"RESONANCES" IN THE DIELECTRIC ABSORPTION
OF DNA?

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ABSTRACT   An attempt was made to confirm previous reports of resonant-like dielectric absorption of plasmid DNA in
aqueous solutions at 1–10 GHz. The dielectric properties of the sample were measured using an automatic network
analyzer with two different techniques. One technique used an open-ended coaxial probe immersed in the sample; the
other employed a coaxial transmission line. No resonances were observed that could be attributed to the sample;
however, resonance-type artifacts were prominent in the probe measurements. The coaxial line technique appears to be
less susceptible to such artifacts. We note two important sources of error in the calibration of the automatic network
analyzer using the probe technique.

INTRODUCTION

Recently, Edwards et al. reported resonant dielectric
absorption in DNA solutions at 1–10 GHz (1–3). Such
behavior is unexpected for two reasons. First, the dielectric
properties of many aqueous polymer solutions, including
DNA (4, 5), have been measured by other investigators
with no evidence of resonant absorption because of the
strong damping by the solvent. Second, the polarizability
of polymers in solution is typically very low in this
frequency range, with the solution permittivity below that
of water due to an excluded volume effect with no observable contribution from the polymer itself. At the comparatively low concentrations of DNA used by Edwards et al.
(<0.1% by volume), even these effects should be unobservable. Much theoretical work has been directed at explaining the resonances in the DNA (6–10).

We have undertaken confirmation experiments with
close attention to the severe measurement problems
involved. As we show below, the change in the measured
quantity in Edwards' study (the reflection coefficient from
a probe that is dipped into the DNA solution) corresponding to the DNA resonance is below the potential errors due
to instrumentation and strongly reminiscent of the characteristic artifacts in such measurements. We repeated the
measurements, with a similar technique and similar samples, but with a greatly improved automatic network
analyzer (ANA). Additional measurements were performed using a second method that avoids some of the
problems with the probe technique. The results do not
suggest resonant absorption by the DNA, and point to
serious problems with the probe technique for precise
dielectric measurements at microwave frequencies.

MATERIALS AND METHODS

The plasmid puC8.c2 was a gift from J. D. Saffer (Jackson Laboratories,
Bar Harbor, ME); this plasmid, a dimer of pUC8, was the same as used in
the earlier studies (3). The plasmid was transformed into *Escherichia coli*
HB101, using transformation competent bacteria purchased from
Bethesda Research Laboratories (Gaithersburg, MD). Transformed cells
(referred to as HB101(puC8.c2)) were grown on modified L-broth (11)
containing ampicillin (100 μg/ml). Individual colonies were selected and
isolated on agar plates containing this medium. Plasmid DNA was
purified from liquid cultures of HB101(pUC8.c2) grown in L-broth
containing ampicillin.

The DNA was extracted using the method of Edwards (3), which
includes opening the cells with lysozyme (Sigma Chemical Co., St. Louis,
MO) and Triton X-100, digesting the RNA with RNase A (Sigma
Chemical Co.), and separating the nucleic acid from most of the protein
by a two-phase separation using phenol-chloroform-isoamyl alcohol. As
with Edwards' method, there was no treatment with protease, or addition
of sodium dodecyl sulfate to the sample to aid removal of protein
components. The plasmid was purified from the lysate by passage through
a Sepharose 4B column. After repeated precipitation in ethanol to remove
salt, the DNA was lyophilized to remove all the ethanol and then
suspended in buffer consisting of 10 mM NaCl, 10 mM Tris-HCl buffer,
1 mM EDTA, pH 7.5. The plasmid concentration ranged from 0.5 to 1.5
mg/ml (determined by UV absorbance), which was comparable to that
reported in the earlier study. Three batches of plasmid DNA were
separately prepared at different times over a 7-mo period.

The plasmid size was determined by agarose gel (0.7%) electrophoresis
of linearized plasmid material produced by digestion with the restriction
endonuclease *Eco*R1. Because pUC8.c2 is a dimer, the monomer (1.8
Md) was observed after the digestion. The migration of the supercoiled
form of the naturally occurring plasmid was consistent with a molecular
weight of 3.6 Md. The DNA material recovered from the Sepharose
column was primarily supercoiled DNA (generally 65–80% of the
material was in the supercoiled form). The samples also contained some
linear and relaxed plasmid molecules and a very minor amount of
chromosomal DNA. The UV absorbance 260:280 ratio of the purified
plasmid preparations was 1.7 (batch 1), 1.6 (batch 2), and 1.6 (batch 3).
Samples monitored using the Coomassie Blue analysis technique (Pierce

Chemical Co., Rockford, IL) indicated that the protein content was 5.7% (wt/wt, referred to dry weight of DNA) (batch 2) and 0.3% (batch 3). Electrophoretic comparison of the plasmid preparations before and after dielectric measurements typically indicated a decrease of $\approx 20\%$ in the amount of supercoiled DNA.

The dielectric properties of the samples were measured in two ways. The first was a variant of the technique employed by Edwards et al., as described by Stuchly et al. (12-14). A probe, consisting of a length of open-ended semirigid 50-ohm coaxial transmission line, is immersed in the sample, and the electrical reflection coefficient at its tip (ρ^*) is measured with an ANA. Two probes were used, which were constructed of 10- and 15-cm lengths of 2.99-mm outer diameter (OD) precision 50-ohm semirigid line. One end of each probe was milled flat and polished with fine crocus cloth; the other end was fitted with a precision type K coaxial connector.

