategy to detect binding of nucleoside to duplexes with 1-nt gaps. Binding of a nucleoside induces a structural change to a structure similar to that of a nicked duplex.

Results and discussion

Pyrene and perylene were selected as a donor and an acceptor, respectively, for our experiments. These dyes were introduced into DNA through D-threoninol since this linker facilitates the intercalation of dyes.[23] The number of residues between the dyes was first set to 8 nucleotides (nts) because in preliminary experiments a large difference of FRET efficiencies between a nicked duplex and a duplex with a 1-nt gap were observed at this distance. Duplexes with 1-nt gaps containing thymine, cytosine, guanine, or adenine as an orphan base were prepared (**T-Gap**, **C-Gap, G-Gap**, and **A-Gap**, respectively; see Table S1 for sequences). Duplexes with orphaned CT or TT bases were also prepared (**CT-Gap** or **TT-Gap**, respectively). A nicked duplex (**Nick**) was used as a control.

[a] *Department of Biomolecular Engineering, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan. E-mail: kashida@chembio.nagoya-u.ac.jp (H.K.), asanuma@chembio.nagoya-u.ac.jp (H.A.)*

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

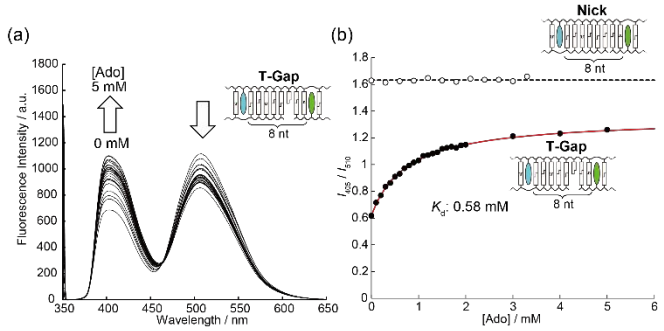


Fig. 2 a) Emission spectra of **T-Gap** with various concentration of Ado. b) Plots of I_{405}/I_{510} of **T-Gap** and **Nick** versus concentration of Ado. Conditions: 0.2 μM pyrene strand, 0.4μM perylene strand, 0.6 μM native strand, 500 mM NaCl, 10 mM phosphate buffer (pH 7.0), 20 °C.

We first monitored the fluorescence change upon the addition of adenosine (Ado) into a solution of **T-Gap** (Fig. 2a). As the concentration of Ado was increased, donor emission increased, whereas acceptor emission decreased. The presence of an isoemissive point indicated that the fluorescent change was caused solely by changes in FRET efficiency. We also confirmed that nucleoside binding to gapped duplex labelled only pyrene or perylene did not affect its fluorescence intensity (Fig. S1). The ratio of donor emission to acceptor emission (I_{405}/I_{510}) was plotted versus Ado concentration (Fig. 2b). I_{405}/I_{510} of **T-Gap** increased monotonically as Ado concentration increased. In sharp contrast, the emission of **Nick** did not change upon the addition of Ado, demonstrating that Ado selectively binds to the orphaned thymine base of **T-Gap**. The dissociation constants (K_ds) are shown in Table 1. The K_d of **T-Gap**/Ado complex was determined to be 0.58 mM. Interestingly, I_{405}/I_{510} of **T-Gap** increased from 0.6 to 1.2 upon addition of Ado, whereas that of **Nick** was about 1.6. These results suggest that the structure of the **T-Gap** duplex after binding to Ado is similar to the structure of the nicked duplex. The complex formation of Ado with **T-Gap** was also investigated via ITC. The K_d was 0.90 mM (Fig. S2), which is similar to that determined by FRET. The K_d of a native **T-Gap** duplex without dyes determined by ITC was 0.99 mM, which agrees with K_d of **T-Gap** with dyes. Circular dichroism spectra also indicated that incorporation of dyes did not disturb the double helical structure of **T-Gap** (Fig. S3). From these results, we concluded that the effect of dye incorporation on nucleoside binding is marginal.

