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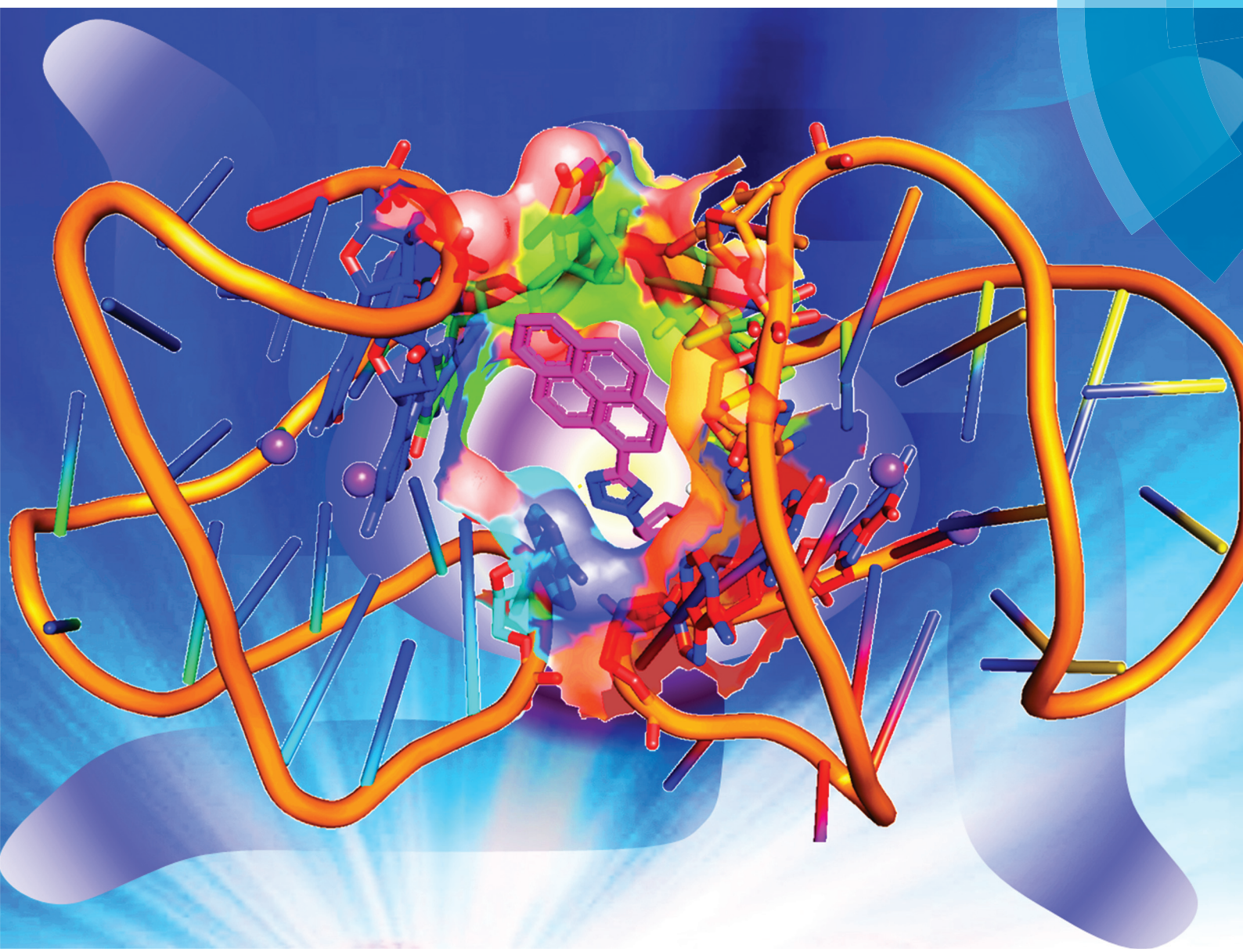

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Tetrazolylpyrene unnatural nucleoside as a human telomeric multimeric G-quadruplex selective switch-on fluorescent sensor†

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We report herein the specific sensing of dimeric H45 G-quadruplex DNA *via* a fluorescence light-up response using fluorescent tetrazolylpyrene nucleoside ($^{TzPy}B_{Do}$) as a probe. The strong binding of the probe *via* an intercalative stacking interaction inside the connecting loop of two G-quadruplex units of H45 and the discrimination to other monomeric and long DNA duplexes are accompanied by a drastic enhancement of the emission intensity without compromising the conformation and stability.

DNA has been recognised as a classical and effective drug target in anticancer chemotherapy.[1] Out of several topological isomers of DNA, the discovery of a guanine-rich G-quadruplex structure has received tremendous attention as a specific and effective drug target at the gene level.[2] The intramolecular G-quadruplexes formed from a single stranded G-rich DNA sequence consist of stacks of several G-quartet planes.[3,4] Being widely populated in the functional region of the human genome, the G-quadruplexes play important roles, such as in (a) gene rearrangement in immunoglobulin, (b) DNA replication and (c) gene transcription. They are also associated with the generation of many human diseases, such as cancer.[5a] Furthermore, they are found to play roles in epigenetics, evolution processes,[5b] and also in DNA damage.[5c] Despite their various conformations,[6a] common planar G-quartet feature, abundance in the functional genome[3,4] and importance in many diseases and biological events,[5] G-quadruplexes have attracted much research interest directed toward understanding their conformation,[3,6a,b] stabilisation,[3,6c,d] and sensing[3,6e,f] and their use as sensors[3,6g,h] and as targets in anticancer drug design.[3,6i–l]

