Guanines are a quartet's best friend:

Impact of base substitutions on the kinetics and stability of tetramolecular quadruplexes.

Julien Gros[1], Frédéric Rosu[1,2], Samir Amrane[1], Anne De Cian[1], Valérie Gabelica[2],

Laurent Lacroix[1] & Jean-Louis Mergny[1*]

SUPPLEMENTARY MATERIAL

Supplementary Figure S1: Association and thermal stability of TG_4T and TG_5T.

A) Isothermal association of TG_4T in ammonium at two different wavelengths: 295 nm (red) and 240 nm (blue). Fits are shown with a black solid line.

B) k_{on} values obtained for TG_5T (left) and TG_4T (right) in 3 different ionic conditions: potassium (black), sodium (blue) and ammonium (red).

C) Melting of the TG_4T quadruplex in ammonium recorded at two different wavelengths: 295 nm (red) and 240 nm (blue). Temperature gradient: 0.48°C/min.

D) Apparent melting temperature values obtained for TG_5T (left) and TG_4T (right) in 3 different ionic conditions: potassium (black), sodium (blue) and ammonium (red). Note that the TG_5T quadruplex has a $T_{1/2} \geq 90$°C in all conditions.

As shown previously (1), the association rate of these quadruplexes is strongly dependent on sequence length: the longer the G-tract, the faster the association. Each additional guanine leads to a ~10-fold larger association rate constant. A stabilizing effect on the apparent melting temperature may also be observed: the longer the G-tract, the higher the $T_{1/2}$.

Supplementary Figure S2: Evidence for tetramolecular quadruplex formation: gel electrophoresis and Absorbance difference spectra.

A) *Non denaturing gel electrophoresis.* Formation of both unmodified and modified tetramolecular quadruplexes was studied by non-denaturing PAGE. To reach a total quadruplex formation, samples « + » were preincubated during at least 48 hours, at 4°C and at high strand concentration (500 µM in 20 µL), in a 10 mM lithium cacodylate pH 7.2 buffer containing 110 mM K^+, Na^+ or NH_4^+. In parallel, samples « - » were incubated in the same conditions omitting cations. Samples « + » and « - » were loaded on a 20% polyacrylamide gel containing TBE 1X and the corresponding cation at 20 mM. Just before loading, samples « - » were briefly heated at 90°C and immediately after put on ice. Electrophoresis was run at 3W in a cold room to control temperature (≤ 15 °C) during migration. Both G4-DNA and single-strands were revealed by UV-shadowing using a UV light source (254 nm) and a digital camera. Quadruplexes (samples « + ») were typically retarded during migration, compared to single-strands (samples « - »). Oligonucleotides dT_n (n = 6/9/12/15/21 or 24) were used as single-strand size markers. Because of high concentration and long incubation time, we have observed in few cases (for example with $[d(TG\underline{\mathbf{X}}GGT)]_4$ in Na^+ and NH_4^+) formation of higher order intermolecular structures in the gel (*data not shown*). This type of structure did not had any influence on the study of the association process (concentrations lower and times shorter than in PAGE experiments), was not observed on CD spectra, but led to supplementary lower transitions during dissociation experiments (*data not shown*). Some of the modifications, such as 8-oxo G (**8**) or 8-Br G (**X**), led to a less important difference between migration of the G4-DNA and the single-strand forms. Incubation for 1 night (top) or one week (bottom) at 300 µM strand concentration.

B) *Isothermal (3°C) difference spectra* of TGGGGGT (in red) and inosine-containing variants (**I**) in 0.11M Na^+

C) *Isothermal (3°C) difference spectra* of TGGGGGT (in red) and 6-methyl-isoxanthopterin-containing variants (**P**) in 0.11M Na^+

Comparison of the absorbance spectra of the totally unfolded (at t=0) and folded conditions allows us to generate an isothermal differential absorbance spectra (2). Its shape is specific for quadruplexes (3).

Supplementary Figure S3: Evidence for tetramolecular quadruplex formation: circular dichroism.

CD spectra were recorded with a Jasco-810 spectropolarimeter using 1 cm pathlength quartz cuvets in a

volume of 350µL. Scans were performed at 4°C over a wavelength range of 230-350 nm with a scanning speed of 200 $nm.min^{-1}$, a response time of 1s, 1 nm pitch and 1 nm bandwidth. For each sample, CD spectrum of buffer was recorded, then the sample was added in the same cuvette, and after that blank was substracted from the collected data. Each CD spectrum represents an average of two scans without any zero correction. Final analysis of the data was carried out using Kaleidagraph 3.6.4 software. Samples were preincubated at high strand concentration to allow quadruplex formation: 300µM of single strand oligonucleotide was heated then immediately added to 10 mM lithium cacodylate pH 7.2, 290 mM KCl, NaCl or NH_4Cl buffer, and finally incubated at 4°C during 72 hours or more.

Examples of CD spectra are provided here in 0.11M K^+ or NH_4^+ for 2 different modifications. As it has already been described (4), the CD spectrum of a parallel tetramolecular quadruplex is characterised by a negative peak around 242 nm and a positive peak around 263 nm. Here we show spectra with quadruplexes bearing two differents modifications : **7** (panel A) and **P** (panel B) at positions 1, 3 or 5 on TG_5T and positions 1 or 4 on TG_4T provided in 0.11M K^+ (left side) and 0.11M NH_4^+ (right side). The shape of each CD spectrum clearly indicates that the corresponding modified quadruplex is parallel. Note that panel B, left is identical to Fig. 3c.

All other oligonucleotides were also tested (not shown). Most oligomers exhibited a similar CD spectrum. However, two oligonucleotides gave a different signature: dTGG**X**GT and dTGGG**X**GT in K^+, Na^+ and NH_4^+ with a totaly inverted CD spectra, as for an antiparallel quadruplex (data not shown). We are currently exploring the conformation of these quadruplexes, which are related to the previously described TGGGT sequence variants (5)) in more details.

Supplementary Figure S4: Evidence for tetramolecular quadruplex formation: ESI-MS results.

ESI-MS experiments were performed as previously described (6-8). All experiments were performed on a Q-TOF Ultima Global (Micromass, now Waters, Manchester, UK) with the Z-spray ESI source. The capillary voltage was set to -2.2 kV and the cone voltage to 35V. The RF lens 1 voltage was set to 74V for all the quadruplexes. The argon pressure inside the collison hexapole (3.0 X 10-5 mbar ± 5%) and the source pressure (2.70 mbar) were carefully kept constant. Methanol (15%) was added to the samples just before injection to obtain a stable electrospray signal.

A) ESI-MS full scan spectra of the quadruplexes (A) $[d(TGGGGT)]_4$ and (B) $[d(TGGGGGT)]_4$ obtained in negative ion mode. The quadruplex concentration was 5 µM. Spectra were recorded in 150mM ammonium acetate. The tetramers are present in three charge states (6-, 5- and 4-). Only a small amount of single strand is detected. close-up allowing determination of the number of ammonium ions

B) Zoom on the 5- charge state of (a) the $[d(TG_4T)]_4$ quadruplex, (b) the $[d(TG_5T)]_4$ quadruplex, (c) the $[d(T\underline{\mathbf{8}}GGGGT)]_4$ quadruplex and (d) the $[d(T\underline{\mathbf{C}}GGGGT)]_4$ quadruplex. 3 and 4 ammonium ions are detected with the model parallel quadruplexes $[d(TG_4T)]_4$ and $[d(TG_5T)]_4$ respectively.

Supplementary Figure S5: Representative examples of isothermal renaturation experiments.

Formation of a quadruplex with inosine-containing oligonucleotides TGG**I**GGT (panel **A**) and TGGGG**I**T (panel **B**) (17.8 and 19.4 µM strand concentration, respectively at 4°C, 0.11 M K^+). Absorbance was recorded simultaneously at two wavelengths (240 nm: blue circles and 295 nm: red inverted triangles). The fitted curves (full lines) are nearly indistinguishable from the experimental data. Fitted k_{on} values are provided for each curve; ± 25% differences are considered acceptable. Often the initial concentration chosen was not optimal. Trial and error allowed the identification of a proper concentration range.

Supplementary Figure S6: Examples of dual wavelength parametric tests.

Dual wavelength parametric tests for :

A) T**G**GGGGT in NaCl 110mM. **B**) T**P**GGGGT in NaCl 110mM. **C**) T**I**GGGGT in NaCl 110mM.

