



Cite this: *Phys. Chem. Chem. Phys.*, 2025, 27, 3341

The role of spatial arrangement of aromatic rings on the binding of *N,N'*-diheteroaryl guanidine ligands to the G2C4/G2C4 motif DNA†

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Non-canonical DNA structures formed by aberrantly expanded repeat DNA are implicated in promoting repeat instability and the onset of repeat expansion diseases. Small molecules that target these disease-causing repeat DNAs hold promise as therapeutic agents for such diseases. Specifically, 1,3-di(quinolin-2-yl)guanidine (**DQG**) has been identified to bind to the disease-causing GGCCCC (G2C4) repeat DNA associated with amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). In this study, we investigate the structure-binding relationships between **DQG** analogs and double-stranded DNA (dsDNA) containing a G2C4/G2C4 unit. Our findings, derived from UV melting temperature, circular dichroism spectra, and surface plasmon resonance (SPR) analyses of **DQG** analogs, highlight the crucial role of the spatial arrangements of aromatic rings in binding to the G2C4/G2C4 unit. Among the tested **DQG** analogs, *N,N'*-di(quinazolin-2-yl)guanidine (**DQzG**) stands out for its ability to form seven planar conformers. These conformers enable ADD–DAA hydrogen bonding with cytosine and multiple spatial arrangements of aromatic rings, including those resembling **DQG**. Our binding analyses revealed that **DQzG** exhibits the highest affinity binding for the G2C4/G2C4 unit. NMR analysis of the **DQzG**-bound G2C4/G2C4-dsDNA further suggested that **DQzG** binds to the G2C4/G2C4 unit *via* hydrogen bonding. Moreover, SPR analysis demonstrated that **DQzG** binds more strongly to G2C4 repeat DNA compared to **DQG**. These results position **DQzG** as a promising lead compound for targeting the G2C4 repeat, offering potential therapeutic avenues for the treatment of ALS/FTD and other repeat expansion diseases.

Received 15th August 2024,
Accepted 10th January 2025

DOI: 10.1039/d4cp03213f

rsc.li/pccp

Introduction

Aberrant expansion of repeat DNA in the human genome is associated with neurodegeneration and repeat expansion diseases.^{1,2} A prominent cause of amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) is the expansion of the GGGGCC (G4C2) repeat DNA sequence located within the first intron of the chromosome 9 open reading frame 72 (C9orf72) gene. This expansion is observed in over 40% of familial and 8% of sporadic ALS/FTD cases.^{3,4} In ALS/FTD patients, the number of G4C2 repeats can surpass 250, while unaffected individuals typically have fewer than 20 repeats.^{5,6} The pathogenesis involves RNA gain-of-function due to the formation of

abnormal RNA aggregates, known as RNA foci, leading to the sequestration of RNA-binding proteins. Additionally, repeat-associated non-AUG translation (RAN translation) produces abnormal peptide repeat protein aggregates.^{7–9} The presence of RNA foci leads to loss of function of RNA-binding proteins, causing impaired splicing and nuclear-cytoplasmic transport. Another reported pathological mechanism is the repression of C9ORF72-coding mRNA expression in affected individuals, termed haploinsufficiency.¹⁰ Reduced levels of the C9ORF72 protein exacerbate the toxicity mediated by the repeat expansions, contributing to ALS/FTD.¹¹ Despite these insights, the molecular mechanisms underlying the root cause of repeat diseases, specifically the aberrant expansion, remain to be fully elucidated.

We have developed mismatch-binding ligands (MBLs) capable of recognizing mismatched base pairs in both DNAs and RNAs through complementary hydrogen bonding with nucleobases.^{12–15} Among these ligands, naphthyridine-azaquinolone (NA) was identified to selectively bind to the CAG/CAG motif, a key component of the hairpin structures formed

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† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d4cp03213f>



by CAG repeats. NMR structural analysis revealed that NA forms hydrogen bonds with nucleobases and engages in stacking interactions with adjacent nucleobases. In preclinical models, NA induced the contraction of expanded CAG repeats in Huntington's disease mice and improved motor phenotypes in dentatorubral-pallidoluysian atrophy (DRPLA) mice.^{12,16,17} These findings suggest that small molecules capable of binding to disease-causing repeat DNA have therapeutic potential by contracting aberrantly expanded repeat sequences.

Previous studies have explored small molecules that target the hairpin and G quadruplex formed by the G4C2 repeat.^{18–21} Our study focused on the antisense repeat GGCCCC (G2C4), which is predicted to form hairpins and i-motif structures.²² Based on a design concept utilizing hydrogen bond complementarity and stacking with adjacent nucleobases, we identified 1,3-di(quinolin-2-yl)guanidine (DQG; Fig. 1a) as a promising core unit for binding to the G2C4 repeat.²³ DQG significantly increased the melting temperature (T_m) of the model duplex 5'-GCAT GGCCCC TACG-3'/3'-CGTA CCCC GG ATGC-5' containing the G2C4/G2C4 unit. Given that the guanidyl group of DQG is protonated at neutral pH, an intramolecular hydrogen bond forms between the N1 nitrogen of quinoline and the guanidinium N-H, resulting in several conformers with donor-donor (DD) hydrogen bonding groups. This pattern partially complements the hydrogen bonding surface of cytosine with donor-acceptor-acceptor (DAA) groups. The lowest energy conformation of DQG (DQG conf-1)

binds to cytosine with two hydrogen bonds without steric repulsion (Fig. 1b). This hydrogen-bonding model suggests that by changing the substitution position of the guanidyl group on quinoline, the aromatic ring arrangement required for stacking interactions with adjacent base pairs can be adjusted. Substitution of quinoline with quinazoline may yield hydrogen-bonded surfaces with an acceptor-donor-donor (ADD) pattern. As the association constant of the ADD/DAA hydrogen-bonded complex is reported to be approximately 50-fold higher than that of the AA/DD hydrogen-bonded complex,²⁴ designing a DQG analog with an ADD hydrogen bonding pattern may enhance its binding property. Here, we present the synthesis of DQG analogs and evaluate their binding properties to the G2C4/G2C4 unit in dsDNA. Our analyses encompassed UV melting, circular dichroism (CD) spectra, surface plasmon resonance (SPR) assays, and nuclear magnetic resonance (NMR) spectra measurements. Notably, among the tested DQG analogs, *N,N'*-di(quinazolin-2-yl)guanidine (DQzG) demonstrated the highest binding affinity. Conformational analysis of DQG analogs, along with spatial alignment of conformers, revealed a similar spatial orientation of aromatic rings for both DQG and DQzG upon hydrogen bonding to cytosine. This similarity underscores the critical role of stacking interactions, complementing hydrogen bonding, in the high-affinity binding of DQzG to the G2C4/G2C4 unit.

Results

Molecular design of DQG analogs

To develop DQG analogs that adopt different orientation of two heteroaromatic rings in planar conformation, we designed *N,N'*-di(isoquinolin-3-yl)guanidine (D3iQG), *N,N'*-di(isoquinolin-1-yl)guanidine (D1iQG), and *N*-(isoquinolin-3-yl)-*N'*-(quinolin-2-yl)guanidine (Q3iQG) as structural isomers of DQG (Fig. 1c). These DQG analogs containing isoquinoline can adopt planar conformations with intramolecular hydrogen bonding between N2 position of isoquinoline and guanidyl group in a manner similar with DQG. *N,N'*-di(naphthalen-2-yl)guanidine (DNpG) and *N,N'*-di(quinolin-2-yl)urea (DQU) were synthesized as control compounds. Furthermore, we designed DQzG, which consisted of two quinazoline rings linked by a guanidine. The ADD hydrogen bonding surface in planar conformations of DQzG can be complementary to DAA hydrogen bonding surface of cytosine. The synthesis of these molecules is described in the Experimental section. Briefly, DQG analogs were synthesized in one or two steps by a Buchwald-Hartwig reaction of guanidine nitrate with the corresponding haloarene.²⁵ DQU was synthesized from triphosgene and 2-aminoquinoline.²⁶ In these reactions, multiple byproducts, including monomers and trimers, were detected by mass spectrometry (MS), along with the presence of unreacted haloarenes, which contributed to the low yield. Further investigation into the synthetic method is required to improve the reaction efficiency.