Several research efforts have been put forth for the design of effective G-quadruplex binding therapeutic drug candidates ranging from natural products to synthetic ligands.[3,6c,d,7] On the other hand, the design of highly sensitive fluorescent probes as sensors for G-quadruplexes has also attracted much research attention in recent years.[3,6e,f,7] However, many of the quadruplex-binding fluorescent dyes have been found to be non-specific[8] and very few have shown specificity for a particular quadruplex over other quadruplex topologies.[9,10]

The challenges for the design of a highly selective fluorescent probe for one type of quadruplex and discrimination of other forms of DNA lie in the common structural features[3,4] of all types of G-quadruplexes. While most of these studies have focused only on monomeric G-quadruplexes,[4] targeting telomeric multimeric G-quadruplexes is most promising for the development of anticancer drug candidates. However, only very few reports exist which target multimeric G-quadruplexes.[10] Therefore, there is high demand to develop a fluorescent probe for the sensing of a particular G-quadruplex topology with high specificity and selectivity.

In a rational approach to design a G-quadruplex selective fluorescent probe, one has to consider factors such as G-quadruplex topology and the binding mode along with end stacking interactions. In this quest, an unnatural nucleoside containing a fluorophoric moiety as a reporter unit would be beneficial over the reported classes of organic/inorganic fluorescent dyes or ligands, possibly because of the electrostatic/H-bonding interactions through the involvement of a sugar unit. Although many nucleosides have been utilised in cancer treatment *via* groove binding and/or stacking interactions with the cancer cell DNA,[11] there is no report of a nucleoside which can bind to a selective G-quadruplex efficiently. In 2009 Tang *et al.* showed a G-quadruplex-groove binding event by two propeimines derivatives *via* H-bond, electrostatic and van der Waals interactions.[12] Therefore, the design of fluorescent nucleosides as specific probes for G-quadruplex DNA is of considerable interest.

As a part of our ongoing research toward the design of fluorescent DNA sensors[13a,b], DNA stabilising/binding fluorescent unnatural nucleosides[13c,d] and the tetrazolyl class of fluorescent nucleosides,[13e,f] we recently observed tetrazolylpyrene nucleoside to be an effective switch-on sensor for DNA

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lesions.[13g] Inspired by our recent result, we thought that the tetrazolylpyrene nucleoside could serve as an efficient probe for sensing G-quadruplex. The fluorescent nucleoside with tetrazolylpyrene as a nucleobase is expected to have the following advantageous properties to interact with G-quadruplex over the other reported classes of ligand: (a) the sugar unit of the nucleoside might interact with the loops and grooves possibly through H-bonding interactions, (b) the specific molecular shape of the tetrazole unit might also offer recognition to the groove regions of the G-quadruplex through H-bonding built on the backbone and within the nucleoside core, and (c) the planar pyrenyl aromatic system along with the tetrazole of comparable size to that of a G-quartet (rise 3.3 Å) could be involved in efficient π–π stacking interactions with the G-quartet. Therefore, we envisaged that the H-bonding/electrostatic/stacking interaction forced mediated binding would be an efficient strategy for the selective sensing of G-quadruplex over duplex DNA with a fluorescent nucleoside similar to the action of the drug, daunomycine.[11c,d]

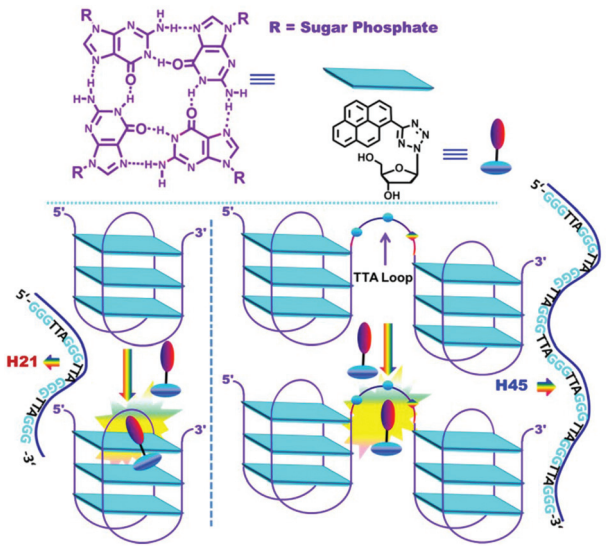

Fig. 1 The structure of the G-quartet, the tetrazolylpyrene nucleoside probe ($^{TzPy}B_{Do}$), the structure of two single strand G-rich DNAs (monomeric and multimeric) and their antiparallel G-quadruplex conformations, and possible binding modes of the tetrazolylpyrene nucleoside probe.