Table 1 Dissociation constants of duplexes with 1-nt gaps with nucleosides or nucleobases.

Sequence	Additive	K_d / mM
T-Gap	Adenosine	0.58
C-Gap	Guanosine	0.89
G-Gap	Cytidine	6.4
A-Gap	Adenosine	29
T-Gap	Deoxyadenosine	0.65
T-Gap	L-Adenosine	1.1
T-Gap	Adenine	0.29

To investigate the structure of the complex, we changed the number of base pairs between the dyes and measured FRET efficiencies (Φ_T) of nicked and gapped duplexes with orphaned T. In the plot of the FRET efficiency versus the number of nucleotides between the dyes, the nicked duplex showed a significant decline of FRET efficiency with 5nt interval, which corresponds to a half-turn of a B-form duplex (Fig. 3, blue circles). We also calculated theoretical FRET efficiency by using cylinder model (Fig. 3, red line; Fig. S4). Observed Φ_T of nicked duplex showed behaviour similar to the theoretical curve, indicating the averaged structure of nicked duplex is B-form. In contrast, the FRET efficiency for the duplex with a 1-nt gap showed better agreement with a theoretical curve calculated assuming an averaged dye orientation (Fig. 3, blue circles and dotted line, respectively). This result suggests that the duplex with a 1-nt gap has high flexibility. We also measured FRET efficiencies of the gapped duplex in the presence of 3 mM Ado (Fig. 3, green circles). Interestingly, the Φ_T of the duplex with the 1-nt gap in the presence of Ado was similar to that of the nicked duplex, suggesting that the structures are similar.

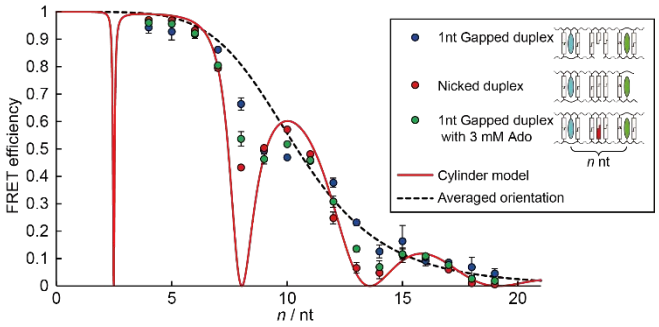


Fig. 3 Plot of FRET efficiency versus the number of nucleotides between the dyes (*n*) for a duplex with 1-nt gap (blue circles), a nicked duplex (red circle), and the duplex with a gap in the presence of 3 mM Ado (green circles). The theoretical curve calculated by assuming a fixed orientation (cylinder model) is shown as a solid red line and the curve obtained assuming an averaged orientation is shown as a dotted line. Error bars show standard deviations of three independent experiments. Conditions: 0.2 μM pyrene strand, 0.4μM perylene strand, 0.6 μM native strand, 500 mM NaCl, 10 mM phosphate buffer (pH 7.0), 20 °C.

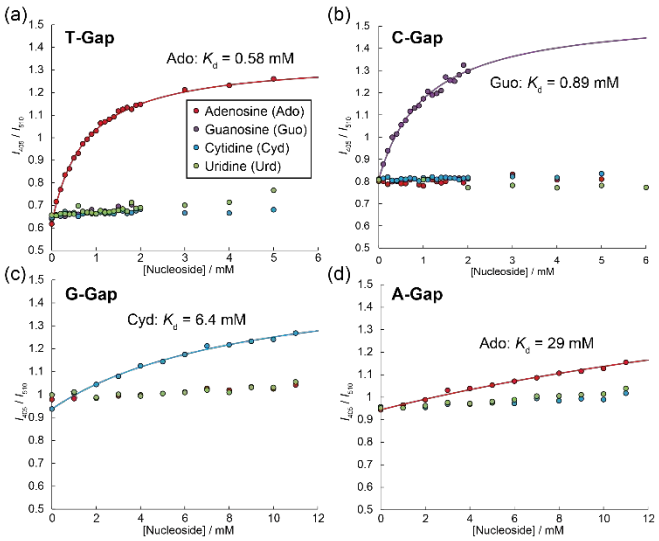


Fig. 4 Plots of I_{405}/I_{510} for (a) **T-Gap**, (b) **C-Gap**, (c) **G-Gap**, and (d) **A-Gap** versus concentration of added nucleosides. Conditions: 0.2 μM pyrene strand, 0.4μM perylene strand, 0.6 μM native strand, 500 mM NaCl, 10 mM phosphate buffer (pH 7.0), 20 °C.

