Liquid Crystalline Phase of G-tetrad DNA for NMR Study of Detergent-Solubilized Proteins

Supporting Information

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Sample Preparation Protocol. The sodium salt of 2′-deoxyguanylyl(3′→5′)-2′-deoxyguanosine – d(GpG) was purchased from Sigma and used without further purification. Efforts are underway to have this material produced in bulk at much lower cost. The powder was dissolved in a buffered solution (25 mM K_2HPO_4, pH 8) at room temperature overnight, followed by mild vortexing to ensure homogeneity. KCl is added to a final concentration of 35 mM to ensure that the complexing potassium concentration is saturating: a 25 mg/ml solution of d(GpG) has a concentration of 40 mM, and a minimum of 20 mM K^+ cation is required to displace the sodium in the G-tetrad. The concentration of d(GpG) is monitored with a UV/Vis spectrophotometer at 260 nm, using the estimated absorbance of ~ 24.5 μg/ml for an $A_{260} = 1.0$. Dilutions of concentrated solutions of d(GpG) require 10-15 minutes to form monomeric d(GpG) and produce accurate absorbance measurements. The d(GpG) threshold concentration for liquid crystal formation increases with DPC concentration and decreases with the addition of K^+ (see Figure S3).

After addition of the protein, the solution is transferred to a Shigemi NMR tube. A uniform and bubble-free sample is obtained by slow centrifugation (80-100g) after transferring the sample to the tube, inserting the plunger to the bottom of the tube and pulling the plunger to the desired height. Sample alignment is confirmed by measuring the ^2H_2O residual quadrupolar splitting with the NMR spectrometer.

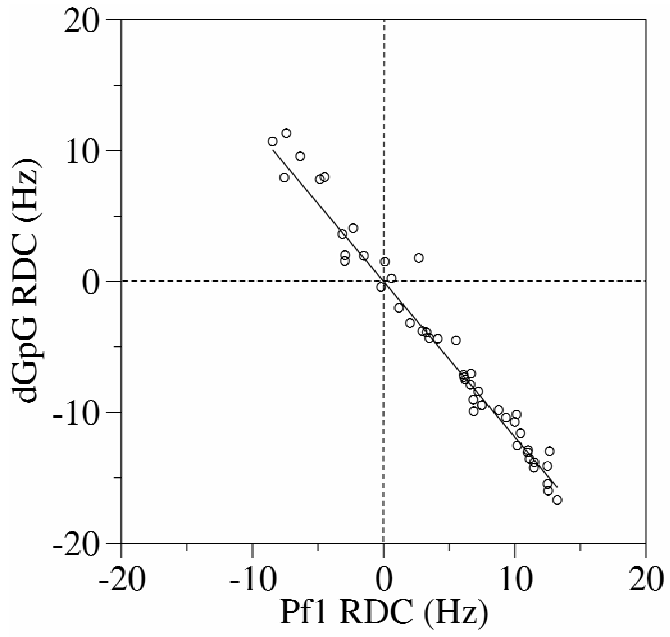

Figure S1. Correlation between the $^1D_{NH}$ RDCs from alignment in d(GpG) (25 mM K_2HPO_4 pH 7.4, 35 mM KCl, 22 mg/ml d(GpG), 25 °C) and Pf1 (22 mg/ml Pf1 [Asla biotech], 25 mM NaH_2PO_4, pH 6.7, 50 mM NaCl). The best-fit linear regression equation is: RDC(dGpG; Hz) = –1.19 * RDC(Pf1; Hz), corr = 0.987. The negative slope reflects the fact that Pf1 aligns with its cylinder axis parallel to the magnetic field, whereas G-tetrad columns orient orthogonal to the field.

