Electronic Supplementary Information for:

Magic-angle spinning NMR spectroscopy provides insight into the impact of small molecule uptake by G-quartet hydrogels

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S1. Multinuclear MAS NMR of G4•Li⁺, G4•Na⁺, G4•K⁺ and G4•Cs⁺ borate gels



Figure S1. Gel-state 1D MAS (5 kHz) NMR spectra acquired at 20 T; (a) $G4 \cdot Li^+$, (b) $G4 \cdot Na^+$, (c) $G4 \cdot K^+$ and (d) $G4 \cdot Cs^+$ borate gels. The narrow and broad signals are assigned to the free cations in the solution-like (sol) and cations bound to G4 networks (gel), respectively. Deconvolution of signal intensities enabled the quantification of cations in soland gel regions, which were found to be 31% and 69% in G4 · Na⁺ gels, and 47% and 53% in G4 · K⁺ gels, respectively.

S2. Calculation of isotropic ¹¹B chemical shifts and quadrupolar product

Nuclear spin interactions can be written by means of their Hamiltonians, with external terms including the interactions with the static magnetic field, \mathcal{H}_Z , radiofrequency pulses, \mathcal{H}_{RF} , and internal terms including chemical shielding, \mathcal{H}_{σ} , *J*-couplings, \mathcal{H}_J , dipole-dipole interactions, \mathcal{H}_D and quadrupolar interactions, \mathcal{H}_Q (spin > ½). The total Hamiltonian is written as,

$$\mathcal{H}_{Total} = \mathcal{H}_{RF} + \mathcal{H}_{Z} + \mathcal{H}_{\sigma} + \mathcal{H}_{J} + \mathcal{H}_{D} + \mathcal{H}_{Q} \tag{S1}$$

NMR interactions are most commonly expressed in terms of Cartesian tensors, for instance, magnetic shielding, σ , can be represented by a second rank tensor (3x3 matrix), diagonalization of the symmetric part of which yields information on its orientation described in terms of the principal axis system (PAS) relative to an external axes system. Chemical shielding, σ , tensors can be defined according to the Haeberlen conversion, $|\sigma_{ZZ} - \sigma_{iso}| \ge |\sigma_{XX} - \sigma_{iso}| \ge |\sigma_{YY} - \sigma_{iso}|$, where σ_{iso} is an average taken over the diagonal elements,

$$\sigma^{iso} = \frac{1}{3} \left(\sigma_{XX} + \sigma_{YY} + \sigma_{ZZ} \right) \tag{S2}$$

and the chemical shift anisotropy is defined as

$$\delta^{ANISO} = \sigma_{ZZ} - \sigma_{iso} = \frac{2}{3}\sigma_{XX} - \frac{1}{3}(\sigma_{XX} + \sigma_{YY})$$
(S3)

The asymmetry is defined as,

$$\eta = \frac{(\sigma_{YY} - \sigma_{XX})}{(\sigma_{ZZ} - \sigma_{iso})}$$
(S4)

Quadrupolar effects (herein ¹¹B, I = 3/2) arise due to interactions between a nuclear electric quadrupole moment, Q and the electric field gradient (EFG) at the nucleus. Similar to the case of the shielding tensor, the EFG tensor may also be diagonalized to provide the principal components (V_{XX} , V_{YY} and V_{ZZ}) of the tensor and orientation of the PAS. The quadrupolar interactions can be defined using two parameters, the quadrupolar coupling constant, C_Q and the asymmetry parameter, η_Q

$$C_Q = \frac{eV_{ZZ}Q}{h} \tag{S5}$$

where e is the fundamental charge and h is Planck's constant.

$$\eta_Q = \frac{(V_{XX} - V_{YY})}{V_{ZZ}} \tag{S6}$$

The quadrupolar product is defined as

$$P_Q = C_Q \sqrt{\left[1 + \frac{\eta_Q^2}{3}\right]} \tag{S7}$$

The experimental shift single-quantum shift, δ_{expt} is given as (for I = 3/2),¹⁻³

$$\delta_{expt} = \delta_{iso}^{CS} - \frac{1}{40} \left(\frac{P_Q}{v_L}\right)^2 10^6 \tag{S8}$$