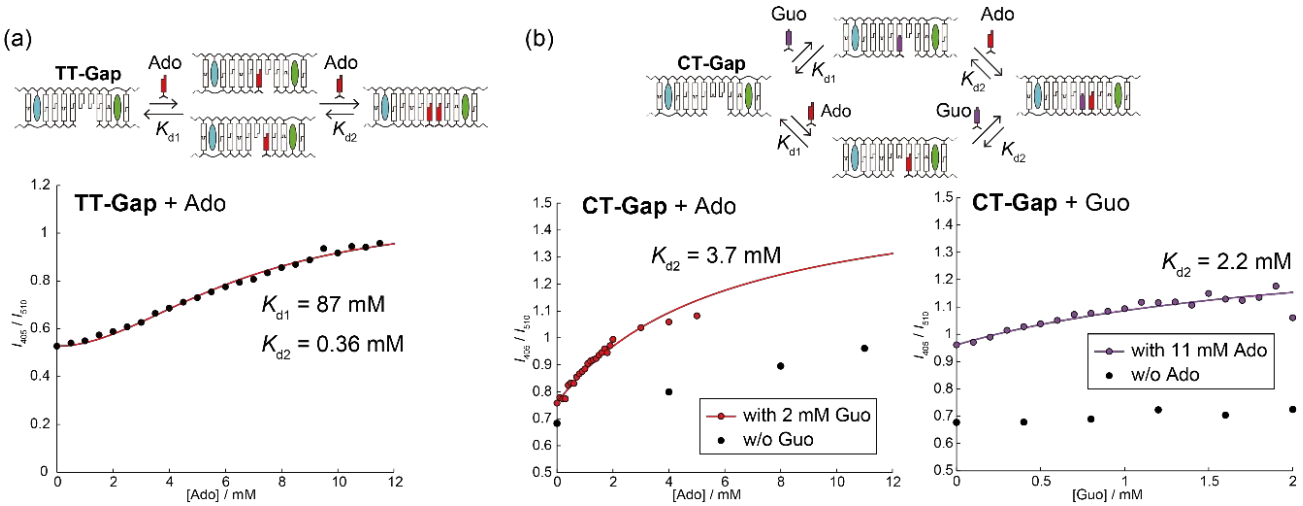


Fig. 5 Analyses of binding of nucleosides to (a) **TT-Gap** and (b) **CT-Gap**. Models used to analyse the binding are shown on the top. Conditions: 0.2 μM pyrene strand, 0.4μM perylene strand, 0.6 μM native strand, 500 mM NaCl, 10 mM phosphate buffer (pH 7.0), 20 °C.

We used this system to evaluate strength and selectivity of complex formation. When guanosine (Guo), cytidine (Cyd), or uridine (Urd) was added to **T-Gap**, the I_{405}/I_{510} did not change significantly (Fig. 4a). These results clearly demonstrate that Watson- Crick base paring between Ado and thymine of **T-Gap**. Similarly, the I_{405}/I_{510} of **C-Gap** increased only in the presence of Guo (Fig. 4b) whereas emission with more than 2 mM Guo could not be measured due to its low solubility. The K_d of **C-Gap**/Guo was determined to be 0.89 mM, which is in the same order as **T-Gap**/Ado. Cyd also bind to **G-Gap**, however, its binding was much weaker than that of Guo; the K_d of the **G-Gap**/Cyd complex was 6.4 mM (Fig. 4c). The weaker binding affinity of the **G-Gap**/Cyd complex compared to the **T-Gap**/Ado complex could be rationalized by the weaker stacking interaction of the pyrimidine base compared to the purine.