In each example, absorbance at 240 nm (left Y-scale, blue circles) and absorbance at 295 nm (right Y-scale, red triangles) are plotted vs. absorbance at 260 nm.

Supplementary Figure S7: Effect of the nature of the monocation on the association process.

Association constants in potassium (panel **A**, left) and ammonium (panel **B**, right): effect of a single guanine substitution on the k_{on} (the corresponding figure for the values obtained in sodium for TG_5T variants is provided in the main manuscrit). The position of the substitution is indicated on the X-axis:

position 1 correspond to the first guanine (5' side), position 5 to the last guanine (3' side). 12 different replacements were tested, each corresponding to a different symbol. The relative k_{on} values (± SD) for the formation of the TG_5T variants are indicated on the left Y-axis (k_{on} for the unmodified TG_5T sequence under the same conditions = 1, corresponding to a horizontal dotted line). Absolute values are shown on the right Y-axis (k_{on} for TG_5T indicated in green). Experiments were performed in 0.11M K^+ or NH_4^+, at a temperature of 3 ± 1 °C. Note the semi-log scale: for many mutants, a single substitution may lead a tremendous decrease in the association. In sodium and ammonium buffer, only a few cases lead to a faster association than TG_5T.

Supplementary Figure S8: Effect of a 3' substitution.

A) *Relative association constant (k_{on}) as compared to TG_5T for oligomers in which the **last** guanine has been replaced by another base* (code as in Table 1 / Figure 1c). Data obtained in potassium (black), sodium (blue) or ammonium (red symbols).

B) *Asymetry effects*: comparison of the k_{on} for oligomers having the substitution on the first or last guanine. A value close to 1 means that no asymetry could be evidenced, as for most modifications (**8**, **I**, **M**, **Q**, **U**, **6**, **T**…); a value >>1 means that the modification is more favourable when inserted in the 5' quartet, as for **X** and **C**; whereas a value <<1 means that the modification is better tolerated when inserted at the 3' end, as for **7**.

Supplementary Figure S9: Kinetics followed by non-denaturing gel electrophoresis.

For the T**X**GGGGT oligonucleotide (panel **A**, top), as compared to TG_5T (panel **B**, bottom) (both strands at 100 µM). Experimental conditions: 0.11 M NH_4^+. Incubation times at 4°C from 5 minutes to 72 hours. M= single stranded markers. See Experimental section for details.

Supplementary Figure S10: Effect of the nature of the monocation on the thermal dissociation process.

Effect of a single guanine substitution on the apparent melting temperature. The position of the substitution is indicated on the X-axis: position 1 correspond to the first guanine (5' side), position 5 (or 4 in the case of TG_4T variants) to the last guanine (3' side). 12 different replacements were tested, each corresponding to a different symbol. The $T_{1/2}$ values (± 1°C) for the preformed quadruplexes with TG_5T and TG_4T variants are indicated on the left and right columns, respectively. Experiments were

performed in 0.11M K^+ (panels **A** and **B**, top), or 0.11 M NH_4^+ (panels **C** and **D**, bottom), using a temperature gradient of 0.5°C/min (data in 0.11M Na^+ is provided in the main manuscrit). In many cases, no melting of the quadruplex could be observed; in that case, is $T_{1/2}$ arbitrary fixed to ≥ 90°C. The apparent melting temperature of the TG_4T canonical quadruplex is indicated by a horizontal dotted line for each condition (no melting of thee TG_5T quadruplex is observed independently of the cation).

Supplementary Figure S11: k_{off} and $t_{1/2}$ determination.

A) Normalized melting profiles of $[d(TG_4T)]_4$ quadruplexes (fraction folded *vs.* Temperature) bearing an 8-Br G (*X, top*) at different positions.

B) Normalized melting profiles of $[d(TG_4T)]_4$ quadruplexes (fraction folded *vs.* Temperature) bearing an 8-oxo G (*8, bottow*) at different positions.

These melting curves are concentration independent, and the apparent melting temperature depends on the heating rate chosen for the experiment (1). They reflect the quasi-irreversible melting of the quadruplex species : depending on strand concentration, one may observe either very slow reformation of the quadruplex upon cooling (hysteresis phenomenon) or no reformation. These profiles reflect the dissociation process, and onemay calculate the kinetic dissociation constant (k_{off}) at each temperature.

C) Arrhenius representation ($\ln(k_{off})$ as a function of 1/T) calculated from the data shown in panel A for 8-bromo G.

D) Arrhenius representation ($\ln(k_{off})$ as a function of 1/T) calculated from the data shown in panel B for 8-oxo G.

Data points may be fitted with a straight line, in agreement with a simple melting process, allowing us to determine a positive activation energy of dissociation (E_{off}). As most fits are nearly parallel, the corresponding structures have comparable E_{off} values (with the sole exception of $[d(TGG\underline{X}GT)]_4$). To illustrate the differences in the dissociation process, one can also calculate the lifetimes of the different quadruplexes ($t_{1/2} = \ln(2) / k_{off}$) at a given temperature. For example, at 60.3°C ($1/T = 3.10^{-3}$), $[d(T\underline{X}GGGT)]_4$ is 50-fold longer lived that the corresponding $[d(TG_4T)]_4$ quadruplex. In contrast, at 44.4°C ($1/T = 3.15.10^{-3}$), $[d(TGGG\underline{X}T)]_4$ has a 20-fold shorter lifetime that the corresponding unmodified $[d(TG_4T)]_4$.

Supplementary Figure S12: Gas phase dissociation profile of 6 quadruplexes.

The fragmentation pattern of the quadruplex TG_4T has been previously described (6). The TG_5T quadruplex with four ammonium ions (black curve) dissociates via the loss of one ammonium, which is the initial step in the unfolding of the quadruplex structure, followed at higher energy by the loss of the 3 remaining ammonium (blue curve) (panel A). The quadruplex then dissociates by losing one strand, thus forming a trimeric species. It has been shown that ammonium ions are labile within the quadruplex structure (6). The gas-phase stability ranking of these quadruplexes is not easily related to thermal stability in solution. Little correlation could be found between the ΔV required for half denaturation and $T_{1/2}$ values. The solvent and counterions interacting with the quadruplex in solution could be major influencing factors. The dissociation profile of six quadruplexes with 4 or 3 ammoniums is presented. The % of ammonium ion in the structure is plotted as a function of the collision energy (black: 4 ammoniums, red: 3, green 2, yellow: 1 and blue: 0). The contribution (%) of ammonium ion is obtained from equation: $\%_{n\mathrm{NH4}} = (\mathrm{IG4}_{\mathrm{with}n\mathrm{NH4}})/(\mathrm{sum}(\mathrm{IG4}_{\mathrm{all}})+\mathrm{I}_{\mathrm{allfragments}})$. The quadruplexes with four NH_4 (TGGGGGT, T**8**GGGGT, TGGGG**8**T) dissociate as the energy increased by losing one ammonium and at higher energy three simultaneous ammonium. The quadruplexes with 3 ammonium (T**C**GGGGT, T**7**GGGGT) lose all the 3 ammoniums simultaneously. T**A**GGGGT shows a intermediate with 2 ammoniums significantly populated.

Only a small percentage of quadruplexes with 2 and 1 ammoniums was populated (green and yellow curves, respectively). Substitution effects were position-dependent: the dissociation profile of T**8**GGGGT is approximately the same than TG_5T (panel B). In contrast, TGGG**8**GT has lost one ammonium and the resulting complexes with 3 ammoniums is the dominant species at high collision energy (panel C). Panels D,E,F shows the ESI-MS/MS spectra on the quadruplexes with 3 ammoniums. The quadruplexes T**7**GGGGT and T**C**GGGGT lose their three ammoniums simultaneously. A substitution effect is presented with T**A**GGGGT in panel D. The MS/MS reveals the presence of a tetramolecular quadruplex with 2 NH_4 at low collision energy. **A** destabilize the next G-tetrad.

Supplementary Figure S13: Dissociation curves obtained on different quadruplexes as a function of collision energy: MS/MS analysis.

The position of the substitution influence the lability of the quadruplex.