Based on the above consideration, we utilised our previously reported tetrazolylpyrene nucleoside ($^{TzPy}B_{Do}$, Fig. 1)[13e,f] for the selective sensing of G-quadruplex DNA. We thought that the tetrazolylpyrene (**TzPy**) moiety of the nucleoside with a large surface area, high polarizability and high stacking propensity could be involved in π–π stacking interactions with G-tetrad while the sugar unit interacts with the groove or loop *via* H-bonding/electrostatic interactions, leading to stabilization of the G-quadruplex. Thus, the binding event would expectedly lead to the generation of a discriminating fluorescence signal and allow for the selective detection of the G-quadruplex. Furthermore, we envisaged that the planar aromatic **TzPy** unit of the probe could be inserted/intercalated between two G-tetrad planes of a multimeric G-quadruplex, leaving the sugar unit for electrostatic/H-bonding interactions in both of the planes. UV-Vis absorption and fluorescence spectroscopic analysis revealed that the nucleoside probe displayed distinctively different spectroscopic characters in the presence of a multimaric G-quadruplex compared to those in the presence of monomeric G-quadruplexes or a DNA duplex or single-stranded DNA. While a drastic enhancement in fluorescence was observed in the presence of **H45** G-quaduplex, almost similar and weak emissions were the results in the presence of duplex or single-stranded DNA or other monomeric G-quadruplexes, indicating that the nucleoside probe is a highly specific switch-on fluorescent probe of **H45** G-quadruplex.

To test whether the $^{TzPy}B_{Do}$ nucleoside could specifically recognize telomeric G-quadruplex and discriminate monomeric G-quadruplexes from multimeric ones, we first studied the interaction of tetrazolylpyrene nucleoside with various DNAs (Table 1) spectroscopically. For that purpose we chose one telomeric multimeric G-quadruplex (**H45**), two single strand DNAs (**SS 1** and **SS 2**), one duplex DNA (**dS** = **SS 1**/**SS 2**) and six monomeric G-quadruplexes, including a chair-type antiparallel G-quadruplex (**TBA**), three parallel G-quadruplexes [(**HIV**), human **CMyc** and Bombyx (**Bom 17**)], a topologically mixed G-quadruplex (**OXY 3.5**) and **H21** antiparallel G-quadruplex. The concentration of all the DNAs studied was 4 μM in 50 mM sodium phosphate buffer at pH 7,

Table 1 DNA sequences used in this study

Sl. no.	Oligo code	Oligo sequence (5′ → 3′)
1	**TBA**	GGTTGGTGTGGTTGG
2	**HIV**	TGGCCTGGGCGGGACTGGG
3	**H21**	GGGTTAGGGTTAGGGTTAGGG
4	**H45**	GGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG
5	**OXY 3.5**	GGGGTTTTGGGGTTTTGGGGTTTTGGGG
6	**Bom 17**	GGTTAGGTTAGGTTAGG
7	**CMyc**	GGGGAGGGTGGGGAGGGTGGGGAAGGTGGGG
8	**SS 1**	CGCAATCTAACGC
9	**SS 2**	GCGTTAGATTGCG
10	**dS**	**SS 1**/**SS 2**
11	**ct-DNA**	—

100 mM NaCl and the probe concentration was 10 μM (Table 1). The G-quadruplex structures are highly polymorphic in nature; however, the conformation of high-order G-quadruplexes in Na^+ solution is clear from the literature. Therefore, from the literature, we have chosen Na^+-buffer for our experiment.[14]

It should be noted that G-quadruplex can be made from one strand (intramolecular) or from multiple strands (intermolecular) in the presence of a monovalent cation. However, the conformation and molecularity strongly depend on the type and concentration of ions used and strand concentration in the solution.[15] While the formation of intramolecular quadruplexes is dependent upon the length of the loops connecting G-runs at low ionic concentrations, oligomeric sequences where G-runs are separated by one or two nucleotides prefer the formation of intermolecular G-quadruplex at a higher concentration of ions.[15a] Furthermore, monomolecular intrastrand G-quadruplexes are thermodynamically more prevalent in the presence of K^+/Na^+ or Li^+ than bi-/tetra-molecular interstrand structures. Moreover, the basic thermodynamic stability parameters such as $\Delta S/\Delta G$ are independent of strand concentration in the case of intramolecular single strand quadruplexes, whereas these are dependent on strand concentration for intermolecular quadruplexes.[15b] From the literature, we considered that the G-quadruplexes were formed out of one oligomeric strand (intramolecular) of low concentration.[15c] All the studies were carried out using a probe concentration of 10 μM.

The UV-visible spectra showed an increase in absorption of the nucleoside tetrazolylpyrene compared to that of the bare nucleoside probe in the presence of any of the target oligonucleotides. The absorption maxima remained at around 347 nm in the absence or in the presence of all the DNA sequences, except for **Oxy 3.5**, **CMyc** and **H45** DNA which experienced a red shift of 5, 6 and 11 nm, respectively (ESI, Table S1†). The appearance of a red shifted new and strong absorption band at 374 nm in the presence of multimeric G-quadruplex **H45** suggested that multimerics can bind to the nucleoside probe selectively. This result also indicated that the strongest ground state complexation, possibly intercalative stacking, took place between the tetrazolylpyrene unit of the nucleoside and **H45** G-quadruplex (Fig. 2a and ESI, Fig. S1a†). It should be noted that the probe displayed high multimeric G-quadruplex selectivity against long-stranded duplex DNA, **ct-DNA**, indicating the suitability of the nucleoside probe to be a good candidate for anticancer drugs targeting G-quadruplexes.