Intriguingly, I_{405}/I_{510} of **A-Gap** did not change upon the addition of Urd. Instead, the I_{405}/I_{510} increased as the concentration of Ado increased, indicating that in the context of a gapped duplex with an orphan A, A:A pairing was more stable than A:U pairing (Fig. 4d). It has been reported that non-canonical base pairs can be formed in the complex of nucleotide and template RNA due to the lack of structural and spatial constraints.[24] The strong stacking interaction of adenosine should likely contributes to the relatively high stability of **A-Gap**/Ado complex,[25] although the structure of this complex is not clear at this stage.

Effects of the sugar moiety on the binding were also assessed using FRET (Table 1 and Fig. S5). The K_d of deoxyadenosine with **T-Gap** was 0.65 mM, which is almost the same as that for the complex with adenosine (0.58 mM). This result indicated that the difference between the ribonucleoside and the deoxyribonucleoside is marginal. The absence of the sugar moiety slightly enhanced the binding probably due to a lower entropic penalty; the K_d of adenine was 0.29 mM. Interestingly, the K_d of L-adenosine was 1.1 mM, which was not much higher than that of D-adenosine. It was reported that non-enzymatic polymerization of activated D-nucleotides is inhibited by the presence of L-nucleotides.[26] The *syn* conformation of the L-nucleotide and the *anti* conformation of the D-nucleotide have similar three-dimensional structures.[27, 28] The relatively high affinity of L-adenosine for **T-Gap** may result from binding in the *syn* conformation.

We next used our strategy to analyse the binding of nucleosides to a duplex with a 2-nt gap. The I_{405}/I_{510} of the **TT-Gap** showed a sigmoidal behaviour upon the addition of Ado, demonstrating cooperative binding of two nucleosides (Fig. 5a). We assumed two-step binding and calculated dissociation constants for each step, K_{d1} and K_{d2}, to be 87 and 0.36 mM, respectively. Thus, binding of the first nucleoside enhanced binding of the second nucleoside by about 240 fold. Two-step binding was also observed for the **CT-Gap** containing CT as orphaned bases (Fig. 5b). The addition of Ado to the solution of **CT-Gap** slightly increased the I_{405}/I_{510} in the absence of Guo, indicating very weak binding of Ado alone. However, in the presence of Guo, the I_{405}/I_{510} upon addition of Ado increased considerably, and K_{d2} was calculated as 3.7 mM. Similar behaviour was observed with titration experiments of Guo. K_{d2} of Guo was 2.2 mM in the presence of 11 mM Ado, whereas almost no binding was observed without Ado (Fig. 5b). These results clearly demonstrated that binding of nucleosides to the duplexes with 2-nt gaps is highly cooperative. To the best of our knowledge, this is the first analysis of cooperative binding of multiple nucleosides to DNA or RNA.

Conclusions

In summary, we successfully detected binding of nucleosides to DNA duplexes with 1- or 2-nucleotide gaps by monitoring the orientation and distance dependence of FRET. Nucleosides selectively bound to duplexes with single nucleotide gaps

through Watson-Crick paring with the exception of the duplex with an orphan A, which bound adenine more tightly than uridine. The FRET efficiencies of the complexes between nucleosides and the duplexes with 1-nt gaps were similar to that of a nicked duplex. Binding of nucleosides to a duplex with a 2-nt gap were also analysed, and highly cooperative binding of two nucleosides was revealed. Interaction between nucleic acids and small molecules has been intensively studied because of the importance of these complexes basic biology and medicine.[29] By using the orientation-dependent FRET, binding constants and structures of complexes can be evaluated with small amounts of samples. Therefore, the system described here could be a versatile tool for analysis of drug-DNA interactions. Moreover, sensors and probes to detect small molecules could be prepared by monitoring FRET efficiencies.