A) 8 oxoguanine substitution: the $[(TG_5T)_4+4NH_4\text{-}9H]^{5-}$ quadruplex is provided as a reference (black circles). One may compare the effect of 8-oxo guanine at the 5' end $[(T\mathbf{8}GGGGT)_4+4NH_4\text{-}9H]^{5-}$

(inverted triangles) with its effect at the 3' end $[(TGGGG\underline{\mathbf{8}}T)_4+4NH_4-9H]^{5-}$ (red circles). When replacing the first guanine by 8-oxo guanine, the quadruplex is slightly destabilized and a lower collision energy is needed to achieved the same degree of dissociation (E_{50} value 51eV vs 53eV for TG_5T). When the last guanine of the G_5 stretch is substituted by **8**, the quadruplex is more stabilized (ΔE_{50} = 4 eV) compared to the canonic TG_5T.

B) Effect of other substitutions

The percentage of intact quadruplex relative to the sum of all fragments was calculated using: %quadruplex=100*I(G4with4,3,2,1,0NH_4)/[I(G4with4,3,2,1,0NH_4)+I(bases lost)+I(single strand lost)+I(triplex)].

Supplementary Table S1: Effect of a 5' modification on quadruplex association and melting.

A: TG_5T variants

Sequence	k_{on}(3°C) $M^{-3}.s^{-1}$			$T_{1/2}$ °C		
(5'->3')	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+
TGGGGGT	$9.8\ 10^{9}$	$2.3\ 10^{12}$	$1.1\ 10^{9}$	≥ 90	≥ 90	≥ 90
T**A**GGGGT	$1.9\ 10^{8}$	$2.0\ 10^{9}$	$1.6\ 10^{7}$	67.5	≥ 90	81.0
T**C**GGGGT	$1.9\ 10^{9}$	$6.0\ 10^{10}$	$4.1\ 10^{8}$	61.0	≥ 90	77.0
T**T**GGGGT	$3.1\ 10^{7}$	$2.4\ 10^{9}$	$5.1\ 10^{6}$	61.0	≥ 90	66.5
T**U**GGGGT	$1.6\ 10^{8}$	$7.0\ 10^{9}$	$1.9\ 10^{7}$	*nd*	≥ 90	81.0
T**I**GGGGT	$2.4\ 10^{8}$	$2.6\ 10^{10}$	$9.4\ 10^{7}$	69.0	≥ 90	79.5
T**6**GGGGT	*nd*	$3.0\ 10^{9}$	$1.1\ 10^{8}$	77.0	≥ 90	≥ 90
T**M**GGGGT	$1.8\ 10^{8}$	$1.7\ 10^{10}$	$4.2\ 10^{7}$	66.2	≥ 90	≥ 90
T**7**GGGGT	$6.1\ 10^{7}$	$2.1\ 10^{9}$	$1.7\ 10^{7}$	67.0	≥ 90	≥ 90
T**8**GGGGT	$1.6\ 10^{9}$	$4.5\ 10^{10}$	$4.4\ 10^{9}$	≥ 90	≥ 90	≥ 90
T**X**GGGGT	**$1.1\ 10^{11}$**	**$1.2\ 10^{13}$**	**$1.3\ 10^{10}$**	≥ 90	≥ 90	≥ 90
T**P**GGGGT	**$8.2\ 10^{10}$**	**$7.0\ 10^{12}$**	**$1.1\ 10^{10}$**	65.5	≥ 90	≥ 90
T**Q**GGGGT	$4.6\ 10^{7}$	$7.8\ 10^{9}$	*nd*	61.5	≥ 90	*nd*

(Sup. Table S1 continued)

B: TG_4T variants

Sequence	k_{on}(3°C) $M^{-3}.s^{-1}$			$T_{1/2}$ °C		
(5'->3')	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+
TGGGGT	3.5 10^8	1.8 10^{10}	1.2 10^8	57.5	≥ 90	77.0
T**A**GGGT	*ts*	*ts*	*ts*	*nd*	*nd*	*nd*
T**C**GGGT	*ts*	1.1 10^8	*ts*	≤ 20	52.5	29.5
T**T**GGGT	*ts*	*nd*	*ts*	≤ 20	58.5	29.5
T**U**GGGT	*ts*	6.0 10^7	1.4 10^6	41.0	76.0	42.0
T**I**GGGT	*ts*	1.6 10^8	4.5 10^6	23.0	63.5	30.5
T**6**GGGT	*ts*	1.3 10^8	5.3 10^6	≤ 20	80.0	40.0
T**M**GGGT	*ts*	*ts*	*ts*	*nd*	*nd*	*nd*
T**7**GGGT	*ts*	*ts*	*ts*	*nd*	75.5	53
T**8**GGGT	4.1 10^7	*nd*	*nd*	59.5	≥ 90	≥ 90
T**X**GGGT	**1.3 10^{10}**	**2.9 10^{11}**	**1.9 10^9**	77.5	≥ 90	≥ 90
T**P**GGGT	*ts*	**7.0 10^{10}**	*ts*	≤ 20	75.5	55.5
T**Q**GGGT	*ts*	*ts*	*ts*	61.5	≥ 90	nd

Association rate constant at 4°C, pH 7, with 0.11 M Na^+, NH_4^+ or K^+. k_{on} is given with a ± 30% accuracy or better. The first and last oligonucleotide correspond to the unmodified TG_4T and TG_5T reference sequences. Many quadruplexes resisted thermal denaturation. For many TG_5T variants, no dissociation of the quadruplex could be observed in potassium ($T_{1/2}$ ≥ 90 °C). Once formed, parallel quadruplexes are very stable. Hence, thermal denaturation data could be collected only for a subset of sequences. Values faster than the reference sequence (TG_5T in panel A, TG_4T in panel B) are shown in bold and green).

$T_{1/2}$ is the non-equilibrium melting temperature of the preformed quadruplex in 0.11 M Na^+, determined with a temperature gradient of 0.5°C/min. ≥ 90 means that no melting is observed in the temperature range studied. ≤ 20 : apparent temperature is too low to be precisely determined.

nd: not done or unreliable

ts: too slow, even at the highest concentration tested (k_{on} (3°C) generally < 10^5 $M^{-3}.s^{-1}$).

U = deoxy uracyl; I = Inosine; 6 = 6-thioguanine; 7 = 7-deaza guanine; 8 = 8-oxo guanine; P = 6MI = 6-methylisoxanthopterin; Q = 3MI = 3-methylisoxanthopterin; M= 6-methyl guanine ; X = 8-bromo guanine.

Supplementary Table 2: Position effect of a substitution.

Sequence	k_{on}(3°C) $M^{-3}.s^{-1}$			$T_{1/2}$ °C		
(5'->3')	Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+
TGGGGGT	9.8 10^9	2.3 10^{12}	1.1 10^9	≥ 90	≥ 90	≥ 90
TXGGGGT	1.1 10^{11}	1.2 10^{13}	1.3 10^{10}	≥ 90	≥ 90	≥ 90
TGXGGGT	5.9 10^9	2.1 10^{11}	8.7 10^8	≥ 90	≥ 90	52.0
TGGXGGT	3.6 10^8	2.1 10^{10}	1.6 10^8	80.0	≥ 90	64.0
TGGGXGT	2.7 10^8	1.1 10^{10}	4.5 10^7	75.5	≥ 90	65.0
TGGGGXT	1.9 10^8	2.5 10^{10}	5.4 10^7	81.0	≥ 90	≥ 90
T7GGGGT	6.1 10^7	2.1 10^9	1.7 10^7	67	≥ 90	≥ 90
TG7GGGT	8.3 10^6	2.0 10^7	3.5 10^5	61.5	≥ 90	62.5
TGG7GGT	2.3 10^7	7.3 10^7	1.9 10^6	29.5	74.0	56.0
TGGG7GT	*nd*	3.4 10^8	2.9 10^6	≤ 20	59.0	33.5
TGGGG7T	1.1 10^9	3.4 10^{10}	1.1 10^8	65.0	≥ 90	78.0

Association rate constant at 4°C, pH 7, with 0.11 M Na^+, NH_4^+ or K^+. k_{on} is given with a ± 30% accuracy or better.
$T_{1/2}$ is the non-equilibrium melting temperature of the preformed quadruplex in 0.11 M Na^+ NH_4^+ or K^+, determined with a temperature gradient of 0.5°C/min.
nd: not determined.
X = 8-bromo guanine; **7** = 7-deaza guanine.

Supplementary Table S3: Effect of 3- and 6-methylisoxanthopterin.

