Supplementary Material (ESI) for Chemical Communications

Electronic supplementary information for the communication

Dynamic Covalent Chemistry on Self-Templating PNA Oligomers – Formation of a Bimolecular PNA Quadruplex.

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Synthesis and Characterization of G_{SH} and T_{SH}:

PNA oligomers were synthesized on tentagel resin with a Wang linker preloaded with Nε,t-Boc-Lysine using standard protocols employing Fmoc chemistry. [(a) L. Christensen, R. Fitzpatrick, B. Gildea, K. H. Petersen, H. F. Hansen, T. Koch, M. Egholm, O. Buchardt, P. E. Nielsen, J. Coull, R. H. Berg, *J. Peptide Sci.*, 1995, **2**, 175 (b) T. Koch, H. F. Hansen, P. Anderson, T. Larsen, H. G. Batz, K. Otteson, H. Orum, *J. Peptide Sci.*, 1997, **49**, 80-88].

DCC Control Experiments:

(A) Oxidation of $G_{SS}G$ and T_{SH}.

In order to confirm that air oxidation of $\mathbf{G_{SH}}$ and $\mathbf{T_{SH}}$ yielded a thermodynamic product distribution, air oxidation was also conducted with 250 μM $\mathbf{G_{SS}G}$ and 500 μM $\mathbf{T_{SH}}$ in 50 mM Tris buffer, pH 7.4, 100 mM KCl. The oxidation was complete in 32 h, and showed a product distribution of 2 : 1 : 2 $\mathbf{G_{SS}G}$: $\mathbf{G_{SS}T}$: $\mathbf{T_{SS}T}$ as shown in ESI-Figure A. This was within error the same distribution obtained by the air oxidation of 500 μM $\mathbf{G_{SH}}$ and $\mathbf{T_{SH}}$.

Nanoelectrospray Ionisation Mass Spectrometry

Nano-ESI-MS was carried out on a Q-TOF-1 mass spectrometer (Micromass, Manchester, U. K.). Complexes of undeuterated **GssG** were prepared at a concentration of 250 uM **GssG** in Milli-Q water, heated to 90 ºC and slow cooled over 3 h to 4 ºC. Prior to injection the samples were diluted 20 fold and used for Nano-ESI-MS. The mass spectrum of undeuterated complex is shown in ESI Figure B(i). Complexes of fully deuterated $\mathbf{G_{SS}G}$ were prepared as described above, but in D_2O [ESI Figure B(iv)].

H/D and D/H exchange:

For H/D exchange experiments, the complex in H_2O was lyophilized and resuspended in an equal volume of D_2O at 4 ºC. An aliquot of the samples were diluted 20-fold in D_2O just prior to injection and used for Nano-ESI-MS [ESI Figure B(ii)]. For D/H exchange, the samples in D_2O were freeze-dried and resuspended in an equal volume of H_2O, diluted 20 fold with H_2O and subjected to nano-ESI-MS [ESI Figure B(iii)].

UV spectrophotometry

All UV measurements were carried out on a Varian Carey 1E UV/Visible Spectrophotometer at 305 nm. Samples were prepared as described in the main manuscript. Heating and cooling rates of 0.5 ºC/ min were employed. Although successive heating curves were super-imposable, cooling curves showed hysteresis characteristic of intermolecular quadruplexes. ESI-Figure C shows a few representative melting curves plotted as the fraction of unmelted quadruplex present as a function of temperature.