Conformational analysis of DQG analogs

To gain insights into the formation of planar conformations in DQG and DQG analogs, conformational analysis was performed

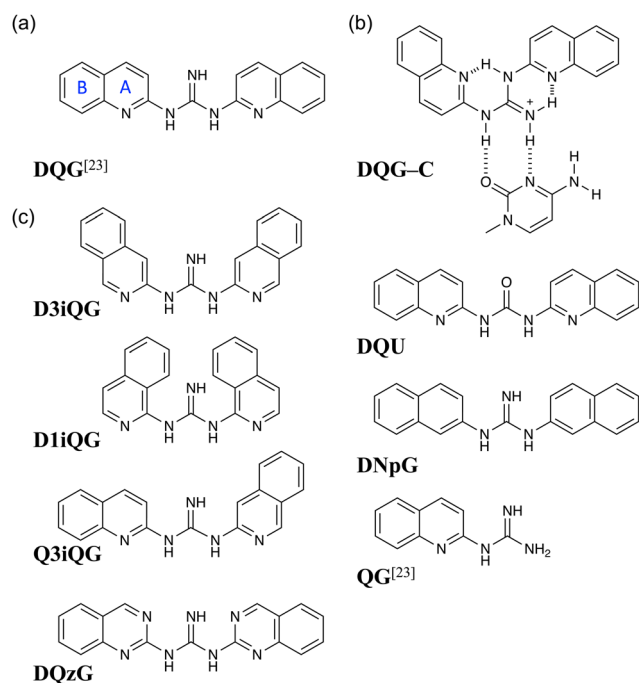


Fig. 1 (a) Chemical structure of DQG. For the discussion of the two aromatic rings, the ring directly attached to the guanidyl group is identified as ring A, and the other is referred to as ring B throughout this study. (b) Plausible hydrogen-bonding scheme of the lowest energy conformation of DQG (DQG conf-1) with cytosine. (c) Chemical structures of DQG analogs used in this study: D3iQG, D1iQG, Q3iQG, DQzG, DQU, DNpG, and QG.



Table 1 Relative potential energies of conformers calculated for **DQG** analogs^a

	DQG	D3iQG	D1iQG	Q3iQG	DQzG
conf-1	0.00	0.00	0.00 ^b	0.00	0.00
2	2.24 ^b	2.17 ^b	3.41 ^b	0.13	0.46
3				2.28 ^b	1.03
4					1.18
5					1.42
6					2.22
7					2.41

^a Relative potential energy (kcal mol⁻¹) of the conformers of **DQG**, **D3iQG**, **D1iQG**, **Q3iQG**, and **DQzG** calculated at the ωB97XD/6-31G+(d,p) level. All conformers listed here have the planar conformation. The entire list of conformational energies can be found in ESI.

^b These conformers are ineligible for stable hydrogen bonds formation with cytosine due to steric repulsion.

using a force field, OPLS4 in an aqueous environment. The conformations of **DQG** analogs within 5 kcal mol⁻¹ of the most stable conformer were obtained from 10 000 conformations generated by molecular mechanics (MM) calculations. Furthermore, quantum mechanical calculations were performed for all conformers obtained by MM calculation using DFT ωB97xd/6-31G+(d,p) in Gaussian 16 (Table 1 and Table S1, ESI[†]). The numbers of conformers within 3.5 kcal mol⁻¹ of the lowest energy conformer obtained for **DQG**, **D3iQG**, **D1iQG**, **Q3iQG**, and **DQzG** were 2, 2, 2, 3, and 7, respectively, and all these conformers are planar structures. The conformations are shown in Fig. S1 (ESI[†]). While **DQG** has two planar conformations, the conf-2 of **DQG** could not form stable hydrogen bonds to cytosine due to the steric repulsion between the amino N-H and aromatic C-H (Fig. S1a, ESI[†]), indicating that the conf-1 of **DQG** would be likely responsible for the binding to C in the G2C4/G2C4 unit to form two hydrogen bonds (*cf.* Fig. 1b). Likewise, **D3iQG** conf-2, **D1iQG** conf-1, **D1iQG** conf-2, and **Q3iQG** conf-3 were ineligible for the hydrogen bonding with cytosine. In contrast, all seven conformers of **DQzG** were eligible to form three hydrogen bonds with cytosine. **DQzG** conf-6 and conf-7 are more than 2 kcal mol⁻¹ above the lowest energy conf-1, and it is reasonable to assume that these conformers have little contribution for the complexation with cytosine. Thus, further discussion on the spatial orientation of heteroaromatic rings in the bound complexes would be focused on the ten conformers of **DQG** conf-1, **D3iQG** conf-1, **D1iQG** conf-1, **Q3iQG** conf-1 and 2, and **DQzG** conf-1, 2, 3, 4, and 5. To discuss the special orientation of heteroaromatic rings, the ring directly attached to the guanidyl group is identified as ring A, and the other is referred as ring B (*cf.* Fig. 1a).

UV melting temperature analysis

To examine the effect of the **DQG** analogs on the thermal stability of a 14-mer double-stranded DNA 5'-d(GCAT GGCCCC TACG)-3'/3'-d(CGTA CCCC GG ATGC)-5' containing the G2C4/G2C4 motif (G2C4/G2C4-dsDNA), we measured the T_m of the G2C4/G2C4-dsDNA without and with **DQG** analogs (Fig. S2, ESI[†]). The differences in the melting temperature (ΔT_m) in the presence and absence of **DQG** analogs are shown in Table 2. As reported previously, the T_m of the G2C4/G2C4-dsDNA in the

Table 2 Melting temperatures of G2C4/G2C4-dsDNA with **DQG** and **DQG** analogs^a

Ligand	T_m (+) ^b	ΔT_m ^c
DQG	49.4 (0.3)	19.5
D3iQG	41.3 (1.7)	11.4
D1iQG	43.2 (0.7)	13.4
Q3iQG	48.5 (0.4)	18.7
DQzG	50.0 (0.7)	20.2
QG	33.2 (0.8)	3.4
DNpG	31.1 (0.4)	1.3
DQU	30.3 (1.0)	0.4

^a Thermal melting curves were measured for dsDNA [5'-d(GCAT GGCCCC TACG)-3'/3'-d(CG TACCCC GGATGC)-5'] (5 μM) in sodium phosphate buffer (10 mM, pH 7) containing sodium chloride (100 mM) and 5% DMSO. ^b T_m values of DNA duplexes in the presence of **DQG** or **DQG** analogs (20 μM). The melting temperature is calculated by using the median method. All measurements were made three times or more and standard deviations are shown in parentheses. ^c ΔT_m (°C) is calculated as the difference of T_m (°C) in the absence T_m (-) (29.8 °C (0.5)) and presence T_m (+) of ligands.

absence and presence of **DQG** were 29.8 and 49.4 °C (ΔT_m = 19.5 °C), respectively.²³ To examine the influence of quinoline and guanidyl group moieties on the interaction between **DQG** and G2C4/G2C4-dsDNA, we performed T_m measurements using **DNpG** and **DQU**. The control compounds, **DNpG** and **DQU**, did not show any significant increase in the T_m of G2C4/G2C4-dsDNA, suggesting that quinoline and guanidyl group are indispensable for **DQG** binding to G2C4/G2C4-dsDNA. The ΔT_m of **D3iQG**, **D1iQG**, and **Q3iQG** were 11.4, 13.4, and 18.7 °C, respectively, showing a decreased T_m compared with **DQG**. In contrast, **DQzG** showed ΔT_m of 20.2 °C, indicating that the substitution of quinoline on **DQG** by quinazoline slightly increased the thermal stability of the ligand-bound complex.