Sequence	k_{on}(3°C) $M^{-3}.s^{-1}$				$T_{1/2}$ °C		
(5'->3')	Na^+ 0.11M	K^+ 0.11M	K^+ 0.05M	NH_4^+ 0.11M	Na^+	K^+	NH_4^+
TGGGGGT	9.8 10^9	2.3 10^{12}	5.3 10^{10}	1.1 10^9	≥ 90	≥ 90	≥ 90
T**P**GGGGT	6.5 10^{10}	*too fast*	2.7 10^{11}	1.1 10^{10}	65.5	≥ 90	≥ 90
T**Q**GGGGT	*nd*	7.8 10^9	*ts*	*nd*	61.5	≥ 90	*nd*
TG**P**GGGT	1.4 10^{11}	*too fast*	3.4 10^{11}	2.1 10^{10}	77.5	≥ 90	≥ 90
TGG**P**GGT	3.7 10^{10}	*too fast*	6.6 10^{10}	1.7 10^{10}	60	≥ 90	≥ 90
TGGG**P**GT	5.6 10^9	1.3 10^{11}	*ts*	5.5 10^8	56.5	≥ 90	≥ 90
TGGGG**P**T	2.8 10^9	2.7 10^{10}	*ts*	1.7 10^8	59.5	≥ 90	77.5
TGGGG**Q**T	*nd*	2.4 10^9	*ts*	*nd*	*nd*	≥ 90	*nd*
TGGGGT	3.5 10^8	1.8 10^{10}	*ts*	1.2 10^8	57.5	≥ 90	77
T**P**GGGT	*nd*	6.0 10^{10}	*ts*	6.0 10^8	≤ 20	75.5	55.5
TGGG**P**T	*nd*	6.8 10^8	*ts*	5.1 10^7	≤ 20	56	28.5

Association rate constant at 3.5 ± 1°C, pH 7, with 0.11 M Na^+, or 0.11 M K^+ or 50 mM K^+. k_{on} is given with a ± 30% accuracy or better.

$T_{1/2}$ is the non-equilibrium melting temperature of the preformed quadruplex in 0.11 M Na^+ NH_4^+ or K^+, determined with a temperature gradient of 0.5°C/min.

nd: not determined.

Quadruplex formation with some of the **P**-containing oligonucleotides was so fast in potassium that we had to decrease ionic strength to obtain reliable data (not shown). Varying the potassium concentration has a dramatic effect on the association process: lowering K^+ concentration from 110 to 50 mM lowered k_{on} ≈ 40-fold, allowing us to slow quadruplex formation.

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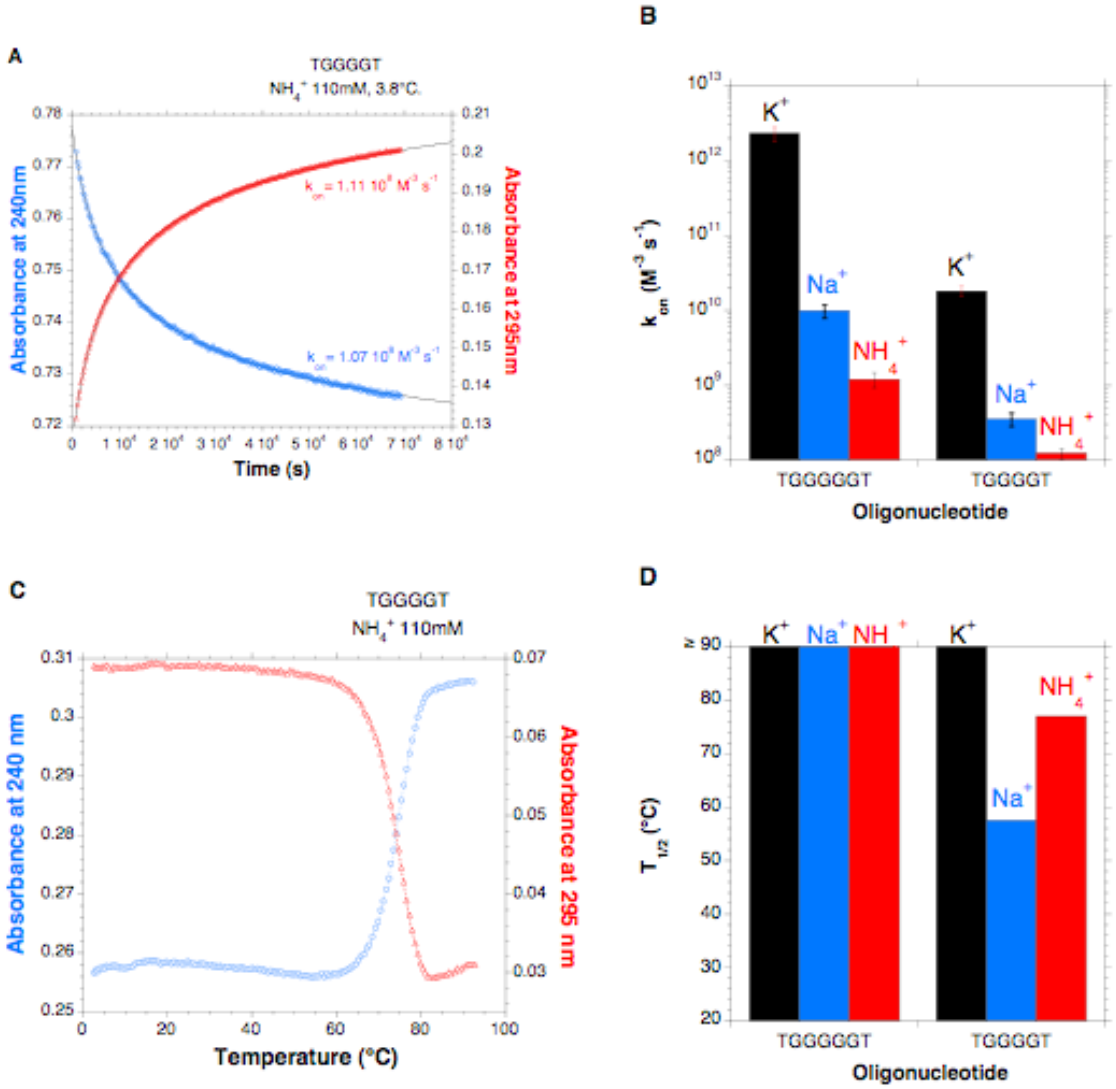

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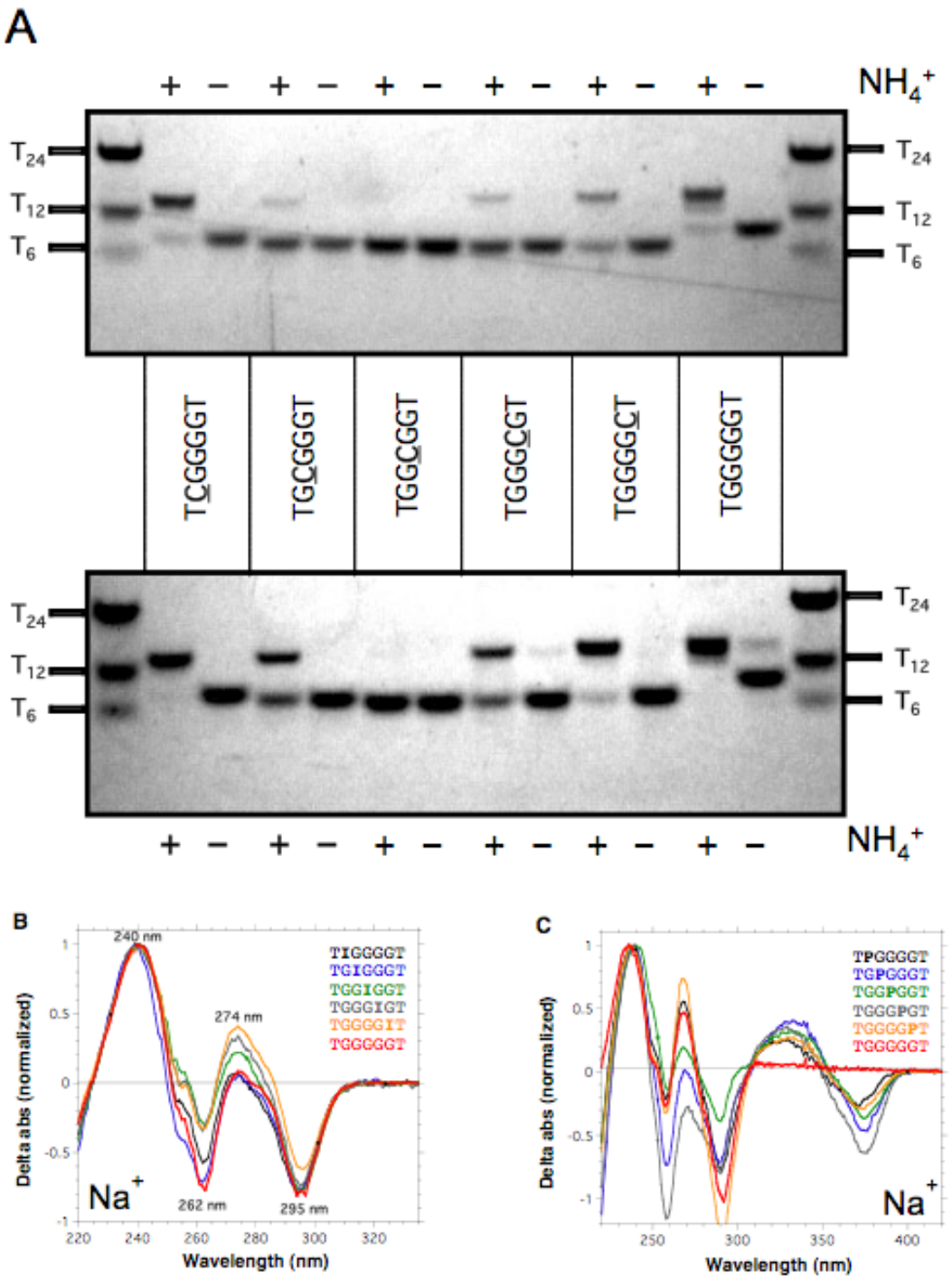