The efficient multimeric G-quadruplex recognition specificity of the nucleoside probe, $^{TzPy}\mathbf{B_{Do}}$, was reflected in the steady state fluorescence spectra of the probe. Thus, upon excitation at the tetrazolylpyrene absorption (350 or 370 nm) the fluorescence emission intensities of the probe at 383, 402 and 422 nm were drastically enhanced in the presence of multimeric **H45** G-quadruplex compared to the single strand, double strand, **ct-DNA** or any of the tested monomeric G-quadruplexes wherein no or negligibly enhanced emission of the probe was observed (Fig. 2b). These observations were

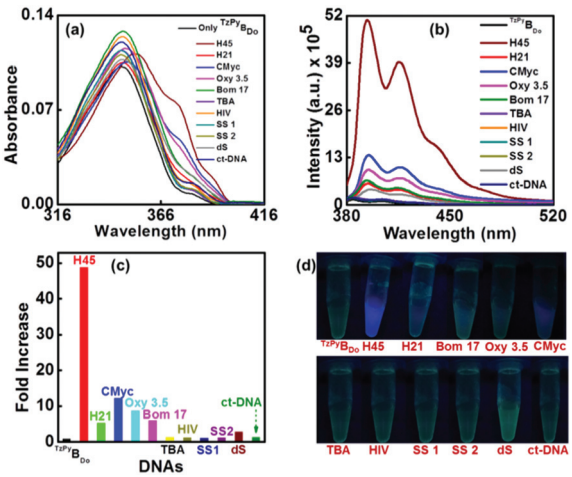

Fig. 2 (a) UV-visible and (b) fluorescence spectra, (c) relative fluorescence enhancement (λ_{ex} = 370 nm) and (d) colour under UV-transilluminator of the nucleoside probe in the presence and absence of various DNAs (probe concentration = 10 μM and DNA concentration = 4 μM in sodium phosphate buffer, pH 7.0, rt).

also reflected in the excitation spectra (ESI, Fig. S1b†). Moreover, all the emission bands experienced a large red shift of 2–15 nm from that of the bare nucleoside probe (ESI, Table S1†). All these observations indicated a strong and special affinity of the probe toward complexation with only the multimeric **H45** G-quadruplex which was also supported by the drastically enhanced and greatest red shifted (291, 357, 370 nm in the presence of and 276, 344 in the absence of **H45**) excitation bands (ESI, section 4†). The relative enhancement of the fluorescence intensity was also found to be higher (49 fold) in the presence of multimeric-**H45** (Fig. 2c) as compared to other DNAs. Following the effects of G-quadruplexes on the fluorescence spectrum of the nucleoside probe, about 980% and 408% multimeric G-quadruplex selectivity against monomeric **H21** and **CMyc**, respectively, was estimated for the probe. The fact that the nucleoside probe specifically and strongly bound with only **H45** was also evident from the discriminating fluorescent blue colour generated in the solution containing the probe and **H45** DNA upon UV-irradiation (Fig. 2d). The steady state fluorescence anisotropy enhancement by 0.15 in the presence of **H45** indicated that the chromophoric unit, **TzPy**, of the nucleoside probe resided in the hydrophobic pocket of the multimeric G-quadruplex DNA, possibly in the TTA pocket of dimeric-**H45** (Fig. 1).[16] There are only very few reports of ligands binding to dimeric G-quadruplex and all are proposed to bind at the cleft between two consecutive G-quadruplexes.[16a–e] Only two reports exist which showed strong binding to dimeric G-quadruplex units *via* stacking to both units – a second mode of binding to dimeric G-quadruplex.[16f,g] Thus, the size of our probe, major literature reports and the fluorescence enhancement led us to propose a cleft binding mode for the present case.

To further demonstrate the multimeric **H45** G-quadruplex recognition specificity of the probe, we titrated a solution of

the nucleoside probe (10 μM) with increasing concentration of the multimeric **H45** G-quadruplex DNA, utilising both UV-visible and fluorescence spectrophotometers. Thus, a continuous enhancement of absorbance at 344 and the appearance of new and increasingly intense bands at 377 and 392 nm, along with a prominent red shift of the band at 344 nm (5–11 nm), were observed in the UV-visible spectra with the gradual addition of increased concentration of **H45** (ESI, Fig. S2, Table S2†). Furthermore, the short wavelength absorbance band of the **TzPy** unit of $^{TzPy}\mathbf{B_{Do}}$ vanished and was buried with the structure-less absorbance band at 254 nm of the multimeric G-quadruplex DNA (ESI, Fig. S2†). However, with the addition of monomeric G-quadruplex DNA, **H21**, the absorption spectrum of the probe experienced an enhancement effect at 346 nm with the appearance of no other absorption bands or a change in band shape (ESI, Fig. S4†). Thus, a DNA concentration-dependent change in absorption suggested a strong binding interaction with the multimeric **H45** G-quadruplex compared to the monomeric G-quadruplex **H21**.