Conflicts of interest

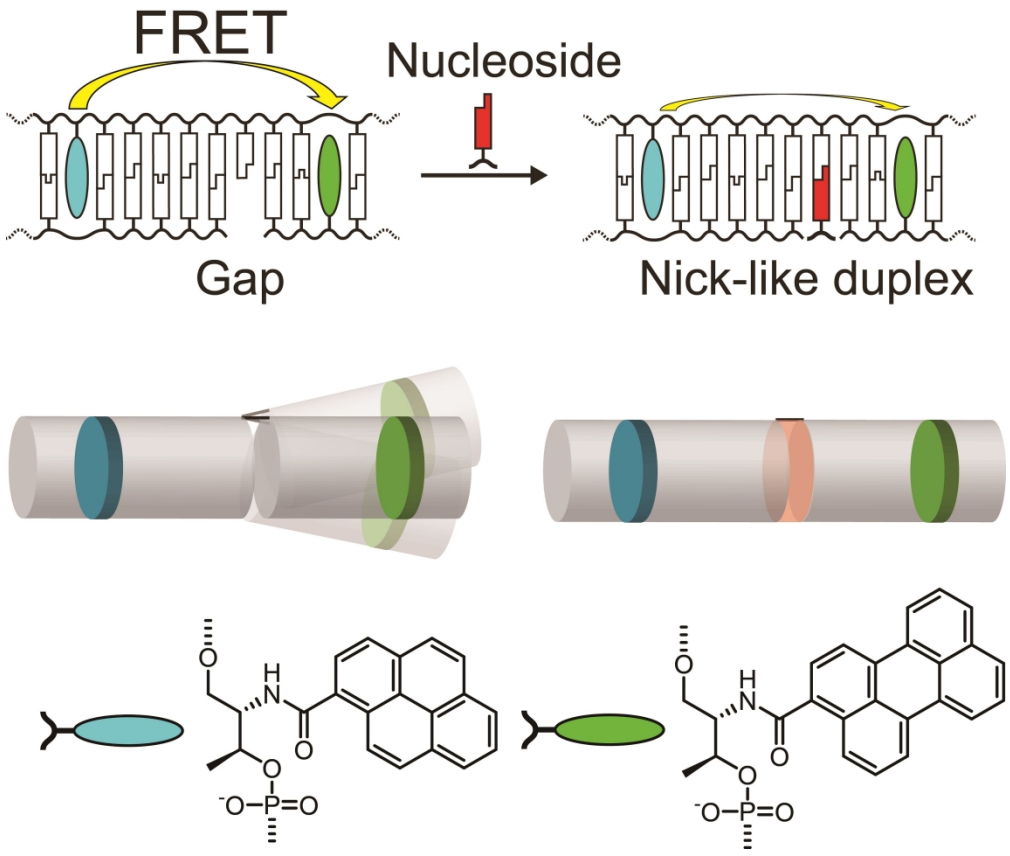
There are no conflicts to declare.

Acknowledgements

This work was supported by JSPS KAKENHI grant numbers JP18H03933 (H.A.), JP16H05925 (H.K.), and JP17H05150 (H.K.) and by A-STEP (H.A.) from Japan Science and Technology Agency. Support from the JSPS A3 Foresight Program and the Asahi Glass Foundation (H.A.) are also acknowledged.