DCC Experiments

Partial Equilibration followed by Oxidation

500 μM $\mathbf{G_{SH}}$ and 500 μM $\mathbf{T_{SH}}$ in buffer were incubated for 10 h to facilitate quadruplex formation. A HPLC profile of the reaction mixture (Trace (ii), ESI-Figure D) indicates that 68% of $\mathbf{G_{SH}}$ has oxidized to $\mathbf{G_{SS}G}$ while $\mathbf{T_{SH}}$ is still unreacted. The trace (i) in Fig 4A (Main Manuscript) shows only the thiols $\mathbf{G_{SH}}$ and $\mathbf{T_{SH}}$ present in the mixture at this time (t = 10h). Sodium perborate was added to this mixture to a final concentration of 20 mM perborate. The HPLC profile of the resultant solution is shown in Trace (i), ESI-Figure D. The amount of disulphides present just prior to perborate oxidation is known from Trace (ii) ESI-Fig D. Thus, the net amount of disulphides formed by perborate oxidation of 1:3.2 $\mathbf{G_{SH}}$: $\mathbf{T_{SH}}$ can be obtained from the difference trace (iii), ESI-Fig D [Trace (i) – (ii)]. The total absorbances in Traces (i) and (ii) were the same within error. The negative peaks in Trace (iii), ESI-Fig. D indicate the ratio of reactant thiols ($\mathbf{G_{SH}}$: $\mathbf{T_{SH}}$ 1:3.2) and the positive peaks indicate the disulphides formed from 1.0:3.2 $\mathbf{G_{SH}}$: $\mathbf{T_{SH}}$. The trace (ii) in Figure 4A (main manuscript) shows the positive peaks of Trace (iii), ESI-Fig D.

Effect of Varying Equilibration times on DCC amplification

The experiment described above was carried out 500 μM $\mathbf{G_{SH}}$ and 500 μM $\mathbf{T_{SH}}$ in buffer which was incubated for various times (t) to facilitate quadruplex formation. Sodium perborate was added to the mixture after time t. The amplification of $\mathbf{G_{SS}G}$ relative to $\mathbf{G_{SS}T}$ is plotted as a function of equilibration time t as shown in ESI-Figure-E.

Amplification of GssG increases along with increase in t. However there is negliglible increase in amplification after t = 8h. This suggests that pre-organization of $\mathbf{G_{SH}}$ is complete by this time.

Kinetics of DCC on PNA Quadruplexes

The rates of disappearance of thiols $\mathbf{G_{SH}}$ and $\mathbf{T_{SH}}$ was monitored individually in buffer at initial concentrations of 500 μM $\mathbf{G_{SH}}$ or $\mathbf{T_{SH}}$. The rate of disappearance of both $\mathbf{G_{SH}}$ and $\mathbf{T_{SH}}$ was also monitored when they were taken as a 1:1 mixture (equimolar $\mathbf{G_{SH}}$ and $\mathbf{T_{SH}}$ at 500 μM each). Thiol oxidation is known to be bimolecular at low thiol concentrations and unimolecular in thiol at high thiol concentrations. In our hands, at concentrations of 500 μM – 1mM thiol, oxidation is bimolecular in thiol. 500 μM $\mathbf{G_{SH}}$ and $\mathbf{T_{SH}}$ showed similar oxidation rates, with bimolecular rate constants shown in ESI-Table 1. As a 1:1 mixture, between t = 0 -24 h, mainly $\mathbf{G_{SH}}$ is consumed first, with a bimolecular rate constant of 1.29×10^{-4} $\mu M h^{-1}$. However, the reaction is bimolecular in thiol because $\mathbf{G_{SH}}$ can react either with another molecule of $\mathbf{G_{SH}}$ or a molecule of $\mathbf{T_{SH}}$ to form a disulphide. Thus, the total initial thiol concentration becomes important and is 1mM in this case (including 500 μM $\mathbf{T_{SH}}$). Therefore the reaction rate of $\mathbf{G_{SH}}$ (when it is present as a 1:1 mixture with 500 μM $\mathbf{T_{SH}}$) roughly doubles compared to 500 μM $\mathbf{G_{SH}}$ or $\mathbf{T_{SH}}$ on their own (Figure 2B). From t = 24 h, only ~500 μM $\mathbf{T_{SH}}$ is present in the reaction mixture of 1:1 $\mathbf{G_{SH}}$: $\mathbf{T_{SH}}$, which now starts oxidizing. The reaction rate of $\mathbf{T_{SH}}$ oxidation now reverts to that obtained when dealing with only 500 mM thiol in buffer. The obtained bimolecular rate constants (k) are shown in ESI-Table 1. Because disulphide exchange occurs much faster ($k \sim 10^3$-10^4 $M^{-1}h^{-1}$)(R. E. Cappel and H. F. Gilbert, *J. Biol. Chem*., 1988, **263**, 12204) than thiol oxidation ($k \sim 10^{-4}$ $M^{-1}h^{-1}$), $\mathbf{T_{SH}}$-related disulphides serve as disulphide carriers to $\mathbf{G_{SH}}$ molecules thus allowing the system to continuously equilibrate while approaching the end point.