where δ_{iso}^{CS} is the isotropic chemical shift (ppm) and v_L is the Larmor frequency (Hz). For mono and diester signals of G4•M⁺ (M= Li, Na, K and Cs) borate gels, the δ_{expt} values are plotted as a function of $\left(\frac{1}{v_L}\right)^2$. The δ_{iso}^{CS} values are obtained from the graphical plots by extrapolating ¹¹B shifts (Figures 2 and S2) and P_Q values are calculated by using the gradient (Table S1),

$$Gradient = -\frac{1}{40} P_Q^2 \ 10^6 \tag{S9}$$

$$P_o = 0.00632\sqrt{Gradient} \tag{S10}$$



Figure S2. Gel-state 1D ¹¹B MAS (5 kHz) spectra of GB•M⁺ (M= Li, Na, K and Cs) borate gels acquired at different magnetic fields, 11.7 T (black), 14.1 T (green) and 20 T (red).

Table S1. Magnitude of gradient extracted from the graphical plots (Figures 2 and S2) of δ_{expt} versus $\left(\frac{1}{v_L}\right)^2$ and P_Q values obtained from Eq. S10 for the mono and diester signals of different GB•M⁺ borate gels.

| Gel | GB monoest | er (6-8 ppm) | GB diester (1 | .1-14 ppm) | GB diester (1 | .1-14 ppm) |
|---------------------|-------------------------------------|--------------|-------------------------------------|------------|-------------------------------------|------------|
| | Gradient | PQ | Gradient | PQ | Gradient | PQ |
| | (10 ¹⁶ Hz ²) | (MHz) | (10 ¹⁶ Hz ²) | (MHz) | (10 ¹⁶ Hz ²) | (MHz) |
| G4∙Li⁺ borate gel | 1.49 | 0.77 | 2.67 | 1.03 | 2.43 | 0.99 |
| G4•Na⁺ borate gel | 1.50 | 0.77 | 1.46 | 0.76 | 2.43 | 0.99 |
| G4∙K⁺ borate gel | 1.81 | 0.85 | 2.13 | 0.92 | 1.97 | 0.89 |
| G4∙K⁺ gel: 3 mM MB | 1.69 | 0.89 | 2.12 | 0.92 | 1.97 | 0.89 |
| G4∙K⁺ gel: 36 mM MB | 2.09 | 0.91 | 3.74 | 1.22 | 2.75 | 1.05 |
| G4•Cs⁺ borate gel | 1.69 | 0.82 | 1.97 | 0.89 | 2.75 | 1.05 |





Figure S3. DFT optimized structures of (a) GB monoester, and GB diesters in (b) *cis* and (c) *trans* conformations are shown in top view and side view representations of purine rings.



Figure S4. A plot of experimental ¹¹B isotropic chemical shifts for GB•Na⁺ (hollow circles) and GB•K⁺ gels (filled circles) against DFT calculated ¹¹B isotropic absolute shielding.

S4. Absorption of adenine into G4•Na⁺ borate gels

Hot saturated solutions of $G4 \cdot Na^+$ gels prepared in D₂O were pipetted into vials containing increasing concentrations of adenine (Table S2). The guanosine concertation was 72 mM. Each vial was then reheated and sonicated until the solid adenine dissolved and the resultant solution was allowed to cool to room temperature. A 'tap test' was carried out whereby each gel was given a sharp tap and then inverted to observe whether it remained stable. 256 mM adenine did not dissolve in a potassium borate gel hence it was not tested in the sodium borate gel. Adenine at 128 mM dissolved in both but started precipitating from potassium borate after cooling, and precipitated in the sodium borate gel after 24 hours. A modest increase in adenine solubility can be achieved in 72 mM G \cdot B(OH)₄Na gel, although at 64 mM adenine it is close to the practical solubility limit.



Figure S5. Photograph (left) of inverted vials of G4•Na⁺ borate gels taken after 24 hours with added adenine at concentrations of (a) 8, (b) 16, (c) 20, (d) 24, (e) 32 and (f) 64 mM. Solution-state ¹H (400 MHz) (middle) and ¹¹B (160.5 MHz) MAS NMR spectra (right) adenine added gels, indicating subtle (or no) changes in the chemical shifts, although there is an increase in the adenine ¹H signal intensities.

| adenine (mM) | Fresh gels | After a week |
|--------------|---------------------------------|-------------------------------|
| 8 | Stable and clear | Stable and clear |
| 16 | Stable and clear | Stable and clear |
| 20 | Stable and clear | Stable and clear |
| 24 | Stable and clear | Stable and clear |
| 32 | Stable and clear | Stable and clear |
| 64 | Stable gel, but slightly cloudy | Little precipitation occurred |
| 128 | Stable gel, but slightly cloudy | Precipitation occurred |
| 256 | Did not dissolve completely | Precipitation occurred |

Table S2. Solubility assay of adenine in G4•Na⁺ borate hydrogel monitored over a week.