When the probe's solution was titrated with increasing concentration of multimeric G-quadruplex **H45** (while exciting either at 350 or 370 nm), a gradually enhanced intensity of emission with a red shift of the bands at 384 → 393 and 403 → 415 nm was observed (Fig. 3a). On the other hand, the addition of a representative monomeric G-quadruplex, **H21**, had a very negligible effect on the variation of fluorescence enhancement (ESI, section 4†). The large enhancement of steady state fluorescence anisotropy upon the gradual addition of the G-quadruplex **H45** indicated the binding of the chromophoric unit of the probe inside a hydrophobic pocket, wherein the probe experienced a restricted rotation (ESI, section 4†). A strong binding event was evident from the binding constant which came out to be 4.7×10^5 M^{-1}, calculated from the fluorescence titration experiment (Fig. 3b). The thermal melting stability of the **H45** G-quadruplex in the presence and in the absence of the nucleoside probe was tested using a UV-visible spectrophotometer equipped with a temperature controller and a microcell of 1 cm path length. Thus, from the absorbance *vs.* temperature graph and applying an in-built average method, the thermal melting temperature in the presence of the nucleoside probe came out to be 2 °C higher (61 °C) compared to that in the absence of the probe (59 °C), indicating slightly higher stability of the multimeric **H45** G-quadruplex upon binding with the probe (ESI, section 3†). On the other hand, binding of the probe with the monomeric **H21** G-quadruplex had no effect on the thermal stability (ESI, section 4†). These results indicated much stronger binding interactions between $^{TzPy}\mathbf{B_{Do}}$ and the telomeric multimeric G-quadruplex **H45** compared to the monomeric **H21** or other duplex DNAs. The global G-quadruplex conformation was also not perturbed in the presence of the probe, as shown in the CD spectra (ESI, section 3†), indicating high stability of the probe-quadruplex complex.

All of the above results suggested that the probe nucleoside $^{TzPy}\mathbf{B_{Do}}$ had better multimeric G-quadruplex (**H45**) recognition specificity against monomeric G-quadruplexes and long double-stranded DNAs or single strand DNA. The discriminating binding specificity could probably be due to the presence of a particular quadruplex–quadruplex interface with a TTA loop in multimeric **H45** that is lacking in monomeric G-quadruplexes. Concentration dependent minimal change in both the absorption and fluorescence intensities of the probe in the presence of an increasing amount of monomeric G-quadruplexes indicated an extremely weak interaction between the probe and **H21** monomeric G-quadruplex. The multimeric G-quadruplex **H45** can afford two binding modes – one is a sandwich-like end-stacking and the other is intercalative stacking along the TTA loop. However, a strong fluorescence enhancement indicated that the G-tetrad has no effect on the quenching of fluorescence and hence the probe most probably bound to the non-quenching TTA loop, bridging the two G-quadruplex units *via* π–π stacking interactions. The regular increase in fluorescence intensity and absorption upon the gradual addition of an increasing amount of multimeric **H45** supported an intercalative stacking mode of the binding interaction inside the TTA loop with a 1 : 1 binding stoichiometry estimated from a double reciprocal Benesi–Hildebrand plot.[17] This is possible as the planar **TzPy** moiety of the nucleoside probe could offer robust stacking with the G-quartets at the interface *via* efficient intercalation into the TTA loop connecting two adjacent G-quadruplex units.

Finally, a molecular docking calculation with the Autodoc 4.2 programme was performed to support the relatively hydrophobic TTA cleft binding mode of the nucleoside $^{TzPy}\mathbf{B_{Do}}$ (ESI, section 5†).[18a,b] The modified dimeric model **H45** G-quadruplex (formed by a Hybrid 1 G-quadruplex at the 5′-end and a Hybrid 2 G-quadruplex at the 3′-end) was taken from the publication by Prof. Huang *et al.*[18c] for a docking study.[18] Among the various conformations of the nucleoside probe docked with **H45** G-quadruplex, the docking pose (rank-2) is shown in Fig. 4. The number of distinct conformational clusters found was 3, out of 10 runs, using an rmsd-tolerance of 2.0 A. The rank-2 contained a maximum of five multimember conformational clusters with an average binding energy of −8.62 kcal mol^{-1} (ESI, section 5†), which is in close agreement with the experimental free energy of binding (−7.73 kcal mol^{-1}) calculated from the Benesi–Hildebrand plot. The nucleoside was found to intercalate into the pocket

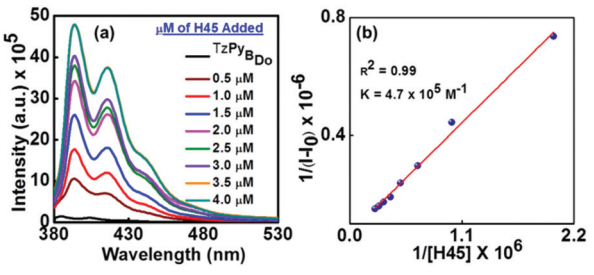

Fig. 3 Fluorescence titration of the probe nucleoside with increasing concentration of H45 G-quadruplex DNA (λ_{ex} = 370 nm) and (b) Benesi–Hildebrand plot from fluorescence titration data (probe concentration = 10 μM in sodium phosphate buffer, pH 7.0, rt).

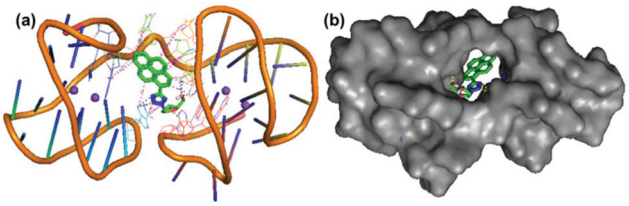

Fig. 4 (a) Interactions of the nucleoside and (b) the surface structure showing the nucleoside inside the TTA cleft.

between two adjacent G-quadruplex units of **H45**. The docking study suggested a clear accommodation of the probe inside the TTA loop with H-bonding and polar interactions through the involvement of both the sugar hydroxyls of the nucleoside with both the G-quadruplex units (Fig. 4a, b and ESI, section 5†). The study of ligand interaction also showed that the pyrene and the triazole unit are involved in T-shaped and parallel, respectively, π–π stacking interactions with the bases of both G-quadruplex units and with bases A and T of the TTA cleft (Fig. 4b and ESI, section 5†).