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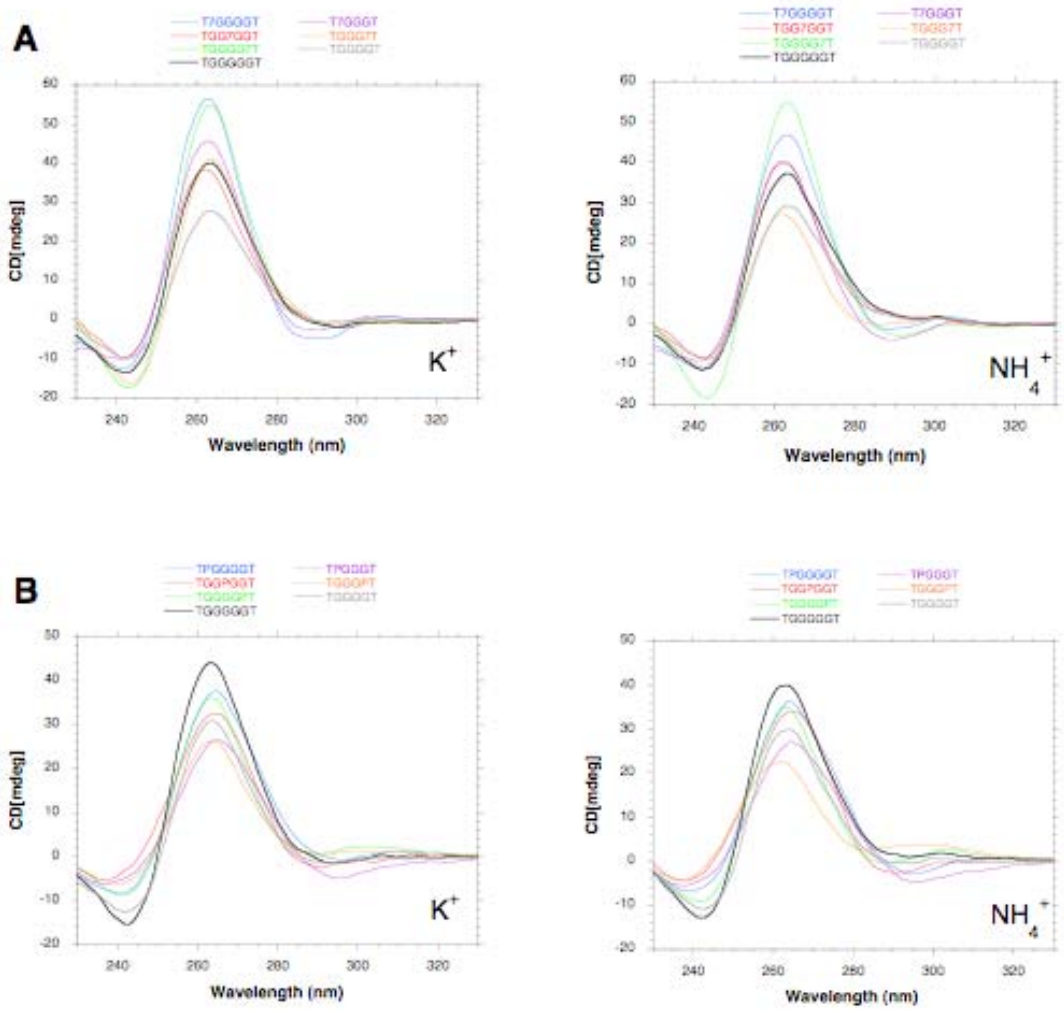

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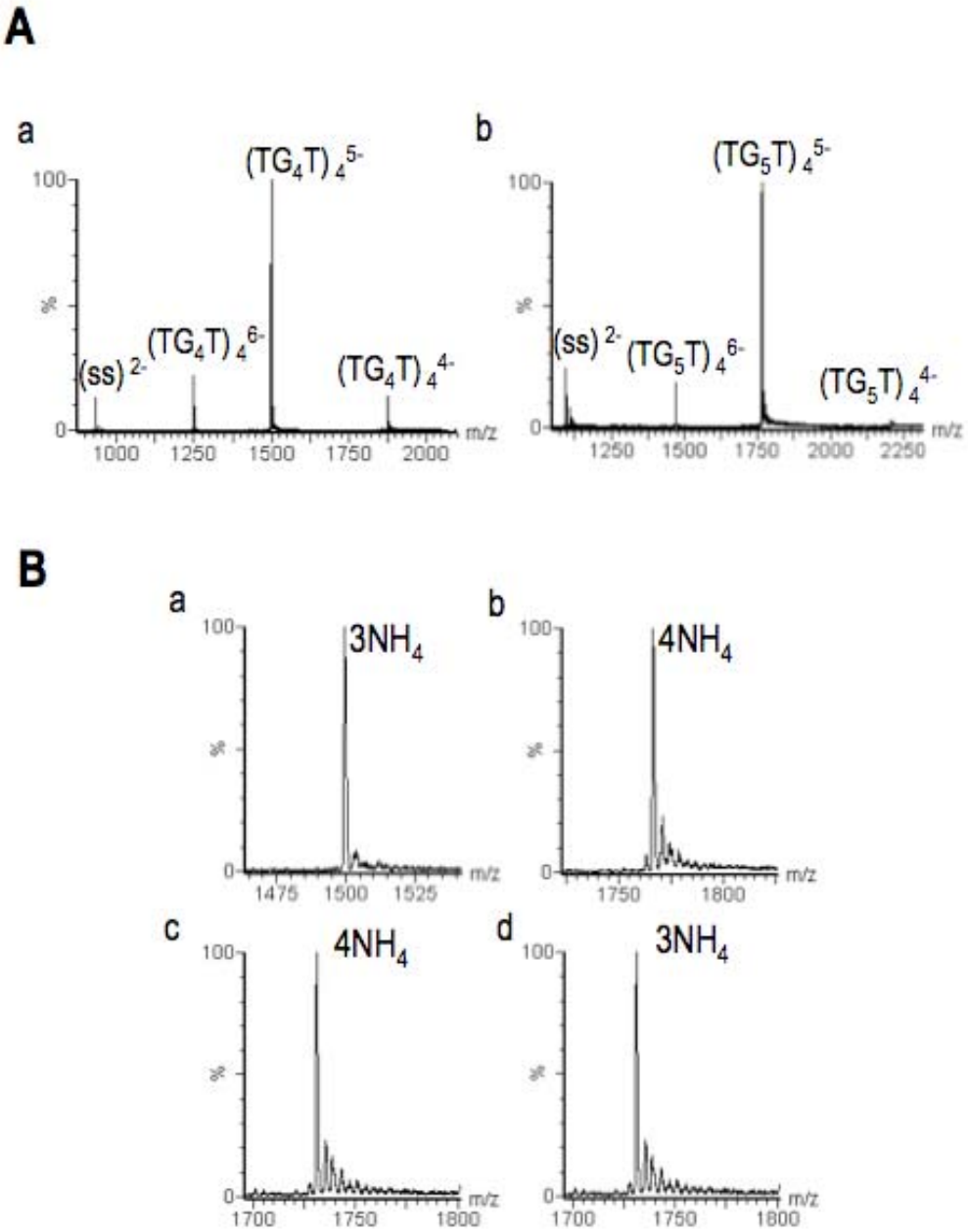

