



NMR Structural Studies of Mass-Limited Modified DNA Using a Protasis/MRM CapNMR Probe



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ABSTRACT

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Exposure to carcinogenic or mutagenic environmental agents often can lead to DNA base modifications that alter the structure and dynamics of the DNA. Possible outcomes, especially if the alterations involve regions important in maintaining proper cell growth and development, would be mutations, cancer, or cell death. While NMR structures and dynamics of many types of DNA lesions have provided detailed insights into the types of distortions that a cellular protein may encounter under biologically relevant conditions, the determination of the structures of numerous DNA adducts has been limited by the need to obtain milligram quantities of sample when using conventional 5 mm NMR probes. In several cases, it has been impossible or has taken several years to synthesize the sufficient quantities needed. The recent development of microcoil NMR detection technology (Protasis/MRM CapNMR probes) to enable high sensitivity detection of low abundance samples has allowed us to address this issue. An advantage of using oligonucleotide DNA is that it is highly soluble due to the negatively charged phosphate groups present, and thus microgram quantities can be readily concentrated into a very small volume without precipitation or aggregation. As proof of principle, we have compared the NMR data collected for a G-T mismatched 11mer duplex using a HCN, 5 μ l volume CapNMR probe with that obtained using a conventional Varian 5 mm, 600 μ l volume indirect ¹H(¹⁵N)-P probe. ¹H-1D, 2D-NOESY and phase sensitive COSY, and ¹³C-HMQC spectra were acquired on 12 mM (CapProbe) or 3 mM (5 mm Probe) sample dissolved in either 100% D₂O or 90% H₂O/10% D₂O 10 mM Na₂PO₄, 0.1 M NaCl, 0.1 mM EDTA buffer, pH 7.0 at 25 °C or 10 °C. The high quality of the data obtained with the CapProbe clearly demonstrates that structural studies of rare, mass-limited DNA adducts are now feasible.

INTRODUCTION

To test the capabilities of the Protasis/MRM TXI CapNMR probe, we used the G-T mismatched 11mer duplex shown in Fig.1. We had previously collected a complete NMR data set for this 11mer using a standard Varian 5 mm ¹H(¹⁵N-³¹P) ID probe because it was used as the unmodified control in another DNA adduct structure study. It had taken several years to synthesize this particular DNA adduct with a cytosine opposite the modified guanine in sufficient quantities to successfully carry out the NMR structure study¹. Many more DNA adducts are of significant interest in the field of chemical carcinogenesis. However, to date, it has not been always possible to obtain enough sample to characterize these adducts structurally by NMR due to the inherent low sensitivity of the method. Today, several options exist that can help address this sensitivity issue, including the use of Shigemitsu tubes and cryogenically cooled detector coils. Nevertheless, structural studies of mass-limited samples in the > μ g range still remain challenging. The triple-resonance (TXI) (¹H(¹³C,¹⁵N)) with z-gradients capillary NMR probe has demonstrated a 10-15 fold increase in mass sensitivity compared with conventional 5 mm probes, and require as little as 5 μ l of sample (Table 1)². Other advantages of these low volume probes are that signals from solvent impurities are much less prominent with decreasing sample volume, high salt tolerance with minimum loss of S/N, and greater field homogeneity over a smaller volume.

REFERENCES

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- Protasis/MRM web site: <http://www.protasis.com/pdf/Catalog%20TXI%20Probe%20-%2020008E.pdf>