In conclusion, we successfully demonstrated that the bare fluorescent tetrazolylpyrene nucleoside served as a versatile fluorescent light-up probe for the label free detection of multimeric G-quadruplex DNA with high specificity. The strong binding of the probe, possibly *via* intercalative stacking, inside the TTA pocket connecting two G-quadruplex units of multimeric **H45** G-quadruplex DNA and discrimination to other monomeric and long DNA duplexes were accompanied by a drastic enhancement of emission intensity without compromising the conformation and thermal melting stability. Thus, the probe had an excellent ability to discriminate between telomeric, multimeric and monomeric G-quadruplexes. To the best of our knowledge, this is the first example of a fluorescent nucleoside as a ligand that can specifically distinguish multimeric G-quadruplexes from monomeric ones. The label free fluorescent light-up sensing by our fluorescent nucleoside probe with a less laborious, simple and cost effective way would be very useful and might find future applications for the specific detection and targeting of multimeric G-quadruplex DNA and help in designing such unnatural nucleosides as multimeric G-quadruplex specific binder/drug like candidates for cancer chemotherapy. The design of multimeric G-quadruplex specific fluorescent probes with enhanced G-quadruplex stabilizing ability is our current research focus.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 R. Palchaudhuri and P. J. Hergenrother, *Curr. Opin. Biotechnol.*, 2007, **18**, 497.
2 (*a*) M. Gellert, M. N. Lipsett and D. R. Davies, *Proc. Natl. Acad. Sci. U. S. A.*, 1962, **48**, 2013; (*b*) J. Choi and T. Majima, *Chem. Soc. Rev.*, 2011, **40**, 5893.
3 Review: (*a*) J. L. Huppert, *Chem. Soc. Rev.*, 2008, **37**, 1375; (*b*) A. K. Todd, M. Johnston and S. Neidle, *Nucleic Acids Res.*, 2005, **33**, 2901; (*c*) J. L. Huppert and S. Balasubramanian, *Nucleic Acids Res.*, 2005, **33**, 2908; (*d*) T. M. Ou, Y. J. Lu, J. H. Tan, *et al.*, *ChemMedChem*, 2008, **3**, 690; (*e*) D. Monchaud, A. Granzhan, N. Saettel, *et al.*, *J. Nucleic Acids*, 2010, **2010**, 19; (*f*) A. Granzhan, D. Monchaud, N. Saettel, *et al.*, *J. Nucleic Acids*, 2010, **2010**, 11; (*g*) T. Vy Thi Le, S. Han, J. Chae, *et al.*, *Curr. Pharm. Des.*, 2012, **18**, 1948.
4 (*a*) J. L. Huppert, A. Bugaut, S. Kumari and S. Balasubramanian, *Nucleic Acids Res.*, 2008, **36**, 6260; (*b*) L. Petraccone, *Top. Curr. Chem.*, 2013, **330**, 23; (*c*) J. M. Nicoludis, S. T. Miller, P. D. Jeffrey, S. P. Barrett, P. R. Rablen, T. J. Lawton and L. A. Yatsunyk, *J. Am. Chem. Soc.*, 2012, **134**, 20446; (*d*) G. W. Collie, R. Promontorio, S. M. Hampel, M. Micco, S. Neidle and G. N. Parkinson, *J. Am. Chem. Soc.*, 2012, **134**, 2723.
5 (*a*) Y. Wu and R. M. Brosh Jr., *FEBS J.*, 2010, **277**, 3470; (*b*) A. Baral, P. Kumar, R. Pathak, *et al.*, *Mol. Biosyst.*, 2013, **9**, 1568; (*c*) K. Paeschke, M. L. Bochman, P. D. Garcia, *et al.*, *Nature*, 2013, **497**, 458.
6 (*a*) S. Balasubramanian, L. H. Hurley and S. Neidle, *Nat. Rev. Drug Discovery*, 2011, **10**, 261; (*b*) N. H. Campbell, M. Patel, A. B. Tofa, R. Ghosh, G. N. Parkinson and S. Neidle, *Biochemistry*, 2009, **48**, 1675; (*c*) S. M. Haider, S. Neidle and G. N. Parkinson, *Biochimie*, 2011, **93**, 1239; (*d*) D. Sun, B. Thompson, B. E. Cathers, M. Salazar, S. M. Kerwin, J. O. Trent, T. C. Jenkins, S. Neidle and L. H. Hurley, *J. Med. Chem.*, 1997, **40**, 2113; (*e*) D. Monchaud and M. P. Teulade-Fichou, *Org. Biomol. Chem.*, 2008, **6**, 627; (*f*) T. Ou, Y. Lu, J. Tan, Z. Huang, K. Wong and L. Gu, *ChemMedChem*, 2008, **3**, 690; (*g*) A. De Cian, G. Cristofari, P. Reichenbach, E. L. Lemos, D. Monchaud, M. P. Teulade-Fichou, K. Shin-ya, L. Lacroix, J. Lingner and J. L. Mergny, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 17347; (*h*) L. Xue, N. Ranjan and D. P. Arya, *Biochemistry*, 2011, **50**, 2838; (*i*) T. M. Ou, Y. J. Lu, J. H. Tan, *et al.*, *ChemMedChem*, 2008, **3**, 690; (*j*) S. Balasubramanian, L. H. Hurley and S. Neidle, *Nat. Rev. Drug Discov.*, 2011, **10**, 261; (*k*) G. Biffi, D. Tannahill, J. McCafferty, *et al.*, *Nat. Chem.*, 2013, **5**, 182; (*l*) E. Y. Lam, D. Beraldi, D. Tannahill, *et al.*, *Nat. Commun.*, 2013, **4**, 1796.
7 (*a*) Y. Yan, J. Tan, T. Ou, Z. Huang and L. Gu, *Expert Opin. Ther. Pat.*, 2013, **23**, 1495 and references therein; (*b*) D. C. Pérez-González, F. Rodríguez-Prieto and J. C. Penedo, *Biophys. J.*, 2015, **108**, 393a; (*c*) S. Xu, Q. Li, J. Xiang, Q. Yang, H. Sun, A. Guan, L. Wang, Y. Liu, L. Yu, Y. Shi, H. Chen and Y. Tang, *Sci. Rep.*, 2016, **6**, 24793;

