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Monitoring NMR Spectrometer Performance during Data Accumulation for Macromolecular Structure Determination

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In multidimensional NMR experiments with biological macromolecules, data sets are typically acquired over a period of several hours to several days (*1, 2*), and the results obtained are critically dependent on high stability of the environment during the entire recording time. Even a short, transient instability in the magnetic field homogeneity or a transient temperature variation can severely distort or even completely corrupt a data set. Another practical aspect of NMR in structural biology is that increasingly the data are handled with computer support, and success of automated analysis can only be expected with NMR spectra of excellent quality. Over the years, we invested more and more heavily into optimizing our NMR laboratories for long-time stability of the experimental conditions (underground rooms with separate air conditioning, ferromagnetic shields for the magnets, elimination of mobile ferromagnetic objects, use of uninteruptable power supplies, etc.), which was prompted by a variety of incidents that interfered with high-quality NMR measurements (Table 1). Thereby, continuous recording of key experimental parameters was essential both for the evaluation of individual experiments and for the design of improved laboratory facilities. The present Communication describes the installations used in our laboratory for continuous monitoring of NMR spectrometer performance and the experience gained with their practical use.

We installed monitoring devices with Bruker AM and AMX instruments and with Varian UNITY*plus* spectrometers. From a variety of observations, it was quite evident that lock level, sample temperature, and room temperature are particularly sensitive experimental variables. Initially, these three parameters were followed with an analog multichannel recorder. For this, the lock signal was branched off from the lock receiver board of the Bruker AM console, the probe temperature was taken from the error signal of the BVT 1000 temperature control unit, and the room temperature was obtained from a temperature-to-voltage converter. This setup served reliably for several years, but, since the recorder had to run continuously, the small scale of the time axis was a limiting factor in the determination of the exact times at which undesirable effects were detected. Therefore, with the Bruker AMX console, we switched to electronic storage of the data and complemented the supervision of the individual NMR experiments with full electronic documentation of all relevant input information. The program package ENREC (*En*vironment *Rec*ording) stores a continuous recording of the environmental variables in a directory of the instrument supervisor, and, for the individual user, a copy of these recordings is included with the individual data sets. This enables the instrument supervisor to inspect the instrument performance for repeated interferences, which can provide a lead to the cause of the malfunctioning. The individual user can detect disturbances related to individual experiments, and, as a result, he may either restart the experiment at the point of interference or rerecord the corrupted FIDs and introduce them into the multidimensional data set. Eventually, the data set is automatically copied to the archiving directory with all files that are relevant for the data acquisition, and, thus, complete electronic documentation of all multidimensional experiments recorded on our instruments is assured. While lock level and sample temperature were obtained in digital form from the AMX console, an eight-channel A/D converter was used to obtain electronic data storage with the UNITY*plus* system, for which the liquid helium and nitrogen levels are also monitored. Lock level and sample temperature are sampled about 30 times (AMX) and 50 times (UNITY*plus*) per minute, respectively, but only the minimum and maximum values within a period of 1 min are stored. This gives good results even for experiments with PFGs, where one has periodical drops of the lock level. The data size of the relevant recordings during a 24 h period is about 30 kbytes, which can be reduced to less than 10 kbytes using a standard compression algorithm, and then transmitted in less than 10 s with a 14.4 kbit/s modem. Thus, efficient remote control of long-term measurements is ensured, either from the individual workstations in the Institute or from terminals in private residences.

Table 1 lists types of instabilities that we have typically encountered. Resulting artifacts (*3*) are invariably either one