S5. Absorption of cytosine into G4•Na⁺ borate gels

Hot saturated solutions of $G4 \cdot Na^+$ gels were prepared in D_2O and pipetted into vials containing increasing concentrations of cytosine (Table S3). Each vial was then reheated and sonicated until the solid cytosine dissolved and the resultant solution was allowed to cool to room temperature and solubility was monitored over a week. A modest increase in cytosine solubility can be achieved in a 24 mM $G \cdot B(OH)_4Na$ gel, although 32 mM cytosine is close to the practical solubility limit.



Figure S6. Photograph (left) of inverted vials of G4•Na⁺ borate gels taken after 24 hours with added cytosine at concentrations of (a) 8, (b) 16, (c) 20, (d) 24, (e) 32 and (f) 64 mM. Solution-state ¹H (400 MHz) (middle) and ¹¹B (160.5 MHz) MAS NMR spectra (right) cytosine added gels, which indicate subtle (or no) changes in the spectral patterns, but cytosine ¹H signal intensities increase as a function of concentration.

| cytosine (mM) | Fresh gels | After a week |
|---------------|--|------------------------|
| 8 | Stable and clear | Stable and clear |
| 16 | Stable and clear | Stable and clear |
| 20 | Stable and clear | Stable and clear |
| 24 | Stable and clear | Stable and clear |
| 32 | Stable and clear | Little precipitation |
| 64 | Stable and clear | Little precipitation |
| 128 | Stable and clear | Precipitation occurred |
| 256 | Stable gel, but precipitation occurred | Precipitation occurred |

Table S3. Solubility of cytosine in G4•Na⁺ borate hydrogel monitored over a week.

S6. Absorption of 1-methylthymine into G4•Na⁺ borate gels

Hot saturated solutions of $G4 \cdot Na^+$ gels prepared in D_2O and pipetted into vials containing increasing concentrations of 1-methylthymine (Table S4). Each vial was then reheated and sonicated until the solid 1-methylthymine dissolved and the resultant solution was allowed to cool to room temperature. A 'tap test' was carried out whereby each gel was given a sharp tap and then inverted to observe whether it remained stable. The addition of 1-methylthymine over 50 mM concentration did not dissolve in either sodium or potassium borate gel.



Figure S7. Photograph (left) of inverted vials of G4•Na⁺ borate gels taken after 24 hours with added 1-methylthymine at concentrations of (a) 21.4, (b) 28.5, (c) 35.8, (d) 42.8, and (e) 50 mM. Solution-state ¹H (400 MHz) (middle) and ¹¹B (160.5 MHz) MAS NMR spectra (right) 1-methylthymine added gels indicate subtle changes in the spectral patterns, but 1-methylthymine ¹H signal intensities increase as a function of concentration.

Table S4. Solubility of 1-methylthymine in G4•Na⁺ borate hydrogel monitored over a week.

| 1-methylthymine (mM) | Fresh gels | After a week | |
|----------------------|--|--------------------------------------|--|
| 21.4 | Solution was clear | Solution was clear | |
| 28.5 | Solution was clear | Solution was clear | |
| 35.8 | Gel was slow to set | Gel was stable | |
| 42.8 | Gel was slow to set | Gel was stable | |
| 50.0 | Gol was stable, and no precipitation occurred | Gel was stable, and no precipitation | |
| | Ger was stable, and no precipitation occurred | occurred | |
| | Gol was stable, and no presiditation occurred | Gel was stable, and no precipitation | |
| 57.1 | Ger was stable, and no precipitation occurred | occurred | |
| 64.2 | Columps stable, and no presidintation occurred | Gel was stable, cloudy, and little | |
| 04.2 | Ger was stable, and no precipitation occurred | precipitation occurred | |
| 71 / | Gol was stable, and no presiditation occurred | Gel was stable, cloudy, and little | |
| /1.4 | Ger was stable, and no precipitation occurred | precipitation occurred | |

References

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