CD spectral change on ligand binding

To obtain insights into structural information of complexes bound by **DQG** analogs, we carried out CD titration experiments with **DQG** analogs against G2C4/G2C4-dsDNA (Fig. 2). The CD spectrum of G2C4/G2C4-dsDNA showed negative and positive bands around 250 and 275 nm, respectively. As previously reported, the addition of **DQG** resulted in the appearance of a positive band around 260 nm and characteristic induced CD bands around 300–345 nm (Fig. 2a).²³ In the case of **D3iQG** and **Q3iQG**, the CD spectra showed two distinctive positive bands around 250 and 275 nm in the DNA region (Fig. 2b and d). The induced CD bands of **D3iQG** and **Q3iQG** were observed around 305 and 300–360 nm, respectively. Broad induced CD bands around 320–360 nm and a positive CD band around 255 nm with shoulder were observed in the presence of **DQzG** (Fig. 2e). In contrast, the CD spectrum in the presence of **D1iQG** showed a ligand-derived induced CD band in the 330–360 nm region and the disappearance of the negative CD band around 250 nm, but the positive CD band in DNA region around 275 nm did not show any significant changes (Fig. 2c). These data suggested that the binding of **DQG** analogs except for **D1iQG** induced large conformational change of G2C4/G2C4-dsDNA. The mode of **D1iQG** binding to the G2C4/G2C4 unit would be different from those of other **DQG** analogs.



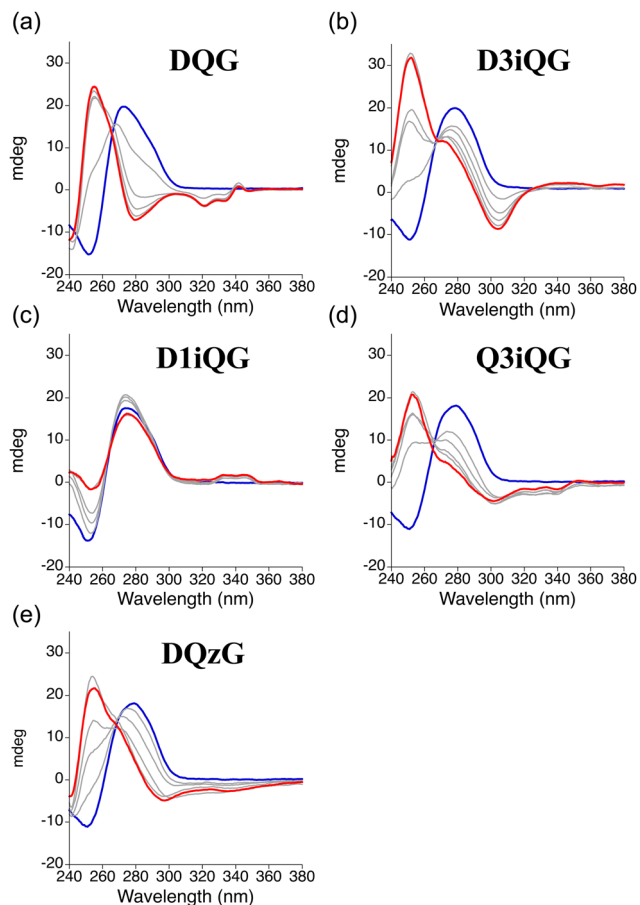


Fig. 2 CD spectra of dsDNA [5'-d(GCATGGCCCCCTACG)-3'/3'-d(CGTACCCCGATGC)-5'] (5 μ M) were measured without (blue) and with (a) **DQG**, (b) **D3iQG**, (c) **D1iQG**, (d) **Q3iQG** (0, 10, 20, 30, 40, 50 (red) μ M), and (e) **DQzG** (0, 5, 10, 15, 20, 25 (red) μ M) in sodium phosphate buffer (10 mM, pH 7) containing sodium chloride (100 mM) and 5% DMSO.

Binding analysis by SPR

SPR measurements were performed to assess the binding affinity of **DQG** analogs for the G2C4/G2C4 unit using sensor chip with immobilized 32-mer hairpin-DNA containing the G2C4/G2C4 unit (Fig. 3). All ligands were sequentially injected at increasing concentrations, resulting in concentration-dependent increases in response unit (RU). The sensorgrams showed a similar shape of SPR response curves among all ligands except for **DQzG**. The injection of **DQG**, **D3iQG**, and **Q3iQG** at a concentration of 4 μ M resulted in the RU of 30–35 (Fig. 3a, b, and d). On the other hand, sequential injection of **D1iQG** reached a plateau of RU at 15 when the concentration was 2 μ M (Fig. 3c). The difference in RU at the saturation point was likely due to a different mode of binding in **D1iQG**. When **DQG** and **Q3iQG** were injected, the RU was almost the same under the condition of same concentrations, suggesting that the binding affinity of **Q3iQG** was comparable with that of **DQG**. In contrast, the injection of **D3iQG** exhibited lower RU than **DQG** under the condition of same concentrations, implying that the binding affinity of **D3iQG** was lower than that of **DQG**. The sensorgram obtained for **DQzG** showed slow

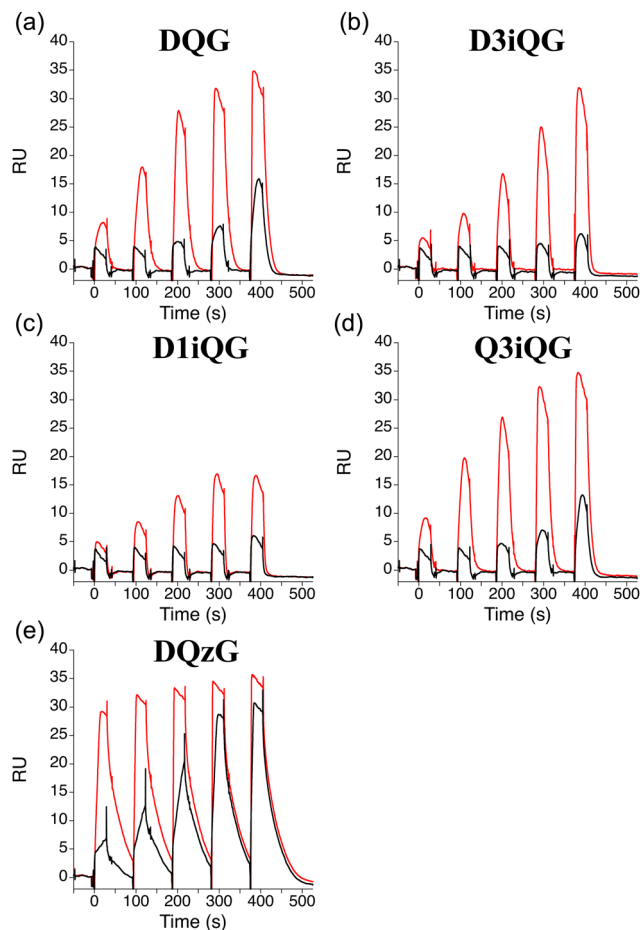


Fig. 3 SPR single cycle kinetic analyses of ligand binding to hairpin DNA containing the G2C4/G2C4 unit. Ligands were applied to the DNA-immobilized surface for 60 seconds, and the sensor surface was subsequently washed by the running buffer for 60 seconds before the next injection of the ligand. The ligands (a) **DQG**, (b) **D3iQG**, (c) **D1iQG**, (d) **Q3iQG**, and (e) **DQzG** were sequentially added at 0.025, 0.050, 0.1, 0.2, 0.4 μ M (black line) and 0.25, 0.50, 1.0, 2.0, 4.0 μ M (red line).

dissociation kinetics, which is different from those of the other **DQG** analogs (Fig. 3e). In the case of **DQzG**, the significant increase in RU was observed under the condition of one order of magnitude lower concentrations than that of **DQG**. We also confirmed that saturation point for RU after injection of **DQzG** was approximately 30 at a concentration of 0.4 μ M. These results suggested that **DQzG** showed the highest binding affinity among **DQG** analogs we tested. We confirmed that the injection of all **DQG** analogs for fully matched DNA duplex-immobilized sensor chip did not show any significant increases in RU, indicating the preferential binding of **DQG** analogs to the G2C4/G2C4 unit (Fig. S3, ESI[†]). The apparent dissociation constants (K_{Dapp}) of ligands binding to the hairpin DNA containing the G2C4/G2C4 unit were determined using BIAevaluation software integrated into the SPR equipment. The K_{Dapp} values were calculated based on the assumption of a 1:1 binding model and are as follows: **DQG**, 1.0 μ M; **D3iQG**, 10 μ M; **D1iQG**, 3.4 μ M; **Q3iQG**, 1.4 μ M; and **DQzG**, 63 nM. These values are fully consistent with the trends of T_m



measurements. It is important to emphasize, however, that this assumption was made solely for the purpose of comparing the affinities of the five molecules. The possibility of other binding stoichiometries cannot be excluded, as discussed later.