(*d*) C.-Q. Zhou, J.-W. Yang, C. Dong, Y.-M. Wang, B. Sun, J.-X. Chen, Y.-S. Xu and W.-H. Chen, *Org. Biomol. Chem.*, 2016, **14**, 191; (*e*) T. F. Anjong, G. Kim, H. Y. Jang, J. Yoon and J. Kim, *New J. Chem.*, 2017, **41**, 377; (*f*) H. Zhao, Q. Liu, M. Liu, Y. Jin and B. Li, *Talanta*, 2017, **165**, 653; (*g*) T. Wu, M. Ye, T. Mao, F. Lin, Y. Hu, N. Gan and Y. Shao, *Anal. Chim. Acta*, 2017, **964**, 161; (*h*) T.-X. Chen, F. Ning, H.-S. Liu, K.-F. Wu, W. Li and C.-B. Ma, *Chin. Chem. Lett.*, 2017, **28**, 1380.

8 (*a*) P. Yang, D. A. Cian, M.-P. Teulade-Fichou, J.-L. Mergny and D. Monchaud, *Angew. Chem., Int. Ed.*, 2009, **48**, 2188; (*b*) H. Yaku, T. Fujimoto, T. Murashima, D. Miyoshi and N. Sugimoto, *Chem. Commun.*, 2012, **48**, 6203; (*c*) R. T. Wheelhouse, D. Sun, H. Han, F. X. Han and L. H. Hurley, *J. Am. Chem. Soc.*, 1998, **120**, 3261.

9 (*a*) S. Sparapani, S. M. Haider, F. Doria, M. Gunaratnam and S. Neidle, *J. Am. Chem. Soc.*, 2010, **132**, 12263; (*b*) J. Dash, P. S. Shirude, S. D. Hsu and S. Balasubramanian, *J. Am. Chem. Soc.*, 2008, **130**, 15950; (*c*) J. Alzeer, B. R. Vummidi, P. J. C. Roth and N. W. Luedtke, *Angew. Chem., Int. Ed.*, 2009, **48**, 9362; (*d*) A. C. Bhasikuttan, J. Mohanty and H. Pal, *Angew. Chem., Int. Ed.*, 2007, **46**, 9305; (*e*) M.-P. Teulade-Fichou, C. Carrasco, L. Guittat, C. Bailly, P. Alberti, *et al.*, *J. Am. Chem. Soc.*, 2003, **125**, 4732; (*f*) P. Yang, A. De Cian, M.-P. Teulade-Fichou, J.-L. Mergny and D. Monchaud, *Angew. Chem., Int. Ed.*, 2009, **48**, 2188; (*g*) H. Arthanari, S. Basu, T. L. Kawano and P. H. Bolton, *Nucleic Acids Res.*, 1998, **26**, 3724; (*h*) J.-H. Guo, L.-N. Zhu, D.-M. Kong and H.-X. Shen, *Talanta*, 2009, **80**, 607.

10 (*a*) K. Shinohara, Y. Sannohe, S. Kaieda, K. Tanaka, H. Osuga, H. Tahara, Y. Xu, T. Kawase and T. Bando, *J. Am. Chem. Soc.*, 2010, **132**, 3778; (*b*) S. M. Haider and S. Neidle, *Biochem. Soc. Trans.*, 2009, **37**, 583; (*c*) A. Cummaro, I. Fottichia, M. Franceschin, C. Giancola and L. Petraccone, *Biochimie*, 2011, **93**, 1392; (*d*) L. N. Zhu, B. Wu and D. M. Kong, *Nucleic Acids Res.*, 2013, **41**, 4324–4335; (*e*) X.-X. Huang, L.-N. Zhu, B. Wu, Y.-F. Huo, N.-N. Duan and D.-M. Kong, *Nucleic Acids Res.*, 2014, **42**, 8719; (*f*) M.-H. Hu, S.-B. Chen, B. Wang, T.-M. Ou, L.-Q. Gu, J.-H. Tan and Z.-S. Huang, *Nucleic Acids Res.*, 2017, **45**, 1606.