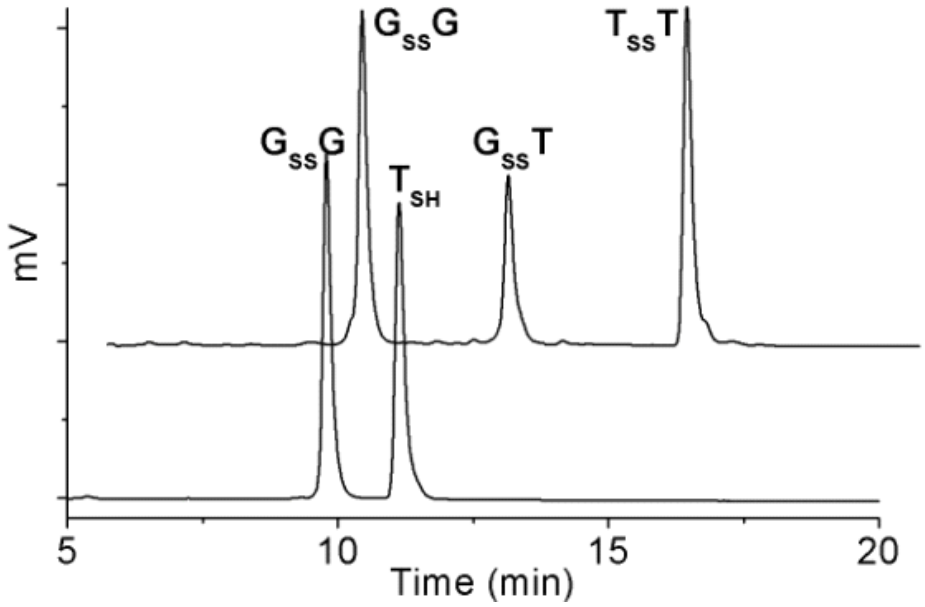
ESI - Table 1. Rate constants[a] of oxidation of 500 μM $\mathbf{G_{SH}}$ and $\mathbf{T_{SH}}$ separately and as a 1:1 mixture in buffer, pH 7.4.

Thiol	Rate constant, k ($\times 10^{-4}$ $\mu M^{-1}h^{-1}$)	Thiol	Rate constant, k ($\times 10^{-4}$ $\mu M^{-1}h^{-1}$)
$\mathbf{G_{SH}}$[b]	1.29 ± 0.08	$\mathbf{G_{SH}}$[d]	1.18 ± 0.09
$\mathbf{T_{SH}}$[c]	1.33 ± 0.12	$\mathbf{T_{SH}}$[d]	1.36 ± 0.11