NMR analysis of DQzG-bound G2C4/G2C4-dsDNA

To obtain further insights into the interaction between **DQzG** and G2C4/G2C4-dsDNA, we performed NMR titration experiments with molar ratios from 1:0 (DNA:**DQzG**) to 1:4 in 20 mM phosphate buffer (pH 6.5) containing 50 mM NaCl and 10% D₂O and monitored the changes in signals of exchangeable protons from 10 to 14 ppm (Fig. 4). In the absence of **DQzG**, the signals of imino protons in A–T and G–C base pairs appeared at 13–14 ppm and 12–13 ppm, respectively. As the concentration of **DQzG** was increased, the appearance of signals around 10–12 ppm was observed with concomitant changes in the signal around 12–14 ppm. These new signals around 10–12 ppm were likely to correspond to guanidine protons of **DQzG**. These results suggested that **DQzG** bound to G2C4/G2C4-dsDNA resulting in the formation of hydrogen bonded complex.

SPR analysis of the binding to G2C4 repeat

Having confirmed the superior properties of **DQzG** in the binding to the G2C4/G2C4 unit in dsDNA compared with the parent molecule **DQG**, we investigated the **DQzG** binding to the d(G2C4)₉ repeat by SPR. **DQzG** showed a significant response at 0.2 μM with a characteristic slow binding and dissociation profile (Fig. 5b). The SPR profile is similar to that obtained for the G2C4/G2C4 unit in dsDNA (*cf.* Fig. 3e). **DQG** showed SPR response only at 1 μM (Fig. 5a). **Q3iQG** showed a similar response to **DQG** (Fig. S4, ESI[†]).

Discussion

Hydrogen bonding and π -stacking play crucial roles in the interactions between small molecules and nucleic acids.^{27,28}

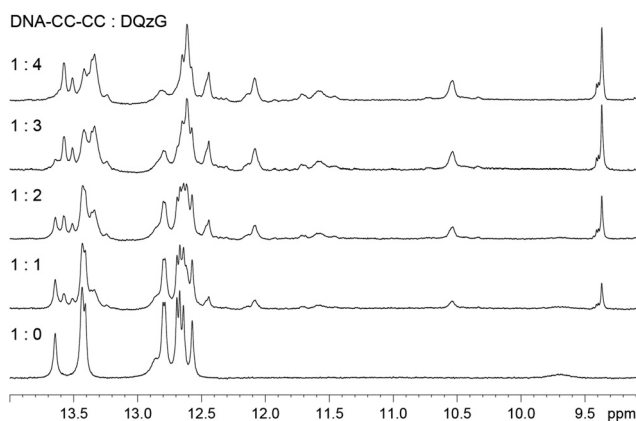


Fig. 4 NMR analysis of **DQzG** binding to G2C4/G2C4-dsDNA [5'-d(GCATGGCCCCTACG)-3'/3'-d(CGTACCCCGGATGC)-5'] (300 μM) in sodium phosphate buffer (20 mM, pH 6.5) containing 50 mM NaCl. Titration of G2C4/G2C4-dsDNA with **DQzG** at molar ratios of 1:0 (dsDNA : **DQzG**) to 1:4.

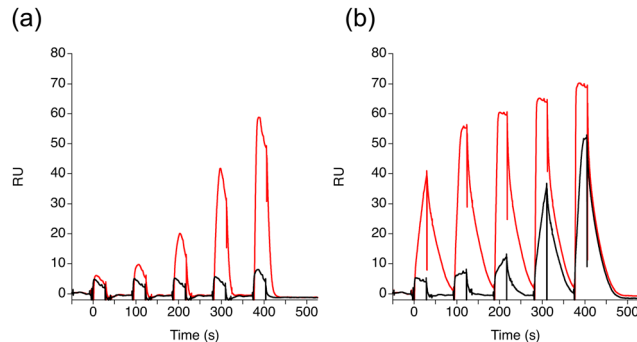


Fig. 5 SPR single cycle kinetic analyses of ligand binding to the G2C4 repeat DNA. Ligands were applied to the DNA-immobilized surface for 60 seconds, and the sensor surface was subsequently washed with the running buffer for 60 seconds before the next injection of the ligand. (a) **DQG** and (b) **DQzG** were sequentially added at 0.25, 0.50, 1.0, 2.0, 4.0 μM (red line) and 0.025, 0.050, 0.1, 0.2, 0.4 μM (black line).

Using molecular design based on complementary hydrogen bonding with mismatched nucleotide bases and π -stacking with adjacent bases, we have developed mismatch-binding ligands (MBLs).^{12–15,29–33} In our ongoing studies focused on nucleic acid-targeted ligands, we previously reported on **DQG**, which binds to the G2C4/G2C4-dsDNA. **DQG** adopts planar conformations due to a π -conjugated system comprising two quinoline rings and the guanidyl group, facilitated by intramolecular hydrogen bonding. Conformational analysis revealed that the **DQG** conf-1 conformation is the only one suitable for complex formation with cytosine in the G2C4/G2C4 unit of dsDNA. To investigate the relationship between hydrogen bonding capability and stacking interactions with adjacent bases, we designed and synthesized six **DQG** analogs.

A critical factor influencing the binding of **DQG** analogs is the planarity of the ligand molecule. Conformational analyses revealed that, except for **DNpG**, the lowest energy state for six analogs is a planar conformer (Fig. S1, ESI[†]). For instance, **DQG** exhibits two planar conformers, conf-1 and conf-2 (Fig. S1a, ESI[†]). The non-planar conformer, conf-3, is 9.37 kcal mol⁻¹ higher in energy than conf-1. **DNpG** does not have a planar conformer due to steric repulsion between the guanidyl N–H and C–H in the naphthyl ring. While this non-planar conformer offers a hydrogen-bonding group with a DD arrangement (Fig. S1g, ESI[†]), UV-melting analyses show a minimal ΔT_m (1.3 °C) for **DNpG** (Table 2). In contrast, **DQG** exhibits a ΔT_m of 19.5 °C under the same conditions, indicating that a planar conformation is essential for binding to the G2C4/G2C4 unit. Among the five analogs with planar conformers, **DQU** fails to bind to G2C4/G2C4-dsDNA. Its lowest-energy conformer is planar but lacks hydrogen-bonding donors with a DD arrangement (Fig. S1f, ESI[†]). Although the next stable conformer has the required hydrogen-bonding arrangement, it is 4.33 kcal mol⁻¹ higher in energy than conf-1, suggesting limited binding potential. Additionally, **DQU** lacks a positive charge essential for nucleic acid binding. The monomeric ligand **QG** has a planar conformation and hydrogen-bonding donors with a DD arrangement but only weakly stabilizes G2C4/G2C4-dsDNA (ΔT_m : 3.4 °C). Based on these findings



for **DNpG**, **DQU**, and **QG**, the requirements for **DQG** analogs to bind effectively to G2C4/G2C4-dsDNA include planar conformations with two heterocycles and a DD arrangement of hydrogen-bonding donors. Five molecules, namely **DQG**, **D3iQG**, **D1iQG**, **Q3iQG**, and **DQzG**, meet these criteria.