11 (*a*) L. P. Jordheim, D. Durantel, F. Zoulim and C. Dumontet, *Nat. Rev. Drug Discov.*, 2013, **12**, 447; (*b*) W. B. Parker, *Chem. Rev.*, 2009, **109**, 2880; (*c*) A. Mukherjee, R. Lavery, B. Bagchi and J. T. Hynes, *J. Am. Chem. Soc.*, 2008, **130**, 9747; (*d*) S. Neidle and D. E. Thurston, *Nat. Rev. Cancer*, 2005, **5**, 285; (*e*) V. A. Sontakke, P. P. Lawande, A. N. Kate, A. Khan, R. Joshi, A. A. Kumbhar and V. S. Shinde, *Org. Biomol. Chem.*, 2016, **14**, 4136 and references therein.

12 Y. Tang, X. Xu and Q. Li, *et al.*, *CN* 101352445, 2009.

13 (*a*) S. S. Bag, R. Kundu and S. Jana, *Tetrahedron Lett.*, 2013, **54**, 2627; (*b*) S. S. Bag, S. K. Das, M. K. Pradhan and S. Jana, *J. Photochem. Photobiol., B*, 2016, **162**, 669; (*c*) S. S. Bag, S. Talukdar, K. Matsumoto and R. Kundu, *J. Org. Chem.*, 2013, **78**, 278; (*d*) S. S. Bag, R. Kundu and S. Talukdar, *RSC Adv.*, 2013, **3**, 21352; (*e*) S. S. Bag, S. Talukdar, S. K. Das, M. K. Pradhan and S. Mukherjee, *Org. Biomol. Chem.*, 2016, **14**, 5088; (*f*) S. S. Bag, S. Talukdar and S. J. Anjali, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 2044; (*g*) S. S. Bag, M. K. Pradhan and S. Talukdar, *J. Photochem. Photobiol., B*, 2017, **173**, 165.

14 (*a*) L. Petraccone, *Top. Curr. Chem.*, 2013, **330**, 23; (*b*) H. Yu, X. Gu, S.-i. Nakano, D. Miyoshi and N. Sugimoto, *J. Am. Chem. Soc.*, 2012, **134**, 20060; (*c*) Y. Wang and D. J. Patel, *Structure*, 1993, **1**, 263; (*d*) Y. Xu, K. Kaminaga and M. Komiyama, *J. Am. Chem. Soc.*, 2008, **130**, 11179; (*e*) R. D. Gray and J. B. Chaires, *Nucleic Acids Res.*, 2008, **36**, 4191; (*f*) J. M. Nicoludis, S. P. Barrett, J.-L. Mergny and L. A. Yatsunyk, *Nucleic Acids Res.*, 2012, **40**, 5432.

15 (*a*) V. Vıglasky, L. Bauer and K. Tluckov, *Biochemistry*, 2010, **49**, 2110; (*b*) A. N. Lane, J. B. Chaires, R. D. Gray and J. O. Trent, *Nucleic Acids Res.*, 2008, **36**, 5482; (*c*) D. N. Edwards, A. Machwe, Z. Wang and D. K. Orren, *PLoS One*, 2014, **9**(1), e80664; (*d*) S. Burge, G. N. Parkinson, P. Hazel, A. K. Todd and S. Neidle, *Nucleic Acids Res.*, 2006, **34**, 5402; (*e*) M. L. Bochman, K. Paeschke and V. A. Zakian, *Nat. Rev. Genet.*, 2012, **13**, 770.

16 (*a*) L. Petraccone, *Top. Curr. Chem.*, 2013, **330**, 23; (*b*) K. Shinohara, Y. Sannohe, S. Kaieda, K. Tanaka, H. Osuga, H. Tahara, Y. Xu, T. Kawase, T. Bando and H. Sugiyama, *J. Am. Chem. Soc.*, 2010, **132**, 3778; (*c*) L. Stefan, F. Denat and D. Monchaud, *J. Am. Chem. Soc.*, 2011, **133**, 20405; (*d*) A. Cummaro, I. Fotticchia, M. Franceschin, C. Giancola and L. Petraccone, *Biochimie*, 2011, **93**, 1392; (*e*) L. P. Bai, M. Hagihara, Z. H. Jiang and K. Nakatani, *ChemBioChem*, 2008, **9**, 2583; (*f*) C. Zhao, L. Wu, J. Ren, Y. Xu and X. Qu, *J. Am. Chem. Soc.*, 2013, **135**, 18786; (*g*) L. Zhang, H. Liu, Y. Shao, C. Lin, H. Jia, G. Chen, D. Yang and Y. Wang, *Anal. Chem.*, 2015, **87**, 730.

17 (*a*) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703; (*b*) S. K. Behera and G. Krishnamoorthy, *Photochem. Photobiol. Sci.*, 2015, **14**, 2225.

18 (*a*) Z. Bikadi and E. Hazai, *J. Cheminf.*, 2009, **11**, 1; (*b*) G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, *J. Comput. Chem.*, 2009, **16**, 2785; (*c*) M.-H. Hu, S.-B. Chen, B. Wang, T.-M. Ou, L.-Q. Gu, J.-H. Tan and Z.-S. Huang, *Nucleic Acids Res.*, 2017, **45**, 1606; (*d*) L. Petraccone, J. O. Trent and J. B. Chaires, *J. Am. Chem. Soc.*, 2008, **130**, 16530.