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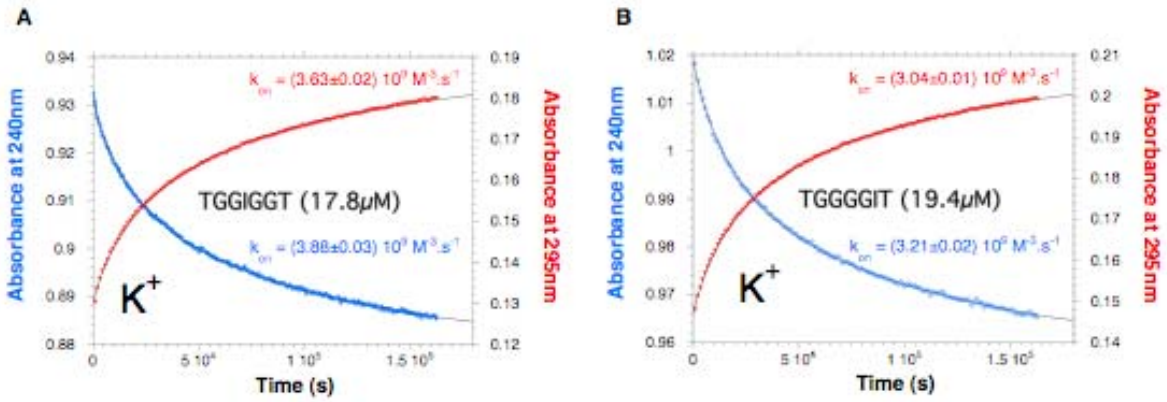

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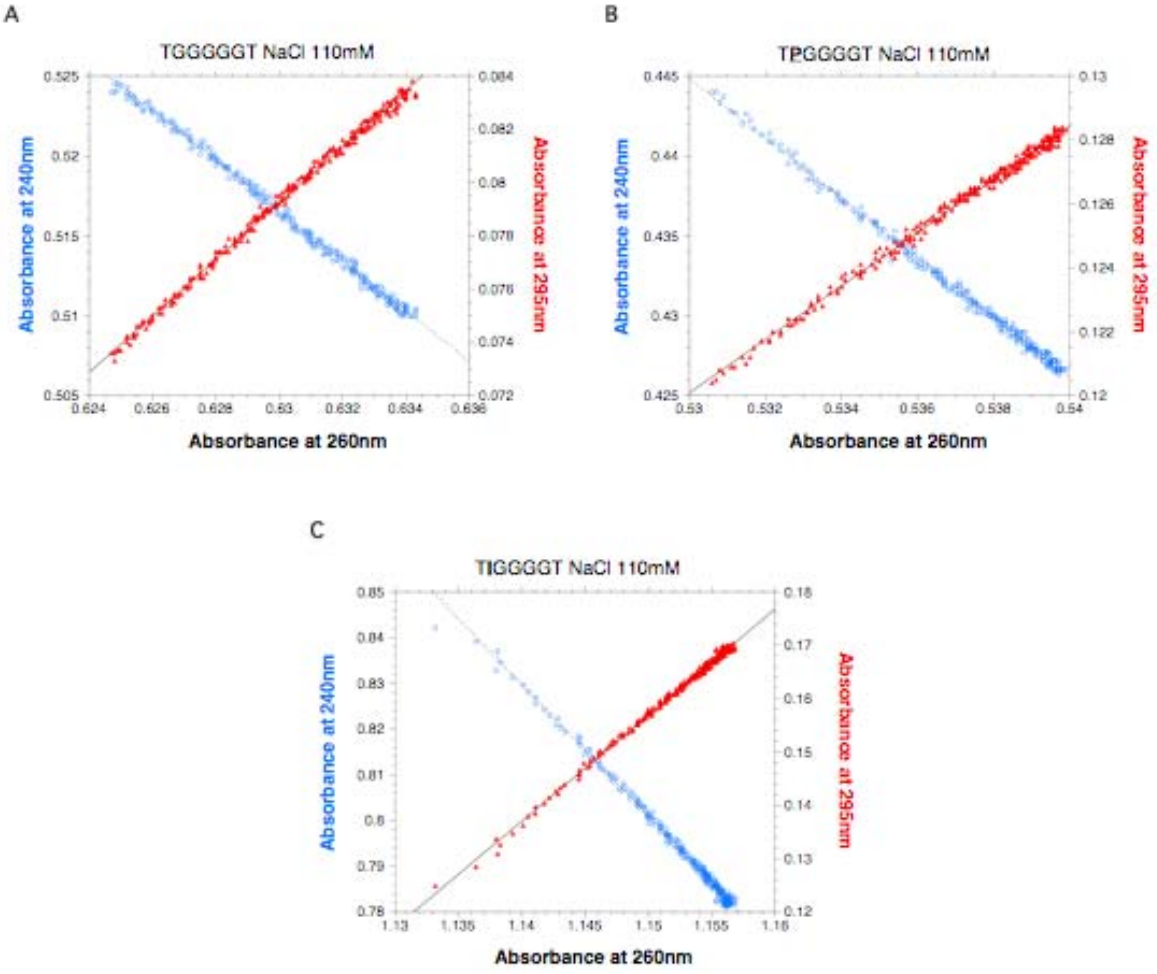

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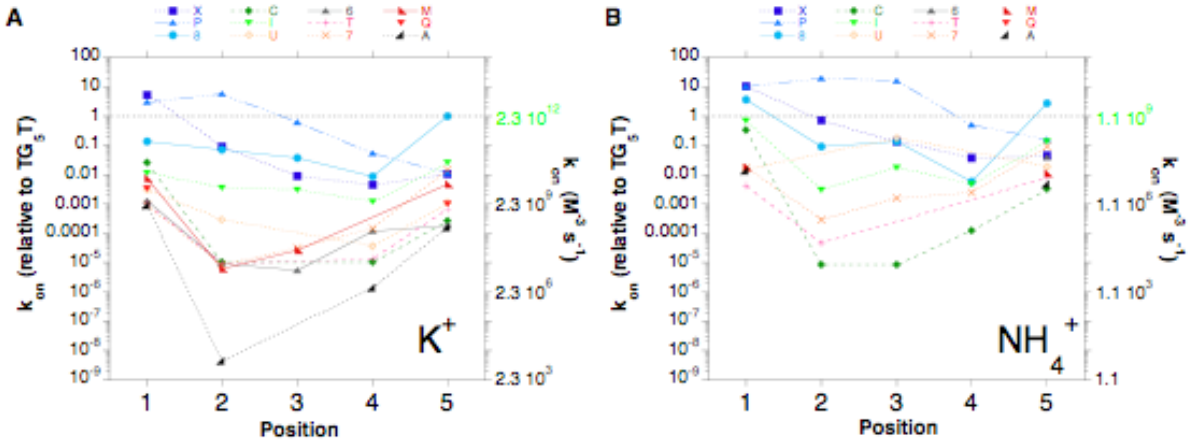