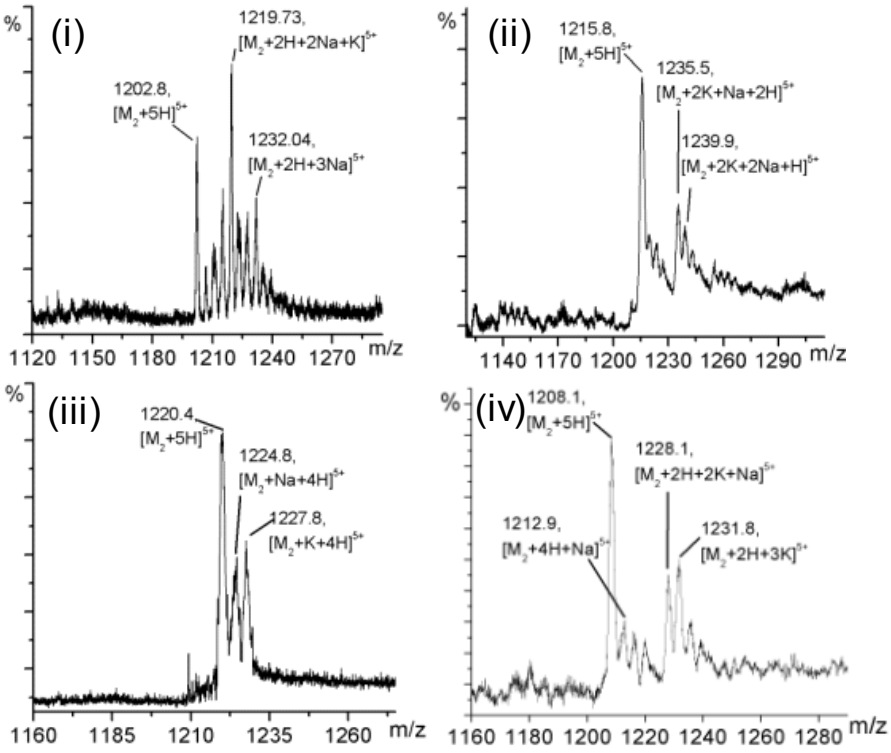
[a] Rate constants, k, were calculated (H. Kuhn, and H.-D. Försterling, in *Principles of Physical Chemistry*, 2000, John Wiley & Sons Ltd, pp. 677-678) assuming oxidation to be bimolecular in thiol, i.e., $[Thiol]_0 = [\mathbf{G_{SH}}]_0+[\mathbf{T_{SH}}]_0$. k is calculated from the slope of the line from a plot of $1/[\mathbf{G_{SH}}]_t$ or $1/[\mathbf{T_{SH}}]_t$ versus time, t. Also $t_{1/2} = (k.[Thiol])^{-1}$; [b] [Thiol] = 1mM as $[\mathbf{G_{SH}}] = [\mathbf{T_{SH}}]$ = 500 μM at t = 0; [c] [Thiol] = 500 μM as at t = 24, $[\mathbf{G_{SH}}]$ = 0 μM, $[\mathbf{T_{SH}}]$ = 500 μM; [d] Oxidation of either $\mathbf{G_{SH}}$ or $\mathbf{T_{SH}}$ at 500 μM in buffer.

Kinetics of Quadruplex formation probed by DCC amplification:
Tetramolecular quadruplex formation is relatively slow with PNA and typically requires a 5-6 h equilibration period. Thus, when a 1:1 mixture of $\mathbf{G_{SH}}$ and $\mathbf{T_{SH}}$ is immediately subjected to perborate oxidation, a statistical product distribution is obtained (Main M/S Figure 2B). However, if the mixture is equilibrated for a time, t and then subjected to perborate oxidation, $\mathbf{G_{SS}G}$ amplification increases with increasing t (ESI Figure E). There is negligible increase in amplification after 8 h suggesting that PNA quadruplex formation is complete at the end of this period.

