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General d(GpG) Preparatory Notes

1. The d(GpG) powder includes water, salt, and organic impurities, and the gravimetric mass will underestimate the amount of d(GpG) added to the sample.
2. The d(GpG) concentration is estimated spectrophotometrically. 1ul of the d(GpG) solution is diluted in 1ml of mQ H_2O, and the A_{260} is monitored. An extinction coefficient of 24.5 $AU_{260}{\cdot}cm{\cdot}mg^{-1}ml$ is used.
3. The viscocity of a > 10mg/ml d(GpG) solution is quite high. Using a 2ul pipetman, pipetting a 1ul aliquot will yield less than the desired volume. When preparing a 1:1000 dilution for spectrophotometric measurement, the actual volume of the aliquot is estimated from the height of the solution in the pipet tip, and the outer surface of the pipet tip is wiped from residual d(GpG) solution. Gel loading tips are useful in properly estimating this volume. Typically, only 0.5-1.0ul of the solution is collected when aliquoting 1ul.
4. The kinetic process for d(GpG) break down is slow – typically 10-20 minutes, or longer. After making the 1:1000 dilution, the A_{260} is monitored every five minutes until it grows to a final, asymptotic value. This value is used to calculate the concentration of the d(GpG). Likewise, diluting a high concentration d(GpG) sample requires time and thorough vortexing for equilibration.
5. The d(GpG) is stored at -20^{o}C and the container is sealed to avoid H_2O contamination.

d(GpG) Sources

Sigma (Lot #012K1855)

1. Catalog # D0770-4MG
2. The spectrophotometric mass is ~65% of the gravimetric mass.
3. Dissolves over 3-4 hours in a microcentrifuge tube shaker @ 1000rpm and 35^{o}C.
4. This is the material used in the JACS 130, 7536 publication. As stated in the publication, compatibility was confirmed with DPC, LPPC and DHPC.
5. $^{2}H_2O$ RQCs as low as a ~5-8 Hz have been measured.

Rasayan (Lot #080418)

1. The spectrophotometric mass is 77% of the gravimetric mass.
2. Dissolves overnight at room temperature – the above protocol for the Sigma material is insufficient.
3. Compatibility was confirmed with DPC.

4. The material is highly alkaline (pH ≥10) and it will likely dominate the pH of a buffered solution. The pH is adjusted by adding microliters of 1% HCl and monitoring the pH with pH paper. The use of a pH electrode is complicated by the high macroscopic viscosity of the solution.
5. ^2H_2O RQCs as low as 1 Hz have been measured.

Rasayan (Lot #081002)

1. The spectrophotometric mass is 38% of the gravimetric mass.
2. Dissolves overnight at room temperature – the above protocol is insufficient.
3. The material is slightly acidic – pH ~6. The pH is adjusted by adding microliters of 0.1M NaOH and monitoring the pH with pH paper. The use of a pH electrode is complicated by the high macroscopic viscosity of the solution.
4. ^2H_2O RQCs from 25 Hz (~22mg/ml) to 1.5 Hz (~8mg/ml) have been measured. The sample consisted of the following components : 22mg/ml K-d(GpG) [Rasayan, Lot #080418], 25mM K_2HPO_4 pH 7.4, 75mM KCl and 5% D2O. This sample was diluted with dd H_2O to a final concentration of ~8mg/ml.

Protocol

Sample Preparation

1. The d(GpG) powder is weighed into a microcentrifuge tube and dissolved. It's preferred to add the K^+ after the solid has dissolved and the concentration has been measured. The K^+ salt of d(GpG) dissolves more slowly.
2. The d(GpG) is allowed to dissolve as noted in the previous section. The d(GpG) is white initially, and the solution becomes clear as it dissolves. The region of higher [d(GpG)] can be seen from the difference in the index of refraction in the solution.
3. The solution is homogenized by mixing with a vortex or a pipetman (see below). There should not be changes in index of refraction throughout the sample – or white solid – if the d(GpG) is completely dissolved. Solutions of >10mg/ml d(GpG) are macroscopically viscous, and they may require a few cycles of votexing and <6000g centrifugation.
4. The concentration of d(GpG) is measured spectrophotometrically, as described before, and before the addition of the K^+ (if possible).
5. The remaining sample components are added and mixed together.
6. The pH is monitored with pH paper and adjusted.
7. Alignment can be observed using crossed polarizers.

Pipettor and Sample Transfer

1. As a consequence of the macroscopic viscosity of the sample, transferring the sample to an NMR tube can be a challenge. A special pipet tip and centrifugation are used.
2. A pipet tip for easy transfer of the solution can be prepared for a 200ul pipetman using a 200ul pipet, some flexible ~1mm I.D. tubing and a 200ul glass capillary – the type used for blotting

samples on a TLC plate. A length of ~3-5cm of the tubing is cut and placed on the pipet tip. A glass capillary can be inserted on the other end of the tubing, and the assembly is used as a pipet tip on a 200ul pipetman. The glass capillary can deliver sample to the bottom of the NMR tube, and it can be replaced between samples.

3. The d(GpG) adheres to the microcentrifuge tube, and the tube should by centrifuged after each sample transfer.
4. The d(GpG) sample is centrifuged in the NMR tube. Centrifugation for 5-20 minutes at 100-200g is useful in removing bubbles from the sample.

Heterogeneous Alignment

1. The alignment may be heterogeneous or partly isotropic, as evidenced by the splitting of the HDO lock signal caused by residual quadrupolar coupling. This is usually remedied by leaving the sample for 20-60 minutes in the spectrometer and/or mixing the sample further in the NMR tube with a pipet.
2. The nematic to isotropic transition temperature for the K^+ salt is about ~44ºC. Near the LC threshold concentration, the transition temperature depreciates, and lowering the sample temperature in the NMR spectrometer is worth trying.
3. We've had difficulty in forming an alignment with one protein in our lab. Alignment was achieved eventually by adding KCl and diluting the protein concentration. Concentrations of KCl over 200mM have not been required.