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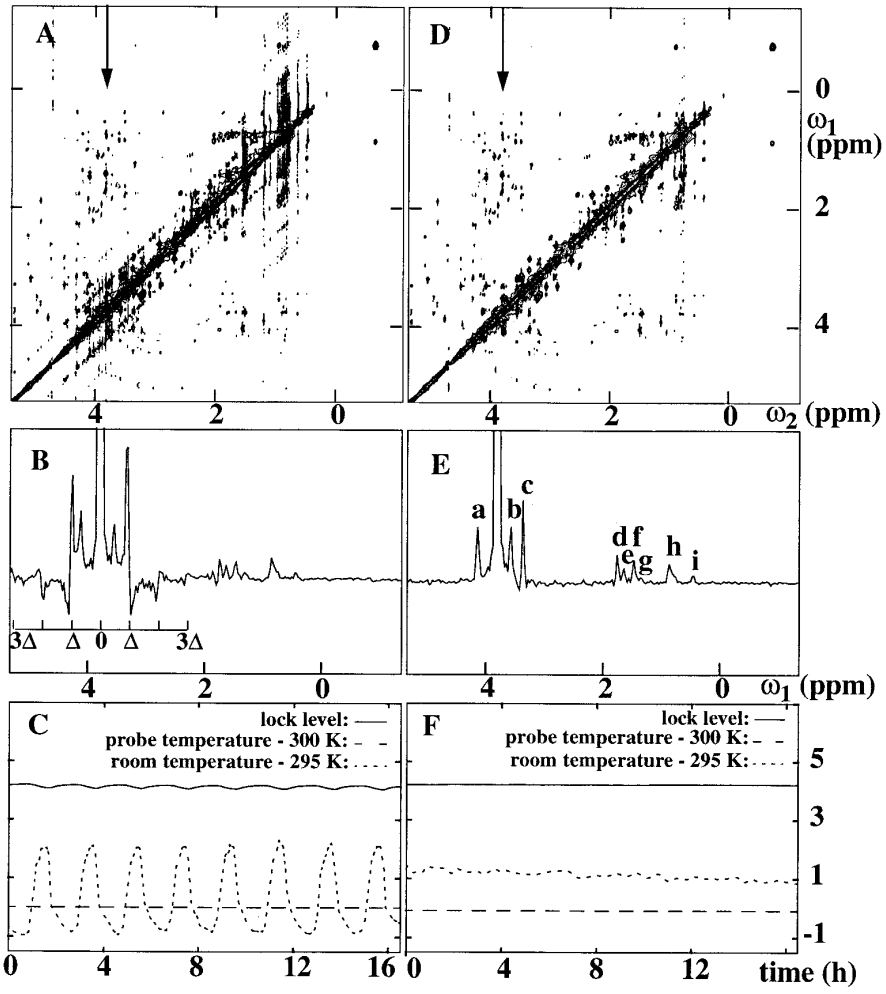

FIG. 1. (A) Contour plot of the region (ω_1, ω_2 = −1.1–5.3 ppm) of a 750 MHz 2D ^{1}H NOESY spectrum of the mating pheromone E*r*-22 from the ciliated protozoan *Euplotes raikovi*, which is a small protein of 37 amino acid residues (solvent H_2O, pH 4.6, T = 27°C, recorded data size 260(t_1) × 2048(t_2) complex points with t_{1max} = 0.024 s and t_{2max} = 0.186 s, total measuring time 16.5 h, digital filtering before Fourier transformation using a cosine-squared window in t_1 and a sine window shifted by 80° in t_2). (B) Cross section along ω_1 of the spectrum shown in (A) taken at the position indicated by the arrow. Strong artifacts can be seen at multiples of Δ = 340 Hz measured from the diagonal resonance. This periodicity corresponds to an environmental variation of 16.5 h/(340 s^{-1} × 0.024 s) ≈ 2 h. (C) Plot from the monitor during the acquisition of the NOESY spectrum. The lock level is displayed in arbitrary units, the temperatures in kelvins, where 300 K have been subtracted from the measured probe temperature and 295 K from the room temperature, respectively. An oscillation of the room temperature with a period of about 2 h and an amplitude of about 3° is readily apparent (different colors of the original plot have been replaced by different line styles). (D–F) Data corresponding to those in (A–C) which were recorded after stabilization of the room temperature. Resonance assignments: within ±0.01 ppm of the position of the cross section [arrow in (A) and (D)], there are the chemical shifts of Ser 11 $H^{\beta 3}$, Ile 23 H^{α}, Gly 30 $H^{\alpha 1}$, and Cys 32 $H^{\beta 2}$. The peaks labeled in (E) correspond to the following NOE cross peaks: (a) Gly 30 $H^{\alpha 1}$–$H^{\alpha 2}$; (b) Ser 11 $H^{\beta 3}$–$H^{\beta 2}$; (c) Cys 32 $H^{\beta 2}$–$H^{\beta 3}$; (d) Ile 23 H^{α}–H^{β}; (e) Ser 11 $H^{\beta 3}$–Leu 14 $H^{\beta 2}$; (f) Ile 23 H^{α}–$H^{\gamma 12}$; (g) Ile 23 H^{α}–$H^{\gamma 13}$; (h) Ile 23 H^{α}–$H^{\gamma 2}$; (i) Ser 11 $H^{\beta 3}$–Val 28 $H^{\gamma 1}$ (A. Liu, P. Luginbühl, and K. Wüthrich, unpublished).