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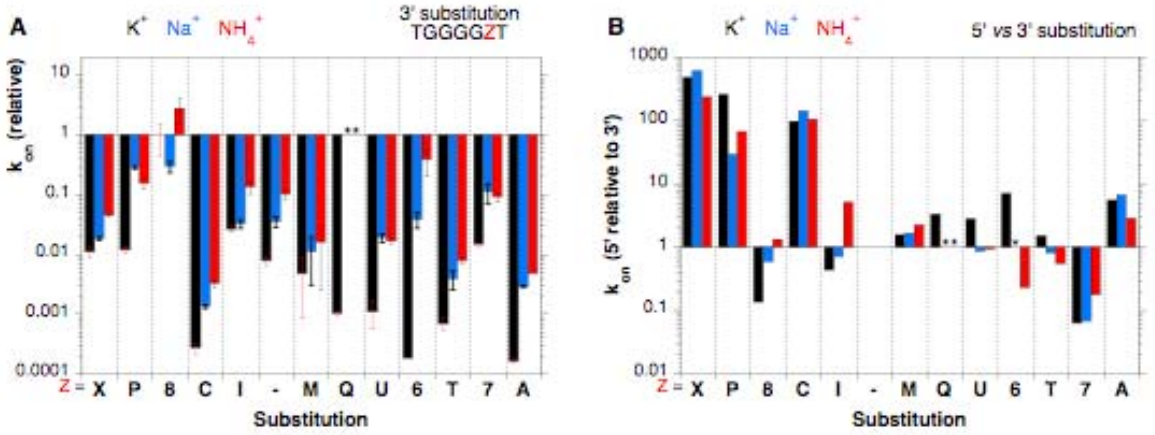

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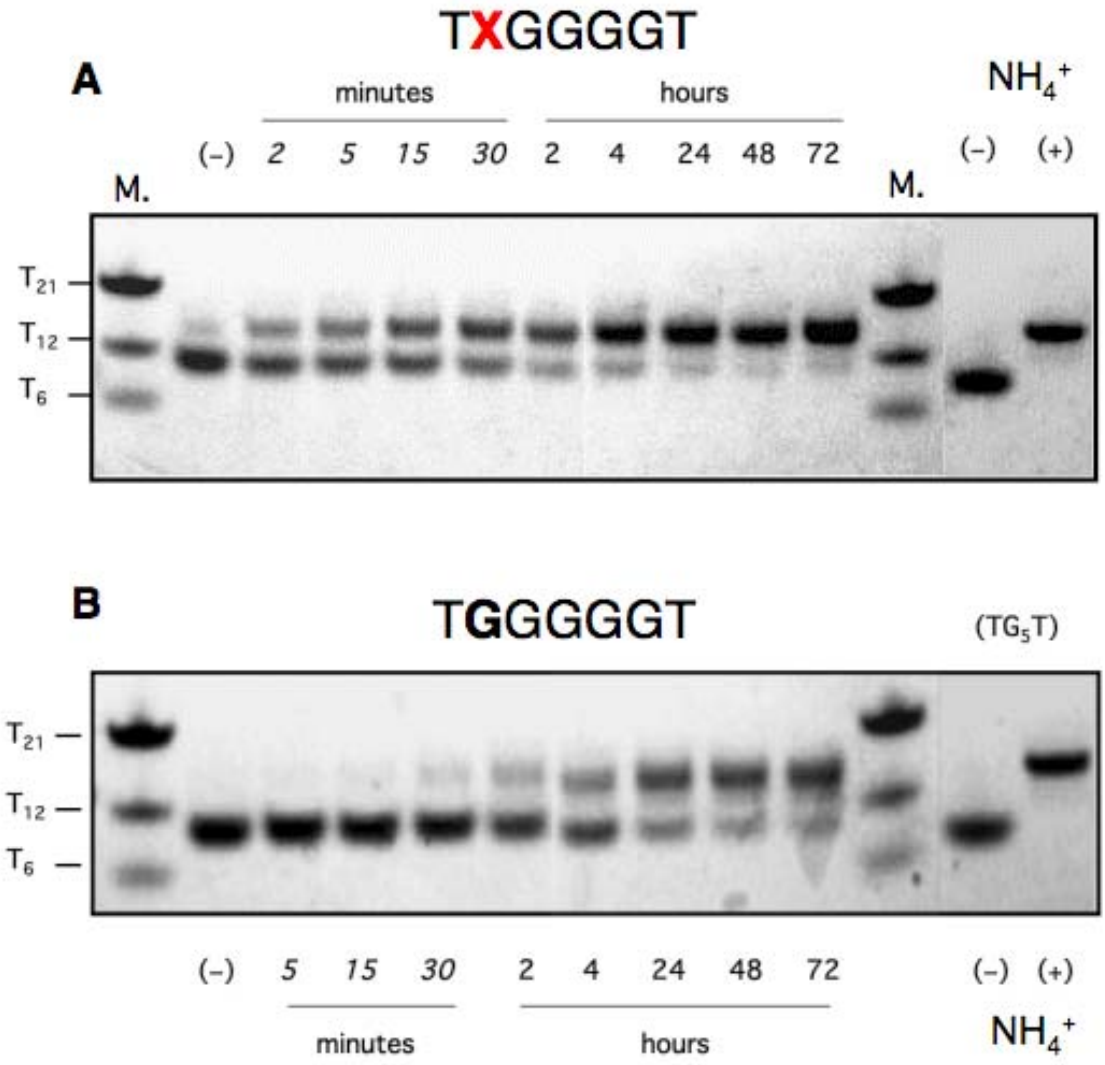

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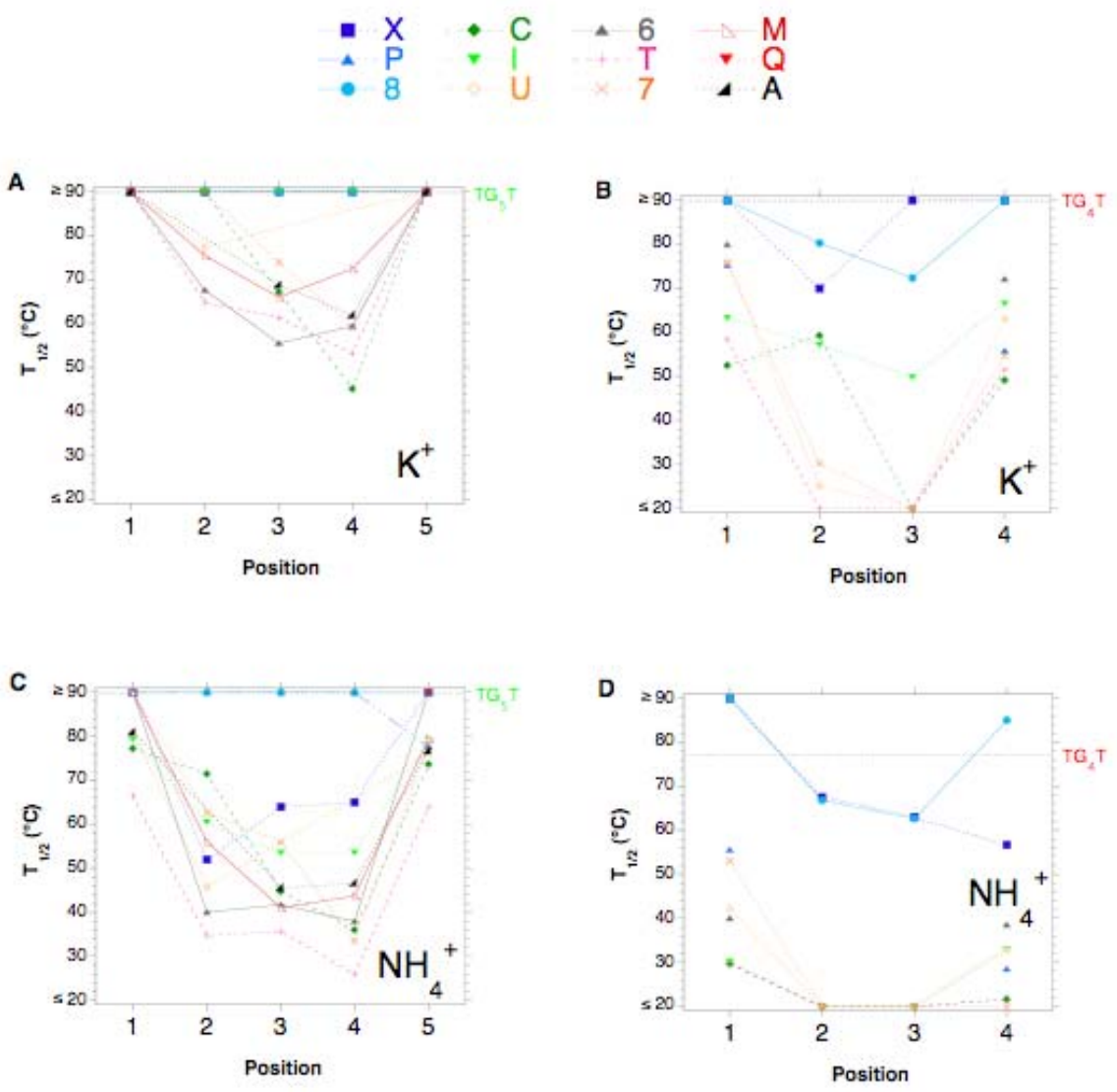