NMR EXPERIMENTS

NMR experiments were performed on a Varian INOVA 600 MHz spectrometer. A combination of through space nuclear and rotational Overhauser effect (150 and 300 ms mixing time NOESY) and through bond correlated (phase sensitive COSY) experiments, as well as ¹³C-HMQC were recorded on ~12 mg (~3 mM) of the G-T 11mer duplex dissolved in 0.6 μ l of D₂O buffer at 25 °C using a regular 5 mm ID probe, or on ~0.2 mg (6 mM) in 5 μ l using the TXI probe at 23 °C. One-dimensional and NOESY (150 ms mixing time) spectra of the G-T 11mer duplex in 90% H₂O/10% D₂O buffer were collected at ~10 °C using a jump-return pulse sequence for solvent suppression. The buffer used was 10 mM Na₂PO₄, 0.1 M NaCl, 0.1 mM EDTA, pH 7.0 for the 5 mm probe sample and 20 mM Na₂PO₄, 0.2 M NaCl, 0.2 mM EDTA, pH 7.0 for the TXI probe sample. Sweep widths of 6000 Hz and 12000 Hz were used for the 11mer duplex dissolved in D₂O buffer and H₂O buffer, respectively, with 1024 complex data points and 300 t_1 increments, each having a recycle delay of 1.8 s. The number of transients used are given in the figure captions. Data sets were processed using the software package VNMR (Varian Inc, Palo Alto, CA), transferred to a SGI workstation, and converted directly into FELIX (Acelerys, San Diego, CA) matrixes for analysis.

Figure 1: The numbering schemes, structure and hydrogen bonding schemes for the dG-dT 11mer duplex used in this study