Among the five molecules tested, **D3iQG** and **D1iQG** exhibited a significant decrease in ΔT_m compared to the other three molecules. **D1iQG** displayed marked differences from the other molecules in both CD spectral changes and SPR analyses. In CD measurements, **D1iQG** induced fewer structural changes in G2C4/G2C4-dsDNA upon binding (Fig. 2c). The SPR response with **D1iQG** was lower than that of other **DQG** analogs at a ligand concentration of 4 μM (Fig. 3c). Additionally, the UV melting profile of **D1iQG** showed a steeper rise in absorbance upon the melting of the ligand-bound DNA duplex compared to the other **DQG** analogs (Fig. S2c, ESI[†]). These observations suggest that the binding mode of **D1iQG** differs from that of the other four molecules. The likely determinant of **D1iQG**'s binding mode is the orientation of its two isoquinoline rings relative to the hydrogen-bonding donors. The lowest-energy conformer of **D1iQG** has DD hydrogen bonding groups but cannot form stable hydrogen bonds with cytosine due to interference by the isoquinoline C–H at C8 (Fig. S1c, ESI[†]). The second lowest-energy conformer experiences even stronger steric repulsion. These steric hindrances likely contribute to the distinct binding mode of **D1iQG** compared to the other **DQG** analogs.

The low ΔT_m observed for **D3iQG** provides valuable insights into the favorable arrangement of aromatic rings in the ligand-bound complex. Both **DQG** and **D3iQG** possess two planar conformers with similar energy differences. However, the conf-2 conformer of both molecules cannot bind to cytosine due to steric repulsion between the cytosine C4–NH₂ group and the C3–H of quinoline in **DQG** conf-2, and between the cytosine

C4–H and the C4–H of isoquinoline in **D3iQG** conf-2 (Fig. S1, ESI[†]). Consequently, **DQG** and **D3iQG** bind to cytosine using their conf-1 conformers. When both molecules bind to cytosine, the spatial arrangement of aromatic ring A is consistent between them, but not for ring B. This discrepancy suggests that the spatial arrangements of rings A and B may contribute differently to the complex stability of **DQG** analogs.

The spatial arrangements of ring A and B of each conformer of **DQG** analogs were compared by superimposing the expected hydrogen-bonded pair with cytosine. Conformers of **DQzG** (conf-1 and 2), **D3iQG** (conf-1), and **Q3iQG** (conf-1 and 2) were superimposed onto **DQG** conf-1 with cytosine as the reference. The three minor conformers (conf-3, 4, and 5) of **DQzG** exhibited properties similar to conf-1 and 2 (Fig. S5, ESI[†]). In Fig. 6, ring A of the six conformers was colored magenta, while **DQG**'s ring A was colored cyan. In the superimposed figures, the overlapped ring A would be shown only in cyan, otherwise both cyan and magenta colored ring As were visible. Consistent with previous discussions, two ring As in **D3iQG** conf-1 are overlapped with those of **DQG** conf-1, therefore only cyan-colored ring A was identified. Interestingly, both conf-1 and conf-2 of **Q3iQG** showed full overlap of ring A with **DQG** conf-1. In contrast, ring As of **DQzG** conf-1 and conf-3 did not overlap with those of **DQG** at all. The conf-2 of **DQzG** showed overlap of one of two ring As.

Fig. 7 illustrates the overlap of ring Bs. Contrary to the results for ring A superimposition, **DQzG** exhibited a notable similarity in spatial arrangement to **DQG** for ring B. Both conf-1 and conf-2 of **DQzG** showed complete overlap of both ring Bs, while conf-3 overlapped with one of its two ring Bs. In contrast, **D3iQG** showed no overlap for ring B. **Q3iQG**'s conf-1 and conf-2 displayed overlap with one of their two ring Bs. The alignment of ring A and B in **DQG** analogs with those in **DQG** is summarized in Table 3.

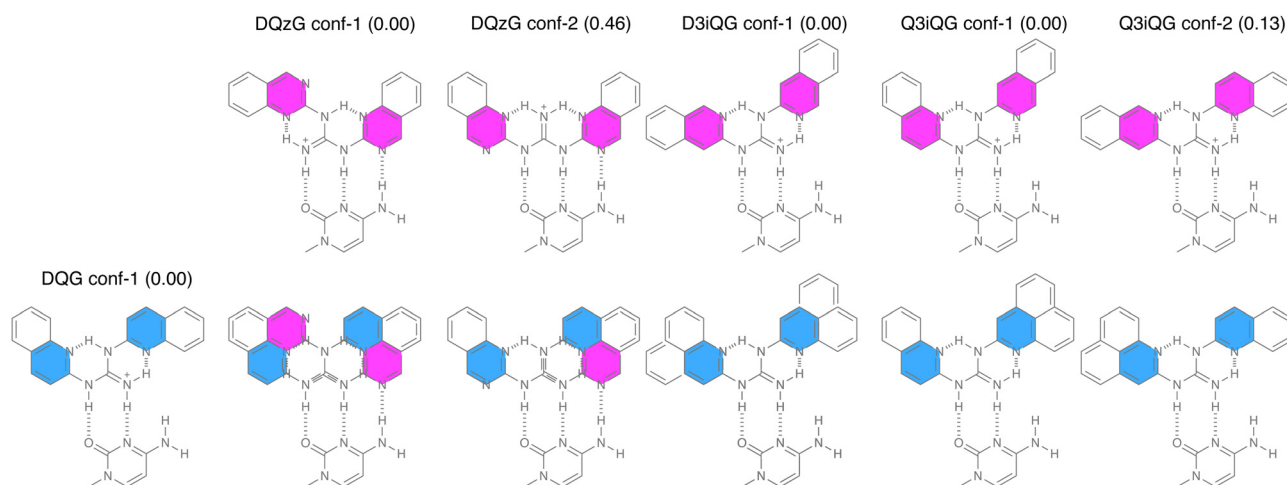


Fig. 6 Spatial alignment comparison of ring A of **DQG** analogs upon hydrogen bonding to cytosine. **DQG** conf-1 was superimposed with **DQzG** conf-1 and conf-2, **D3iQG** conf-1, and **Q3iQG** conf-1 and conf-2 with respect to cytosine. Ring A of **DQG** conf-1 was colored in cyan, whereas those of others are colored in magenta. In the superimposed figures, the overlapped ring A is shown only in cyan; otherwise, both cyan and magenta-colored ring A are visible. The numbers in parentheses indicate the energy difference (kcal mol^{-1}) from the lowest energy conformation. Plus signs (+) were omitted from the superimposed structures for clarity.