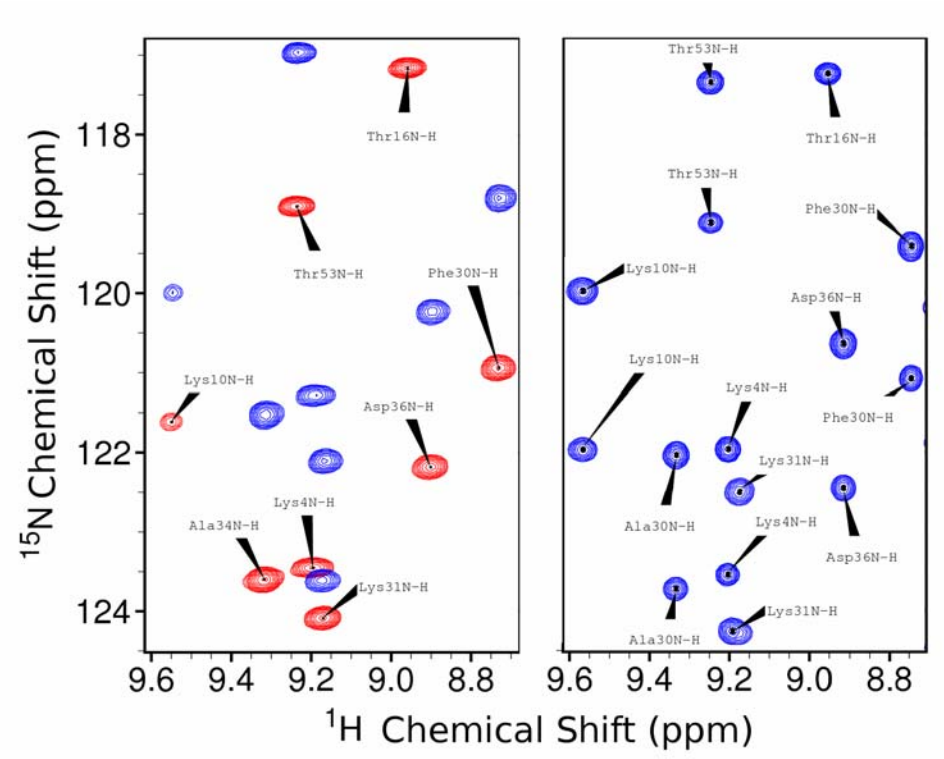

Figure S2. ^{1}H-^{15}N IPAP-HSQC spectrum of d(GpG) (left) and ^{1}H-^{15}N projection spectrum from an $^{1}J_{NH}$-coupled HNCO 3D spectrum of Pf1 (right) for U-{^{13}C,^{15}N}-[K19E,D40N,V42E]-GB3. The spectra containing the upfield (blue) and downfield (red) components of the ^{15}N-{^{1}H} doublets are superimposed, with the downfield component labeled. The $^{1}J_{NH}$-coupled HNCO 3D spectrum shows both doublet components in blue.

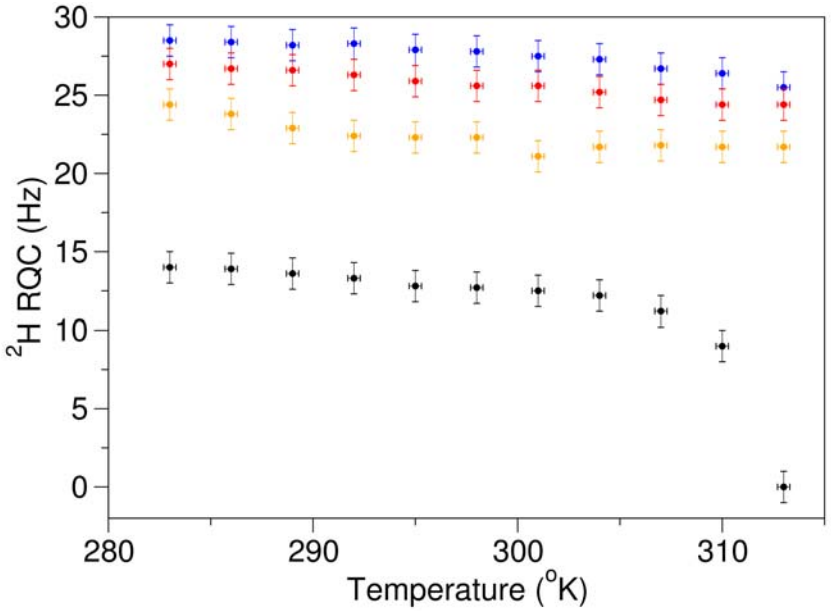

Figure S3. Liquid crystalline phase of d(GpG) under different conditions as monitored by the ^2H_2O deuterium RQC. Black: 12.5 mg/ml d(GpG), 25 mM K_2HPO_4 pH 8.0 and 12% ^2H_2O. Blue : 24.3 mg/ml d(GpG), 25 mM K_2HPO_4 pH 8.0 and 12% ^2H_2O. Red: 23.6 mg/ml d(GpG), 25 mM K_2HPO_4 pH 8.0, 35 mM KCl, 100 mM DPC and 12% ^2H_2O. Orange: 22.8 mg/ml d(GpG), 25 mM K_2HPO_4 pH 8.0, 70 mM KCl, 214 mM DPC and 12% ^2H_2O. No efforts were made to remove the Na^+ ions present in the purchased d(GpG) sample. Measurements were conducted on a Bruker DRX 600 MHz spectrometer.