or several of the following: t_1 noise, wiggles in the indirect dimensions, baseline roll in the indirect dimensions, or line broadening in the indirect or direct dimensions. The table also shows that different origins of a given type of artifact can in most instances be identified by monitoring the three parameters used here. The table is largely self-explanatory, and, therefore, we illustrate the functioning of our surveillance system with just one example (Fig. 1): Long-term instabilities of the room temperature have at times occurred in each of the rooms that we installed for NMR spectroscopy. Besides transient one-time changes of the temperature (for example, because of external variation of the cooling water temperature), which we could not have unambiguously identified as the source of spectral artifacts without continuous monitoring, oscillation of the room temperature (Fig. 1C) due to unfortunate choice of the set point and the regulation

TABLE 1
Types of Instabilities of the NMR Systems Encountered in Our Laboratories during Acquisition of Multidimensional Data Sets with Proteins

Type of instability	Resulting spectral artifacts	Manifestation on monitor[a]	Comments
Room temperature variation	t_1 noise	Direct recording	Illustrated in Fig. 1
External magnetic disturbance	Baseline roll, wiggles	Dip in lock trace	Moving ferromagnetic items one or two floors above the NMR laboratory were detected
Electric spikes (e.g., operation of elevator)	Baseline roll, wiggles	Spikes in lock trace	Was reliably stabilized by installation of an uninterruptable power supply (UPS)
Magnet instability	t_1 noise	Fluctuating lock trace	Typical after liquid He or N_2 refilling
Precipitation of solute	Loss of signal intensity, line broadening	Monotonous decrease of lock level	
Solvent evaporation and falling drops	t_1 noise	Unstable lock level	
Change of He back pressure	t_1 noise	Unstable lock level	Critical point: Valve used for pressure regulation
Autoshim failure	t_1 noise, wiggles	Monotonous decrease of lock level	Typical for multiple local extrema in the lock settings
Sample heating due to RF dissipation	Line broadening in directly acquired dimensions	Temperature increase and decrease of lock level at start of the experiment	Monitor recordings show time needed to reach equilibrium
Unstable temperature control unit (gas flow fluctuations, misadjustment of regulation parameters)	t_1 noise	Unstable probe temperature	
Heater failure in temperature control unit	Wiggles, t_1 noise	Monotonous temperature variation	

[a] The monitor records the time course of the lock level, the probe temperature and the room temperature (Figs. 1C and 1F).

parameters was a rather frequent nuisance. Figure 1C shows that the oscillation of the room temperature is paralleled, with a time shift of about 20 minutes, by small oscillations of the lock level. In a NOESY spectrum recorded during this temperature instability, there are two lines of artifactual peaks on each side of and parallel to the diagonal (Figs. 1A and 1B). From the separation of these peaks from the diagonal, one calculates that there must be a modulation in t_1 with a period of about 2 hours, which fits with the observations in Fig. 1C. After resetting the regulation parameters so that a stable temperature was achieved (Fig. 1F), a clean spectrum was obtained (Figs. 1D and 1E). It is readily seen that some of the lines identified in Fig. 1E (see legend to Fig. 1) are overlapped with artifactual lines and could not be analyzed in Figs. 1A and 1B.

We decided to share these laboratory details with others in the expectation that similar problems might come up elsewhere. Moreover, we would be very pleased if this Communication could initiate plans with the commercial suppliers to furnish a long-term monitoring device with the basic outfit of spectrometers designed for biomacromolecular studies. Although it appears that the presently described setup monitors most disturbances that lead to spectral distortions, an even more rigorous surveillance could then be envisaged, possibly including the z_0 current, the pressure at the helium outlet, the air flow for the temperature regulation, and an indicator for the RF power at the amplifier output.

Versions of the program ENREC for Bruker AMX and Varian UNITY*plus* consoles can be obtained from the authors.

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