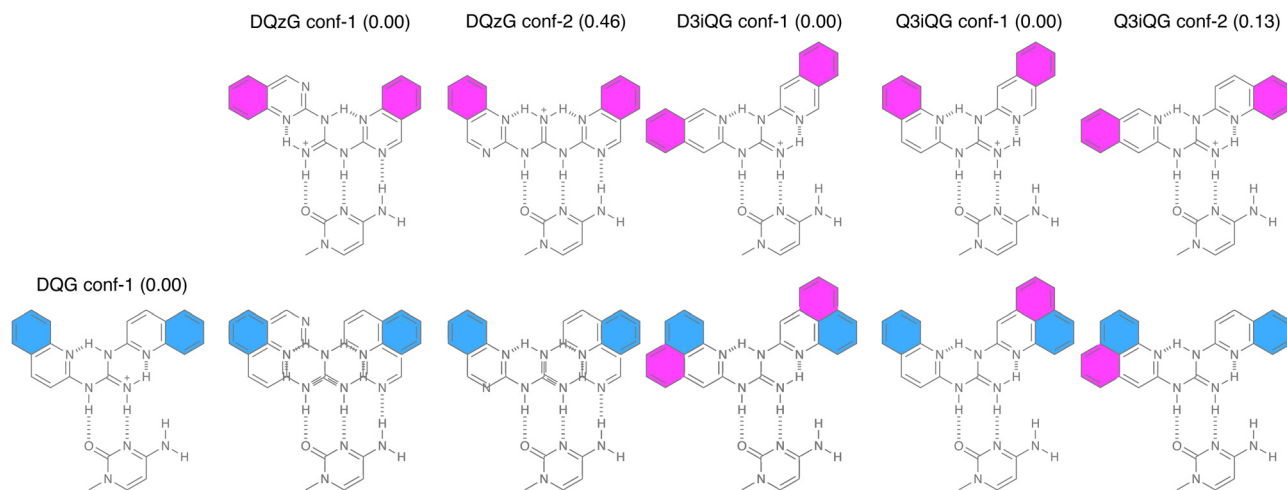


Fig. 7 Spatial alignment comparison of ring B of **DQzG** analogs upon hydrogen bonding to cytosine. **DQzG** conf-1 was superimposed with **DQzG** conf-1 and conf-2, **D3iQG** conf-1, and **Q3iQG** conf-1 and conf-2 with respect to cytosine. Ring B of **DQzG** conf-1 was colored in cyan, whereas those of others are colored in magenta. In the superimposed figures, the overlapped ring B is shown only in cyan; otherwise, both cyan and magenta-colored ring A are visible. The numbers in parentheses indicate the energy difference (kcal mol^{-1}) from the lowest energy conformation. Plus signs (+) were omitted from the superimposed structures for clarity.

Table 3 Summary of the superimposition of ring A and B and the expected H-bonding pattern

	DQzG	DQzG		D3iQG	Q3iQG	
	conf-1	conf-1	conf-2	conf-1	conf-1	conf-2
Ring A ^a	—	0	1	2	2	2
Ring B ^a	—	2	2	0	1	1
H-bonding ^b	DD/AA	ADD/DAA	ADD/DAA	DD/AA	DD/AA	DD/AA
ΔT_m ($^{\circ}\text{C}$)	19.5	20.2		11.4	18.7	

^a The number of ring A and B overlapped with that of **DQzG** conf-1. ^b Hydrogen bonding pattern between **DQzG** analogs and cytosine.

Table 3 highlights the relationships between the spatial orientation of rings A and B, the hydrogen-bonding pattern with cytosine, and the stability of the ligand-bound complex. Both **D3iQG** and **DQzG** share the orientation of ring A relative to cytosine and the DD/AA hydrogen-bonding pattern, but differ in ring B arrangement. The significant ΔT_m difference ($19.5\text{ }^{\circ}\text{C}$ vs. $11.4\text{ }^{\circ}\text{C}$) suggests that the spatial arrangement of ring B likely accounts for this variation. **Q3iQG**, which overlaps one of its two ring Bs with **DQzG** and shares the ring A orientation and hydrogen-bonding pattern with **D3iQG**, exhibits only a slight decrease in stability compared to **DQzG**. This underscores the importance of ring B arrangement in the **Q3iQG**-bound complex. **DQzG**'s conformers feature ADD hydrogen-bonding groups and are susceptible to hydrogen bonding with cytosine. The lowest-energy conf-1 of **DQzG** shares the ring B spatial arrangement with **DQzG**, while conf-2 also shares ring A arrangement. Conf-3 of **DQzG** shared the arrangement of one of two ring Bs similarly to conf-1 and conf-2 (Table S2, ESI[†]). A slight increase in stability can be attributed to the ADD–DAA hydrogen-bonding with cytosine. Thus, **DQzG** has an advantage over **DQzG** due to its ADD hydrogen-bonding groups while maintaining the spatial arrangement of heteroaromatic rings. Conf-4 and conf-5 also share similar features with conf-3.

Further discussion on these minor conformers of **DQzG** is provided in ESI[†].

In summary, the pronounced and consistent SPR responses of **DQzG** to G2C4 repeat DNA, when compared to those of **DQzG**, strongly indicate the potential of **DQzG** as a superior lead compound for targeting G2C4 repeat DNA. The SPR response of **DQzG** towards G2C4 repeat DNA closely parallels that observed for the G2C4/G2C4 unit in double-stranded DNA. This suggests that double-stranded DNA containing the G2C4/G2C4 unit is a suitable model for drug discovery studies focused on G2C4 repeat.

In the SPR data analysis of **DQzG** binding to the hairpin DNA containing G2C4/G2C4, the binding stoichiometry could be estimated using the following equation:

$$R_{\text{max}} = MW_{\text{A}} \times R_{\text{I}}/MW_{\text{L}} \times n,$$

where n is stoichiometric ratio (number of binding sites per immobilized DNA); R_{max} (RU) is maximum binding response (30 RU); R_{I} (RU) is immobilization level of DNA (515 RU), MW_{L} (Da) is molecular weight of DNA (10927 Da); and MW_{A} (Da) is molecular weight of **DQzG** (315 Da). This analysis suggests a binding stoichiometry of 2 for **DQzG** binding to G2C4/G2C4. (Fig. 3 and Table S3, ESI[†]) In contrast, CD titration experiments



showed saturation at a DNA:DQzG ratio of 1:4 (Fig. 2), and NMR titration experiments similarly indicated a DNA:DQzG ratio of 1:4 as peaks corresponding to the initial DNA duplex disappeared (Fig. 4). These discrepancies likely arise from differences in DQzG concentrations used in each measurement: $\sim 4 \mu\text{M}$ for SPR, $50 \mu\text{M}$ for CD titration, and $1200 \mu\text{M}$ for NMR titration. This suggested that at lower concentrations (below $4 \mu\text{M}$), the binding stoichiometry is 2, while at higher concentrations it increases to 4. The G2C4/G2C4 unit contains four mismatched cytosines, which could potentially bind up to four DQzG molecules *via* hydrogen bonding. At lower concentrations, two of these cytosines are likely bound by DQzG to form the initial complex. At higher concentrations, the binding transitions to a complex involving all four DQzG molecules. The interaction between two DQzG molecules in the initial complex and their potential stacking interactions with neighbouring base pairs are key questions. However, structural determination of the complex will be necessary for further discussion and validation of these hypotheses.

Conclusions

In conclusion, our comparative analysis of binding characteristics among DQG analogs underscores the importance of the spatial arrangement of aromatic rings. The interplay of electrostatic attraction, hydrogen bonding, and stacking interactions with neighboring bases demonstrates a compensatory relationship, with DQzG exhibiting an optimal combination of these interactions, resulting in enhanced binding to the G2C4 repeat compared with the parent ligand, DQG. This study offers valuable insights into the molecular design of ligands targeting GGCCCC repeats, leveraging guanidine and heteroaromatic rings. Such insights pave the way for developing next-generation ligands with potential applications in the study and treatment of ALS/FTD and other repeat diseases. The elucidation of the DQzG-bound structure to G2C4 repeat DNA is pivotal for deeper understanding of the critical factors influencing molecule binding to the repeat. Ongoing research in our laboratories is focused on further exploring these aspects, promising further advancements in this field.

Experimental section

Synthesis of ligands

Reagents and solvents were purchased from standard suppliers and used without further purification. Reactions were monitored with TLC plates precoated with Merck Silica Gel 60 F254. Spots were visualized with UV light or ninhydrin. Wako gel C-200 was used for silica gel flash chromatography. High performance liquid chromatography (HPLC) was performed by a Gilson 811C Dynamic Mixer system with a UV detector set at 254 nm using a Cosmosil 5C₁₈-MS-II column ($150 \times 20 \text{ mm}$) with a dual solvent system of 0.1% AcOH/H₂O (solvent A) and CH₃CN (solvent B). ¹H NMR and ¹³C NMR spectra were measured with ECS400 (JEOL), ECA600 (JEOL), and Avance III 700 (BRUKER). The chemical shifts are expressed in parts per

million (ppm) relative to a residual solvent as an internal standard. Chemical shifts were reported in ppm and coupling constants (J values) in hertz. ¹H NMR chemical shifts were referenced to the residual solvent peak at 3.31 ppm in CD₃OD and at 2.50 ppm in DMSO-d₆. ¹³C NMR chemical shifts were referenced to the center solvent peak at 49.00 ppm for CD₃OD and at 39.52 ppm for DMSO-d₆. High-resolution mass spectra (HRMS) were recorded on a Thermo LTQ Orbitrap XL mass spectrometer in electron spray ionization mode.