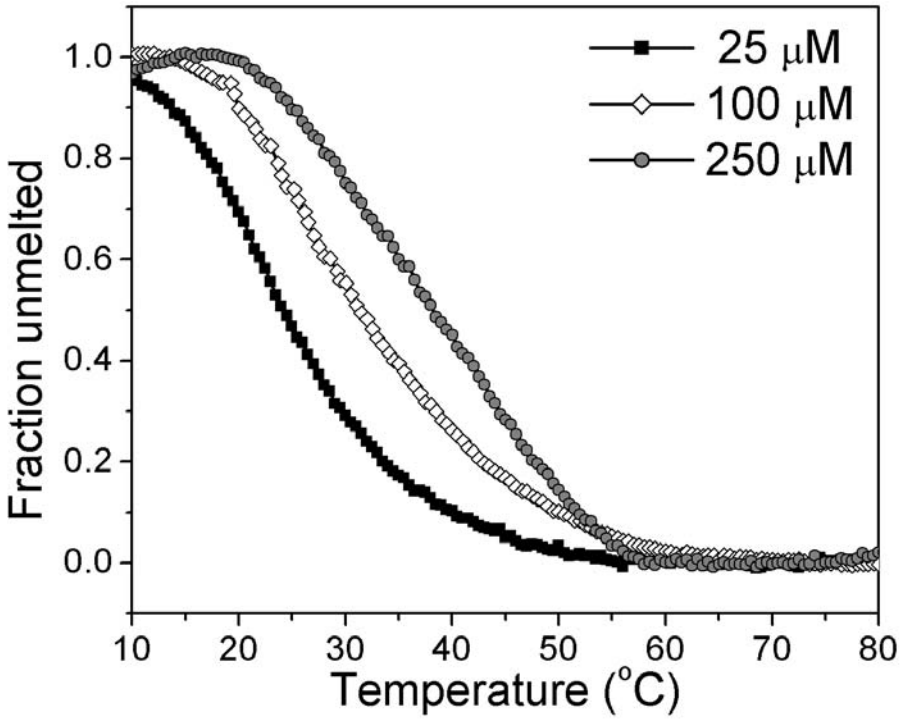
Circular Dichroism to address strand polarity in $(\mathbf{G_{SS}G})_2$
CD spectra were recorded on a JASCO J-810 circular dichroism spectrometer. 250 μM $\mathbf{(G_{SS}G)_2}$ in 100 mM Tris, pH 7.4, 100 mM KCl was prepared as described and spectra recorded between 350 nm and 200 nm and have been presented as an average of 20 successive runs. All spectra were corrected for buffer. The trace (ESI Figure F) shows a negative band centering at 260 nm and a positive band centered at 290 nm, resembling that obtained for a tetramolecular PNA_4 quadruplex and an antiparallel DNA quadruplex. For a more detailed discussion on the use of CD as a probe of strand polarity see P. J. Bates, *et al.*, *Nucleic Acids Res.*, 2003, **31**, 2097. The CD is weak compared to that of DNA quadruplexes. This is in accord with the CD of complexes of PNA duplexes (P. Wittung *et al.*, *J. Am. Chem. Soc.*, 1995, **117**, 10167) and quadruplexes (Y. Krishnan-Ghosh *et al.*, *J. Am. Chem. Soc.*, 2004, **126**, 5944). This is because the PNA backbone is achiral and the only sources of chirality are the terminal Lys and Cys. It appears that the PNA bimolecular quadruplex is antiparallel, and indeed such an arrangement would favourably position the charged N and C termini in $\mathbf{(G_{SS}G)_2}$. $\mathbf{(G_{SS}G)_2}$ could thus adopt an arrangement analogous to a chair or basket form of a bimolecular DNA quadruplex.