Notes and references

1. R. M. Clegg, *Methods Enzymol.*, 1992, **211**, 353-388.
2. J. Zhang, R. E. Campbell, A. Y. Ting and R. Y. Tsien, *Nat. Rev. Mol. Cell Biol.*, 2002, **3**, 906-918.
3. S. Preus and L. M. Wilhelmsson, *ChemBioChem*, 2012, **13**, 1990-2001.
4. H. Asanuma, K. Murayama, Y. Kamiya and H. Kashida, *Bull. Chem. Soc. Jpn.*, 2018, **91**, 1739-1748.
5. F. D. Lewis, L. Zhang and X. Zuo, *J. Am. Chem. Soc.*, 2005, **127**, 10002-10003.
6. A. Iqbal, S. Arslan, B. Okumus, T. J. Wilson, G. Giraud, D. G. Norman, T. Ha and D. M. J. Lilley, *Proc. Natl. Acad. Sci. USA*, 2008, **105**, 11176-11181.
7. K. Börjesson, S. Preus, A. H. El-Sagheer, T. Brown, B. Albinsson and L. M. Wilhelmsson, *J. Am. Chem. Soc.*, 2009, **131**, 4288-4293.
8. S. Preus, K. Kilså, F.-A. Miannay, B. Albinsson and L. M. Wilhelmsson, *Nucleic Acids Res.*, 2013, **41**, e18.
9. T. Fessl and David M. J. Lilley, *Biophys. J.*, 2013, **105**, 2175-2181.
10. T. Kato, H. Kashida, H. Kishida, H. Yada, H. Okamoto and H. Asanuma, *J. Am. Chem. Soc.*, 2013, **135**, 741-750.
11. M. S. Wranne, A. F. Füchtbauer, B. Dumat, M. Bood, A. H. El-Sagheer, T. Brown, H. Gradén, M. Grøtli and L. M. Wilhelmsson, *J. Am. Chem. Soc.*, 2017, **139**, 9271-9280.
12. H. Kashida, H. Kawai, R. Maruyama, Y. Kokubo, Y. Araki, T. Wada and H. Asanuma, *Commun. Chem.*, 2018, **1**, 91.
13. H. Kashida, A. Kurihara, H. Kawai and H. Asanuma, *Nucleic Acids Res.*, 2017, **45**, e105.
14. L. E. Orgel, *Crit. Rev. Biochem. Mol. Biol.*, 2004, **39**, 99-123.
15. J. W. Szostak, *J. Syst. Chem.*, 2012, **3**, 2.
16. M. P. Robertson and G. F. Joyce, *Cold Spring Harb. Perspect. Biol.*, 2012, **4**, a003608.
17. M. Sosson and C. Richert, *Beilstein J. Org. Chem.*, 2018, **14**, 603-617.
18. S. Nakane, H. Ishikawa, N. Nakagawa, S. Kuramitsu and R. Masui, *J. Mol. Biol.*, 2012, **417**, 179-196.
19. E. Kervio, B. Claasen, U. E. Steiner and C. Richert, *Nucleic Acids Res.*, 2014, **42**, 7409-7420.
20. E. Kervio, M. Sosson and C. Richert, *Nucleic Acids Res.*, 2016, **44**, 5504-5514.
21. E. C. Izgu, A. C. Fahrenbach, N. Zhang, L. Li, W. Zhang, A. T. Larsen, J. C. Blain and J. W. Szostak, *J. Am. Chem. Soc.*, 2015, **137**, 6373-6382.
22. C. P. Tam, A. C. Fahrenbach, A. Björkbom, N. Prywes, E. C. Izgu and J. W. Szostak, *J. Am. Chem. Soc.*, 2017, **139**, 571-574.
23. H. Asanuma, K. Murayama, Y. Kamiya and H. Kashida, *Polymer J.*, 2016, **49**, 279-289.
24. W. Zhang, C. P. Tam, J. Wang and J. W. Szostak, *ACS Cent. Sci.*, 2016, **2**, 916-926.
25. K. M. Guckian, B. A. Schweitzer, R. X. F. Ren, C. J. Sheils, D. C. Tahmassebi and E. T. Kool, *J. Am. Chem. Soc.*, 2000, **122**, 2213-2222.
26. G. F. Joyce, G. M. Visser, C. A. A. van Boeckel, J. H. van Boom, L. E. Orgel and J. van Westrenen, *Nature*, 1984, **310**, 602-604.
27. G. F. Joyce, A. W. Schwartz, S. L. Miller and L. E. Orgel, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 4398-4402.
28. H. Urata, Y. Ueda, H. Suhara, E. Nishioka and M. Akagi, *J. Am. Chem. Soc.*, 1993, **115**, 9852-9853.
29. M. Sirajuddin, S. Ali and A. Badshah, *J. Photochem. Photobiol. B*, 2013, **124**, 1-19.



300x254mm (300 x 300 DPI)