Conformational searches

Conformational searches were performed for the initially selected structures of DQG, D3iQG, D1iQG, Q3iQG, DQzG, DQU, and DNpG by using Maestro version 13.0.135 (Schrödinger Inc.) under the following conditions: low-frequency-mode conformational search with probability of TORS/MOLS steps of 0.5 and OPLS4 force field in solvent water. Among 10000 conformations generated, the lowest conformer within 5 kcal mol^{-1} after energy minimization were saved. The saved conformers of DQG, D3iQG, D1iQG, Q3iQG, DQzG, DQU, and DNpG were found 21, 19, 32, 36, 11, 9, and 32 conformers, respectively. Structure optimization of DQG, D3iQG, D1iQG, Q3iQG, DQzG, DQU, and DNpG was performed by Gaussian 16 using parameter of ωB97XD/6-31G+(d,p). All conformers saved in conformational searches of DQG, D3iQG, D1iQG, Q3iQG, DQzG, DQU, and DNpG were used as the initial structures.

UV-melting analysis

All samples were mixed with $5 \mu\text{M}$ solutions of DNA, 10 mM sodium phosphate buffer (pH 7.0), 100 mM NaCl, $20 \mu\text{M}$ ligand, and 5.0% DMSO. The melting temperature was determined by measuring the absorbance of samples at $\lambda = 260 \text{ nm}$ from 2 to $80 \text{ }^\circ\text{C}$ ($1 \text{ }^\circ\text{C min}^{-1}$). Thermal denaturation profiles were monitored on a UV-2700 spectrophotometer (Shimadzu) equipped with a 10 mm path-length cell and a TMSPC-8 temperature controller. T_m was calculated by using the median method.

Circular dichroism (CD) measurements

CD spectra were recorded with a J-725 CD spectrometer (JASCO) using a 10 mm path length cell. CD spectra of DNA ($5 \mu\text{M}$ DNA duplex) in the absence and presence of ligand (DQG, D3iQG, D1iQG, Q3iQG ($10, 20, 30, 40, 50 \mu\text{M}$), and DQzG ($5, 10, 15, 20, 25 \mu\text{M}$)) were measured in sodium phosphate buffer (10 mM, pH 7.0), NaCl (100 mM), and 5.0% DMSO.

Surface plasmon resonance (SPR) analysis

5'-Biotinylated DNAs (G2C4/G2C4, (G2C4)₉, and fullmatch) were immobilized on the streptavidine-coated surface of Series S Sensor chip SA (GE Healthcare, Life Science). All immobilization reactions were performed in HBS-EP + buffer (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM ethylenediaminetetraacetic acid (EDTA), 0.05% (v/v) surfactant P20) by using a Biacore T200 instrument (GE Healthcare, Life Science) at $25 \text{ }^\circ\text{C}$. The amounts of immobilized G2C4/G2C4, (G2C4)₉, and fullmatch on the chip surface were 515, 521, and 490 response units (RU), respectively. Sensorgrams were obtained in the ligand



concentrations of 0.25, 0.50, 1.0, 2.0, 4.0 μM and 0.025, 0.050, 0.1, 0.2, 0.4 μM in single-cycle mode (flow rate 60 $\mu\text{M min}^{-1}$, contact time 30 s, and dissociation time 120 s). All sensorgrams were corrected by reference subtraction of blank flow cell response and buffer injection response. The data collected were analyzed with the Biacore T200 evaluation software (version 2.0), and kinetic parameters were derived through the application of both the kinetic analysis and curve fitting techniques, assuming a 1:1 binding interaction.

Sample preparation and NMR measurement

The chemically synthesized DNA oligomers 5'-GCATGGCC CCTACG-3'/3'-CGTACCCCGGATGC-5' were purchased from commercial suppliers (FASMAC). DQzG at molar ratios of 0, 1, 2, 3, and 4 was dissolved in 20 mM sodium phosphate buffer (pH 6.5), 100 mM NaCl, 300 μM DNA duplex, and 10% D_2O . The solution was heated at 80 $^\circ\text{C}$ for 1 h and cooled to 4 $^\circ\text{C}$ overnight. Using these solutions, $^1\text{H-NMR}$ were measured on BRUKER Avance III 700.

Data availability

Raw data are available from the corresponding author upon request.

Conflicts of interest

Conflict of interest statement. None declared.

Acknowledgements

This work was supported by Grant-in-Aid for Scientific research (A) [19H00924], [22H00351] to K. N., for Scientific Research (B) [20H02880] and for Challenging Exploratory Research [21K19049] to T. S. We thank for Ms Yu Kawakami for experimental assistance.