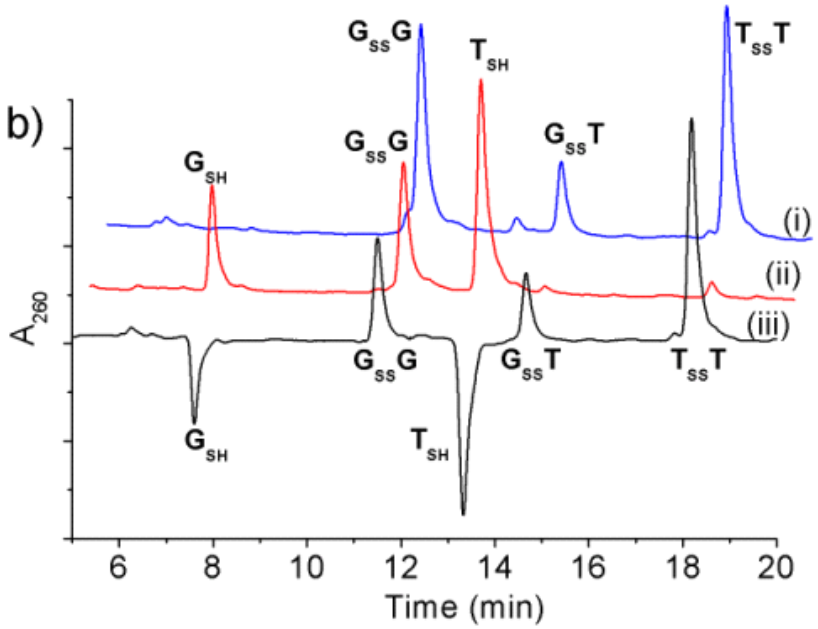
ESI-Figure A



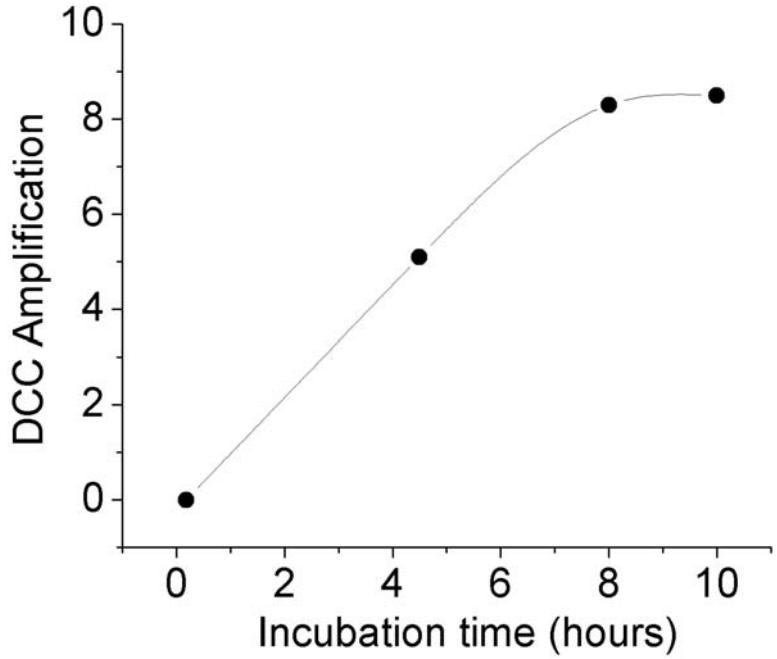
ESI-Figure B



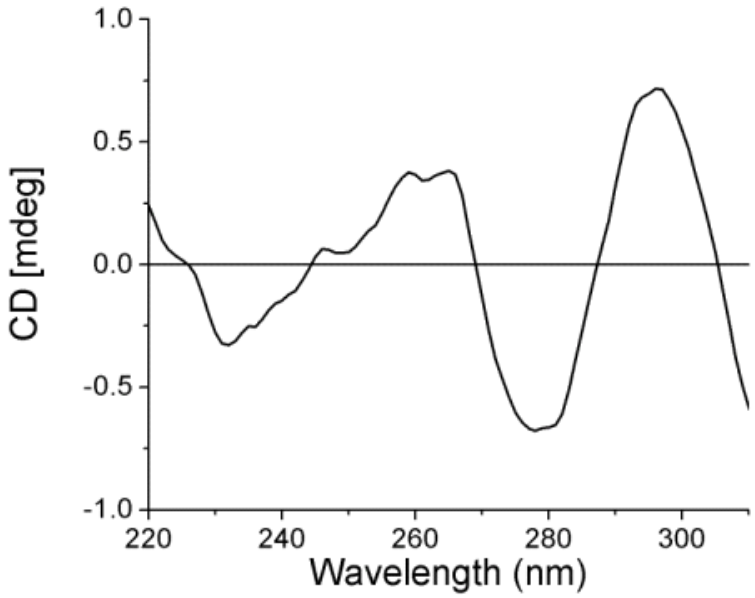
ESI-Figure C



ESI – Figure D



ESI-Figure E



ESI – Figure F