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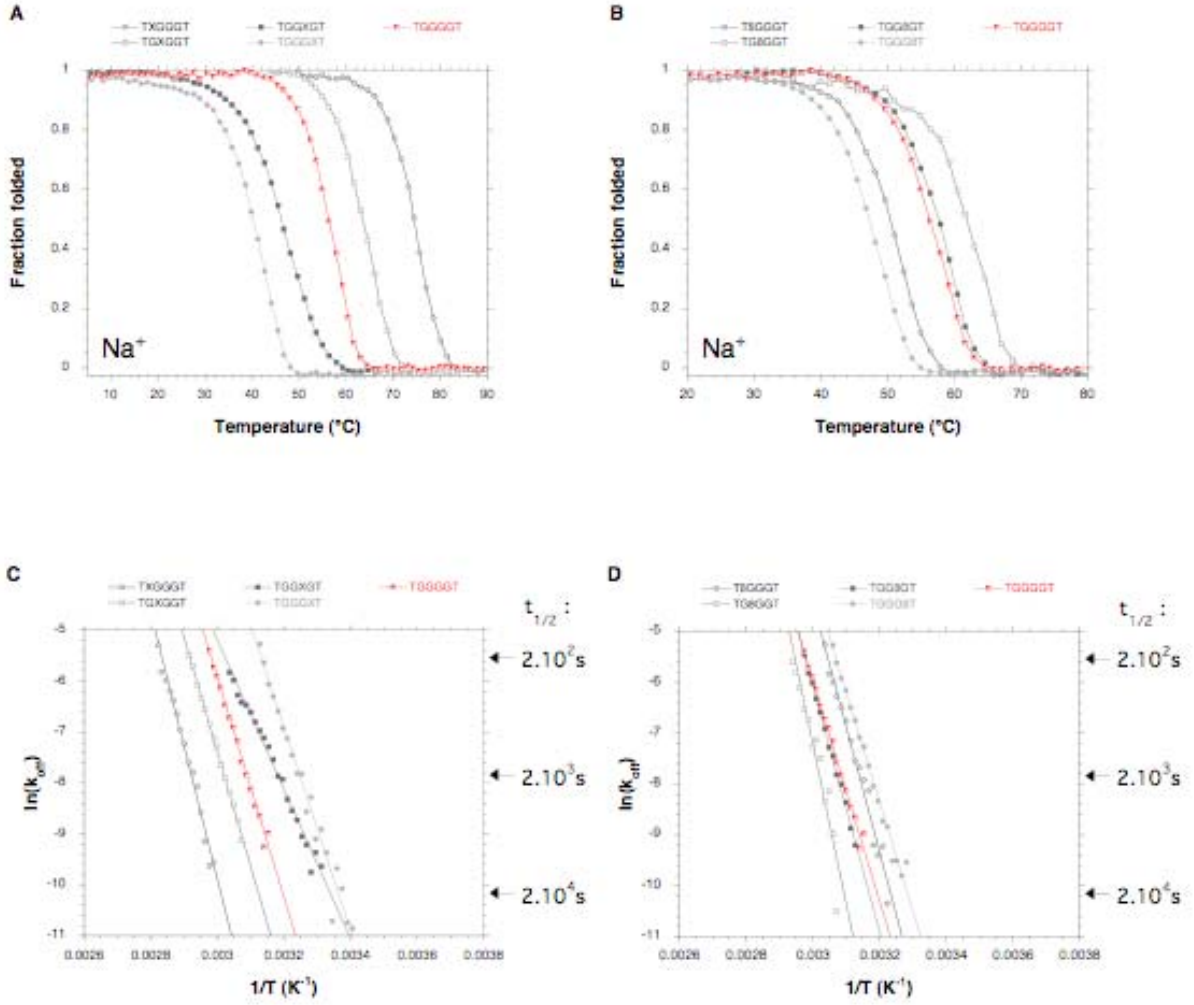

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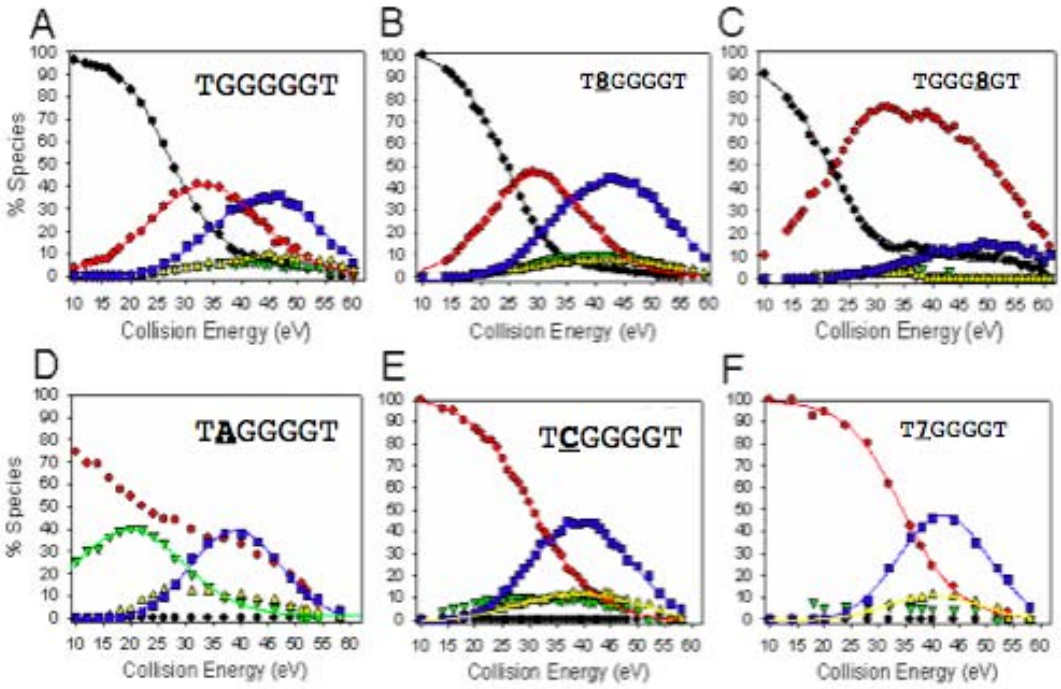

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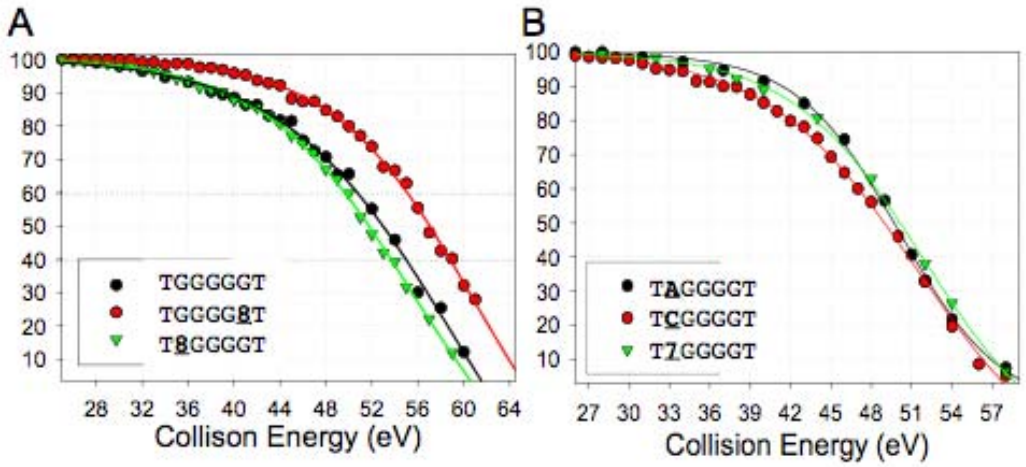

Supplementary figure S13