Notes and references

- C. E. Pearson, K. N. Edamura and J. D. Cleary, *Nat. Rev. Genet.*, 2005, **6**, 729–742.
- I. Malik, C. P. Kelley, E. T. Wang and P. K. Todd, *Nat. Rev. Mol. Cell Biol.*, 2021, **22**, 589–607.
- M. DeJesus-Hernandez, I. R. Mackenzie, B. F. Boeve, A. L. Boxer, M. Baker, N. J. Rutherford, A. M. Nicholson, N. A. Finch, H. Flynn, J. Adamson, N. Kouri, A. Wojtas, P. Sengdy, G.-Y. R. Hsiung, A. Karydas, W. W. Seeley, K. A. Josephs, G. Coppola, D. H. Geschwind, Z. K. Wszolek, H. Feldman, D. S. Knopman, R. C. Petersen, B. L. Miller, D. W. Dickson, K. B. Boylan, N. R. Graff-Radford and R. Rademakers, *Neuron*, 2011, **72**, 245–256.
- A. E. Renton, E. Majounie, A. Waite, J. Simón-Sánchez, S. Rollinson, J. R. Gibbs, J. C. Schymick, H. Laaksovirta, J. C. van Swieten, L. Myllykangas, H. Kalimo, A. Paetau, Y. Abramzon, A. M. Remes, A. Kaganovich, S. W. Scholz, J. Duckworth, J. Ding, D. W. Harmer, D. G. Hernandez, J. O. Johnson, K. Mok, M. Ryten, D. Trabzuni, R. J. Guerreiro, R. W. Orrell, J. Neal, A. Murray, J. Pearson, I. E. Jansen, D. Sondervan, H. Seelaar, D. Blake, K. Young, N. Halliwell, J. B. Callister, G. Toulson, A. Richardson, A. Gerhard, J. Snowden, D. Mann, D. Neary, M. A. Nalls, T. Peuralinna, L. Jansson, V.-M. Isoviita, A.-L. Kaivorinne, M. Hölttä-Vuori, E. Ikonen, R. Sulkava, M. Benatar, J. Wu, A. Chiò, G. Restagno, G. Borghero, M. Sabatelli, D. Heckerman, E. Rogaeva, L. Zinman, J. D. Rothstein, M. Sendtner, C. Drepper, E. E. Eichler, C. Alkan, Z. Abdullaev, S. D. Pack, A. Dutra, E. Pak, J. Hardy, A. Singleton, N. M. Williams, P. Heutink, S. Pickering-Brown, H. R. Morris, P. J. Tienari and B. J. Traynor, *Neuron*, 2011, **72**, 257–268.
- M. Van Blitterswijk, M. DeJesus-Hernandez, E. Niemant-sverdriet, M. E. Murray, M. G. Heckman, N. N. Diehl, P. H. Brown, M. C. Baker, N. A. Finch, P. O. Bauer, G. Serrano, T. G. Beach, K. A. Josephs, D. S. Knopman, R. C. Petersen, B. F. Boeve, N. R. Graff-Radford, K. B. Boylan, L. Petrucelli, D. W. Dickson and R. Rademakers, *Lancet Neurol.*, 2013, **12**, 978–988.
- B. Swinnen, W. Robberecht and L. Van Den Bosch, *EMBO J.*, 2020, **39**, e101112.
- K. Mori, S.-M. Weng, T. Arzberger, S. May, K. Rentzsch, E. Kremmer, B. Schmid, H. A. Kretschmar, M. Cruts, C. Van Broeckhoven, C. Haass and D. Edbauer, *Science*, 2013, **339**, 1335–1338.
- Y.-B. Lee, H.-J. Chen, J. N. Peres, J. Gomez-Deza, J. Attig, M. Štalekar, C. Troakes, A. L. Nishimura, E. L. Scotter, C. Vance, Y. Adachi, V. Sardone, J. W. Miller, B. N. Smith, J.-M. Gallo, J. Ule, F. Hirth, B. Rogelj, C. Houart and C. E. Shaw, *Cell Rep.*, 2013, **5**, 1178–1186.
- J. Cooper-Knock, M. J. Walsh, A. Higginbottom, J. Robin Highley, M. J. Dickman, D. Edbauer, P. G. Ince, S. B. Wharton, S. A. Wilson, J. Kirby, G. M. Hautbergue and P. J. Shaw, *Brain*, 2014, **137**, 2040–2051.
- Y. Shi, S. Lin, K. A. Staats, Y. Li, W.-H. Chang, S.-T. Hung, E. Hendricks, G. R. Linares, Y. Wang, E. Y. Son, X. Wen, K. Kisler, B. Wilkinson, L. Menendez, T. Sugawara, P. Woolwine, M. Huang, M. J. Cowan, B. Ge, N. Koutsodendris, K. P. Sandor, J. Komberg, V. R. Vangoor, K. Senthilkumar, V. Hennes, C. Seah, A. R. Nelson, T.-Y. Cheng, S.-J. J. Lee, P. R. August, J. A. Chen, N. Wisniewski, V. Hanson-Smith, T. G. Belgard, A. Zhang, M. Coba, C. Grunseich, M. E. Ward, L. H. Van Den Berg, R. J. Pasterkamp, D. Trotti, B. V. Zlokovic and J. K. Ichida, *Nat. Med.*, 2018, **24**, 313–325.
- Q. Zhu, J. Jiang, T. F. Gendron, M. McAlonis-Downes, L. Jiang, A. Taylor, S. Diaz Garcia, S. Ghosh Dastidar, M. J. Rodriguez, P. King, Y. Zhang, A. R. La Spada, H. Xu, L. Petrucelli, J. Ravits, S. Da Cruz, C. Lagier-Tourenne and D. W. Cleveland, *Nat. Neurosci.*, 2020, **23**, 615–624.
- K. Nakatani, S. Hagihara, Y. Goto, A. Kobori, M. Hagihara, G. Hayashi, M. Kyo, M. Nomura, M. Mishima and C. Kojima, *Nat. Chem. Biol.*, 2005, **1**, 39–43.
- K. Nakatani, S. Sando, H. Kumasawa, J. Kikuchi and I. Saito, *J. Am. Chem. Soc.*, 2001, **123**, 12650–12657.



- 14 T. Yamada, K. Furuita, S. Sakurabayashi, M. Nomura, C. Kojima and K. Nakatani, *Nucleic Acids Res.*, 2022, **50**, 9621–9631.
- 15 K. Nakatani, *Proc. Jpn. Acad., Ser. B*, 2022, **98**, 30–48.
- 16 M. Nakamori, G. B. Panigrahi, S. Lanni, T. Gall-Duncan, H. Hayakawa, H. Tanaka, J. Luo, T. Otabe, J. Li, A. Sakata, M.-C. Caron, N. Joshi, T. Prasolava, K. Chiang, J.-Y. Masson, M. S. Wold, X. Wang, M. Y. W. T. Lee, J. Huddleston, K. M. Munson, S. Davidson, M. Layeghifard, L.-M. Edward, R. Gallon, M. Santibanez-Koref, A. Murata, M. P. Takahashi, E. E. Eichler, A. Shlien, K. Nakatani, H. Mochizuki and C. E. Pearson, *Nat. Genet.*, 2020, **52**, 146–159.
- 17 Y. Hasuike, H. Tanaka, T. Gall-Duncan, M. Mehkary, K. Nakatani, C. E. Pearson, S. Tsuji, H. Mochizuki and M. Nakamori, *Neurobiol. Dis.*, 2022, **163**, 105604.
- 18 Y. Lu, C. Dohno and K. Nakatani, *Chem. Commun.*, 2020, **56**, 754–757.
- 19 C.-R. Jhan, R. Satange, S.-C. Wang, J.-Y. Zeng, Y.-C. Horng, P. Jin, S. Neidle and M.-H. Hou, *Nucleic Acids Res.*, 2021, **49**, 9526–9538.
- 20 R. Simone, R. Balendra, T. G. Moens, E. Preza, K. M. Wilson, A. Heslegrave, N. S. Woodling, T. Niccoli, J. Gilbert-Jaramillo, S. Abdelkarim, E. L. Clayton, M. Clarke, M. Konrad, A. J. Nicoll, J. S. Mitchell, A. Calvo, A. Chio, H. Houlden, J. M. Polke, M. A. Ismail, C. E. Stephens, T. Vo, A. A. Farahat, W. D. Wilson, D. W. Boykin, H. Zetterberg, L. Partridge, S. Wray, G. Parkinson, S. Neidle, R. Patani, P. Fratta and A. M. Isaacs, *EMBO Mol. Med.*, 2018, **10**, 22–31.
- 21 A. Cheng, C. Liu, W. Ye, D. Huang, W. She, X. Liu, C. P. Fung, N. Xu, M. C. Suen, W. Ye, H. H. Y. Sung, I. D. Williams, G. Zhu and P.-Y. Qian, *J. Med. Chem.*, 2022, **65**, 12825–12837.
- 22 A. Kovanda, M. Zalar, P. Šket, J. Plavec and B. Rogelj, *Sci. Rep.*, 2015, **5**, 17944.
- 23 T. Shibata, E. Murakami and K. Nakatani, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 2364–2368.
- 24 S. C. Zimmerman and T. J. Murray, *Philos. Trans. R. Soc., A*, 1993, **345**, 49–56.
- 25 B. P. Fors, K. Dooleweerd, Q. Zeng and S. L. Buchwald, *Tetrahedron*, 2009, **65**, 6576–6583.
- 26 P. S. Corbin, S. C. Zimmerman, P. A. Thiessen, N. A. Hawryluk and T. J. Murray, *J. Am. Chem. Soc.*, 2001, **123**, 10475–10488.
- 27 J. Rebek, B. Askew, P. Ballester, C. Buhr, S. Jones, D. Nemeth and K. Williams, *J. Am. Chem. Soc.*, 1987, **109**, 5033–5035.
- 28 A. D. Hamilton and N. Pant, *Chem. Commun.*, 1988, 765–766.
- 29 J. Li, A. Sakata, H. He, L.-P. Bai, A. Murata, C. Dohno and K. Nakatani, *Chem. – Asian J.*, 2016, **11**, 1971–1981.
- 30 A. Murata, T. Otabe, J. Zhang and K. Nakatani, *ACS Chem. Biol.*, 2016, **11**, 2790–2796.
- 31 T. Shibata and K. Nakatani, *Chem. Commun.*, 2018, **54**, 7074–7077.
- 32 S. Hagihara, H. Kusama, Y. Goto, G. Hayashi, A. Kobori, I. Saito and K. Nakatani, *Nucleic Acids Res.*, 2004, **32**, 278–286.
- 33 T. Shibata, K. Nagano, M. Ueyama, K. Ninomiya, T. Hirose, Y. Nagai, K. Ishikawa, G. Kawai and K. Nakatani, *Nat. Commun.*, 2021, **12**, 236.