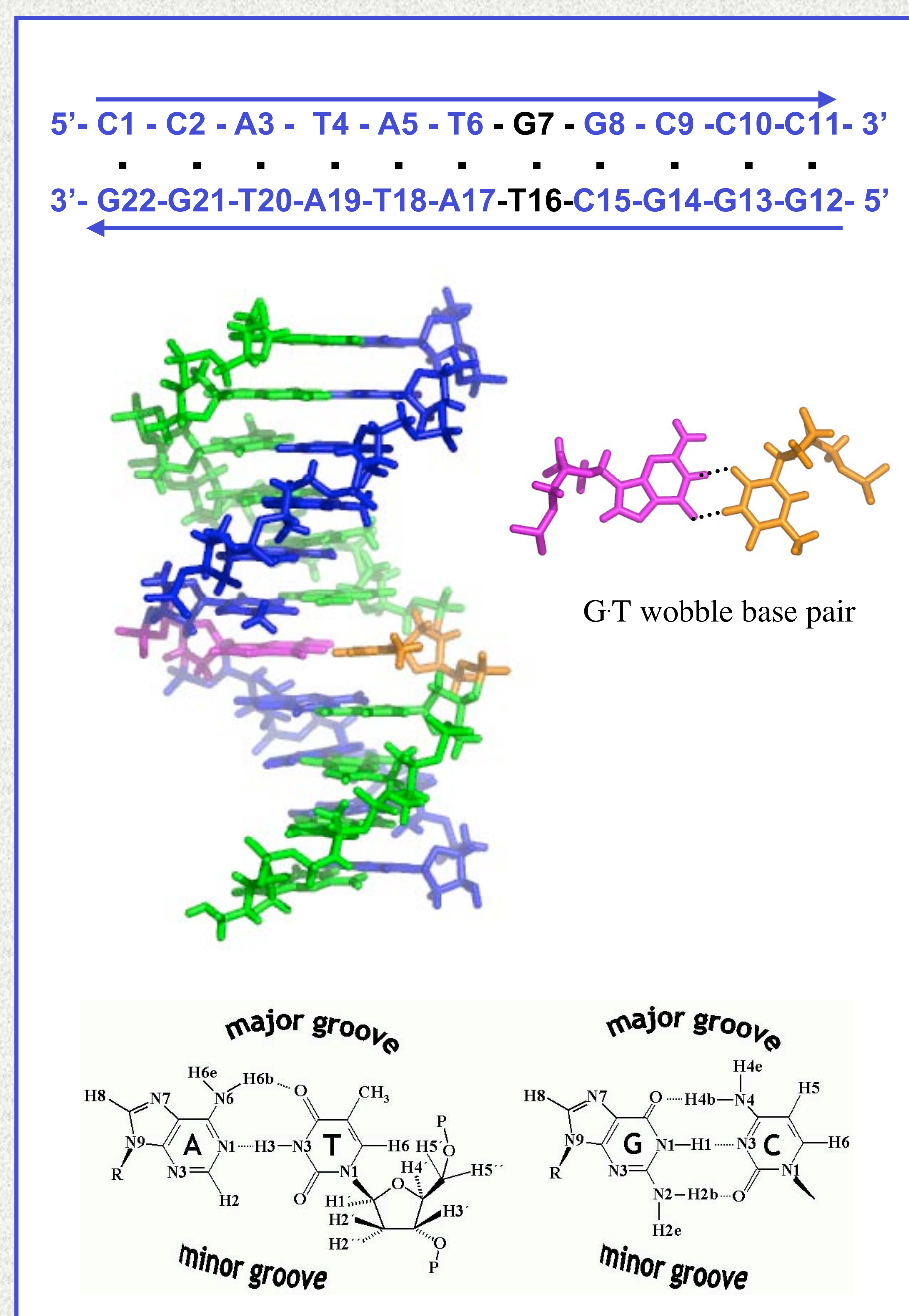


Table 1. Mass sensitivity comparison of common NMR probes

Single Scan Figure of Merit*	5mm ¹	LC Flow ²	Specialty Trace ²	CapNMR ³
Spectral Resolution (Hz)	< 1	< 1	< 1	< 1
Relative S/N, Fixed Mass	1	1.3	2	10
Relative Data Acquisition Time, Fixed Mass	100	59	25	1

*Sucrose anomeric proton; ¹Line broadening 1 Hz, 600 MHz, measured by MRM staff; ²Derived from manufacturer's promotional literature; ³Line broadening 1 Hz, 600 MHz

SIGNAL to NOISE definition

$$S/N \sim \pi^2(1+\gamma)(QV_s v_0 T_2 \beta T_1)^{1/2}, \quad \text{eq. 1}$$

where z is the filling factor of the coil (including sample concentration), Q is the quality factor of the probe (circuit inductive reactance/equivalent series resistance of the circuit), V_s is the sample coil volume, ν_0 is the resonance frequency, T_2 is the spin-spin relaxation time, T_1 is the spin-lattice relaxation time, and b is the receiver-detector bandwidth.

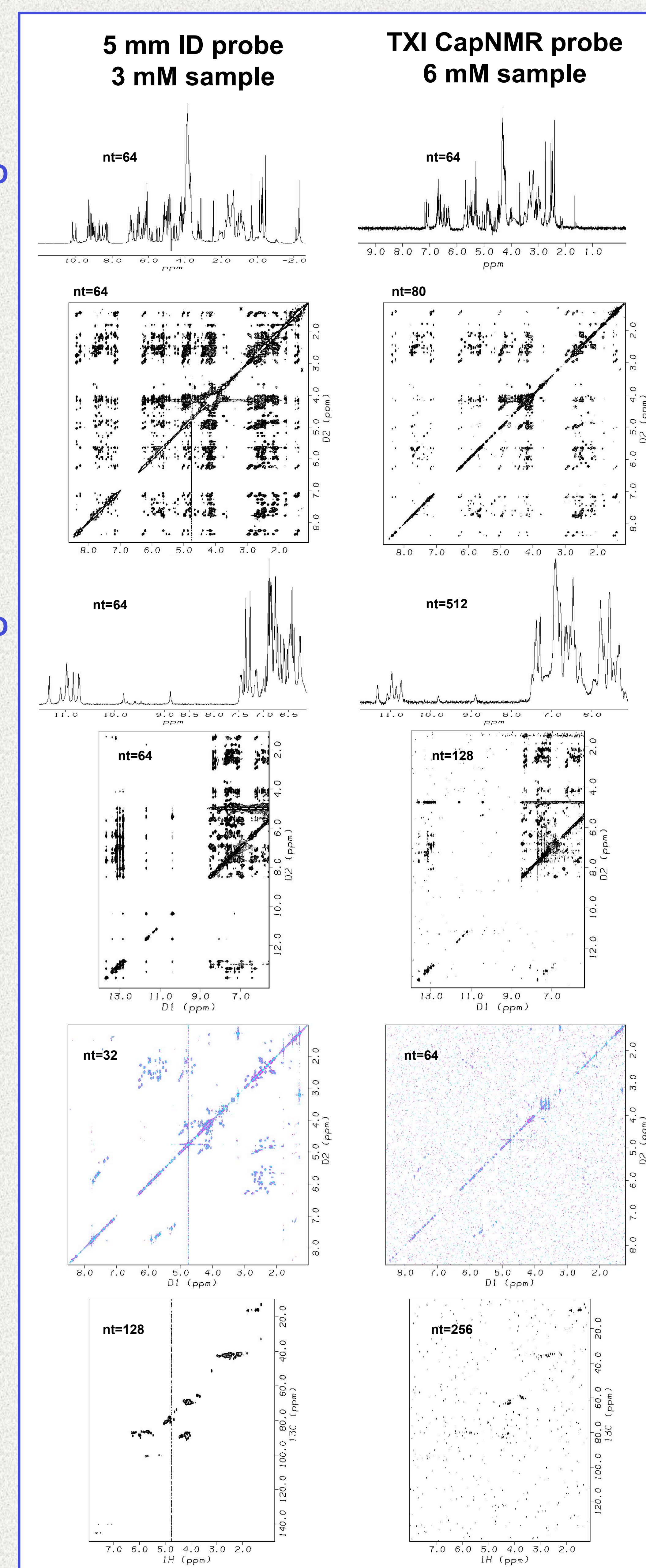
In order to maximize the S/N, this equation implies that one should use a:

- Large γ - biggest $\gamma = ^1\text{H}$
- High frequency ν_0 - largest B_0 , highest magnetic field
- Large z - highest concentration, fraction of the coil detection volume filled with sample
- Large volume
- High Q - hence minimize salt to load coil
- Large T_2 - narrow lines
- Short T_1
- Small β - narrow bandwidth receiver/detector

The most important parameters that can affect a probe's sensitivity are: Coil length, coil-filling factor, probe Q , dielectric value of sample, and % of the overall coils inductance in the coil leads.

The first and second items in the sensitivity parameters list determine how much sample the probe coil can see. Microcoils are ~ 10 times more sensitive (at equivalent mass) than conventional 5 mm probes for samples soluble at 1 mM or more because of the large coil filling factor, the lower noise associated with the smaller NMR coils, and the ~ 3x more sensitivity of solenoid coils compared to the open saddle coils required to accept NMR tubes.

Figure 2: Side-by-side comparison of the spectra obtained with each probe



CONCLUSIONS

The volume (obs) in the 5 mm ID probe is ~ 250 μ L, while it is only 1.5 μ L in the TXI microcoil probe. Therefore, according to Eq. 1, decreasing the volume of sample with all other factors being equal would, by theory, reduce the sensitivity by 12.9 times (250/1.5)^{1/2}. Recent comparisons of proton S/N between a 5 mm and 5 μ L probe indicate that for a constant concentration the relative S/N is 15, indicating that other factors, such as filling factor and Q increase the S/N of the TXI probe despite the large decrease in volume. Twice the concentration of the DNA 11mer duplex, as used in these experiments shown here, would then only provide ~13% of the S/N, explaining why even with a larger number of transients, the spectra for the TXI microcoil are much weaker than those acquired with the 5 mm ID probe. However, the quality of the data with respect to observing very sharp resonances and very good water suppression is excellent, especially considering that the amount of salt used was double that used in 5 mm probe sample. The H₂O data is broad, however, because of a problem encountered with keeping the temperature stable at 10 °C. Also, more careful calibration of the power levels and other parameters for the ¹³C-HMQC are needed for future experiments. In particular, the standard Varian parameters that are loaded for HMQC's have a dmf which is much smaller than the 20k-30k appropriate for ¹³C.

A set of experiments with optimized parameters will be conducted in the future to determine the quality of the spectra of the G-T mismatched 11mer duplex at same mass, but different concentrations. Using 10 times the concentration in a 5 μ L probe should leave one with ~ 66 % the S/N, while 15 times the concentration should be at 100 %. At higher concentrations, increased viscosity of the sample would tend to decrease the S/N again. DNA oligomers remain very soluble at very high concentrations because of the presence of the negatively charged phosphate groups, so the samples are not concentration limited.

The amount of mass of the 3 mM 11mer duplex dissolved in 600 μ L is 12 mg, while the mass of the 6 mM sample in 5 μ L is 0.2 mg. For rare DNA adducts that can only be obtained in micromolar quantities, dissolving it in as small a volume as possible and using a microprobe with increased number of transients may be the only option available to characterize these samples, other than using a cryoprobe.

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