The reflection coefficient of the probe was measured using a model 8510 automatic network analyzer (ANA; Hewlett-Packard Co., Palo Alto, CA) equipped with a flexible precision test line. Measurements were performed with the instrument in the step mode, between 0.045 and 18 GHz in intervals of 0.045 GHz, with ten measurements averaged per reading. The system was calibrated at the end of the test line according to the manufacturer's recommendations using precision standard loads consisting of a short and open circuit, and either a single or sliding 50-ohm load, depending on the frequency range. All connectors were precision types (K or APC 3.5), and care was taken to minimize any bending of the flexible test line during a measurement.

After the ANA was calibrated, the probe was attached to the end of the test line. The reference plane of the ANA was set at the distal tip of the probe, by short circuiting the tip and adjusting the time delay of the ANA to produce a constant 180° reflection coefficient. The small reflection from the connector between the probe and ANA was removed using the time domain gating method, in which the reflection coefficients are transformed into the time domain, gated to remove connector artifact, and transformed back into the frequency domain.

The measured complex reflection coefficient ρ^* is related to the complex admittance y^* of the probe:

$$y^* = \frac{(0.02)(1 - \rho^*)}{(1 + \rho^*)}, \qquad (1)$$

which is a function of the complex dielectric permittivity (ϵ^*) of the sample. In the limit of low frequencies,

$$y^* = j\omega(C_o\epsilon^* + C_f), \qquad (2a)$$

where C_o and C_f are constants that depend on the geometry of the line and ω is the measurement frequency in radians per second. These constants were empirically measured for the different probes at 0.5 GHz using water-dioxane solutions of known permittivity (15).

Unfortunately, the range of frequencies for which Eq. 2a applies is, for the probes used in the present and in Edwards' studies, below that in which the resonances were reported. Above 1 GHz (for these probes) radiation effects become significant and the conductance of the probes increases rapidly with frequency and with the permittivity of the medium (the corresponding changes in the capacitance of the line are much smaller). While numerical studies have been reported for coaxial lines of the sort we used (16), no closed form expression is available for the admittance of the probe which includes radiation effects. This problem was not discussed by Edwards et al., whose analysis was based on Eq. 2a over the entire frequency range of their measurements.

Approximately, radiation effects can be modeled as a conductance (the radiation conductance) in parallel with the sample. The radiation conductance was estimated from measurements on water-dioxane solutions, using dielectric dispersion data of Hasted et al. (17). Additional measurements were performed with probes made from the slightly larger (3.6-mm OD) lines used by Edwards et al. Up to 10 GHz the admittance of the

probes could be fitted approximately by

$$y^* = j\omega(C_o\epsilon^* + C_f) + kf^3[Re(\epsilon^*)]^2, \qquad (2b)$$

where $k = 3.6\ (10^{-9})$ and $8.9\ (10^{-9})$ for the 2.9- and 3.6-mm lines, respectively, and f is the frequency in gigahertz. The conductivity of water increases quadratically with frequency in this range, and the real part of the first term in the right side of Eq. 2b (which is proportional to the conductivity of the solution) and the second term are comparable at low-gigahertz frequencies. We estimate that errors of the order of 100% will occur in calculating the conductance of the probe between 2 and 10 GHz due to neglect of radiation effects.

The effect of such errors on the dielectric measurements will depend on the details of the technique used. Since the correction for radiation effects is large and uncertain, large errors might be introduced in measuring the dielectric properties of the samples. However, this error is a smooth function of frequency that will not introduce a sharp frequency dependence when comparing samples of similar dielectric properties. On the other hand, if measurements on the probe are used to calibrate the system (as in the study by Edwards et al.) neglect of radiation effects will lead to imprecise correction for resonance-type artifacts that arise elsewhere in the system. We show in the Appendix that such calibration errors exceed the change in reflection coefficient of the probe attributed to resonances in the DNA.

Finally, a small correction was applied to compensate for losses in the probe itself. The radiation conductance of the probe in air is negligible at 1-10 GHz, and its reflection coefficient ρ^* will have unit amplitude. The measured reflection coefficient ρ_m^* decreased as the inverse square root of the frequency because of losses in the line. From such measurements an empirical function was obtained and subsequently used to correct the data:

$$\rho^* = \rho_m^*/(1 - af^{1/2}), \qquad (3)$$

where a is an adjustable parameter and f is the frequency. The maximum correction was at most 0.05 in magnitude at 10 GHz.

Dielectric measurements on the DNA solutions were performed blind, using samples in coded vials containing plasmid DNA of several different concentrations, commercial DNA, or buffer solution. To test for instrumental artifacts, all measurements were repeated using the two probes of different length. In other experiments, measurements were repeated with different gating parameters in the ANA. Samples were reanalyzed after completion of the dielectric measurements to rule out the possibility of significant changes caused by handling during the experiments.

Additional measurements were performed on one sample from the third batch of plasmid DNA using the method introduced by Roberts and von Hippel (18), below referred to as the transmission line technique. The method and instrumentation are described in detail elsewhere (19, 20). Briefly, the liquid was placed inside a length of 7-mm coaxial transmission line between a thin Teflon disk and a short circuit terminating the line. The reflection coefficient from the sample was then measured using an HP8410 ANA over an appropriate range of frequencies. This process was repeated using several samples of varying thickness, after which the data were sorted by frequency and fitted by computer to the appropriate theoretical expression for a transmission line to obtain the complex permittivity of the sample. In performing these measurements, the ANA and sample cell had been calibrated with factory-standard loads (short and open circuit and sliding 50-ohm load) and care was taken to ensure that the frequency range included the frequency corresponding to one-quarter of a wavelength in the sample, a prerequisite for accurate measurements.

The transmission line method has important advantages over the probe technique. The ANA can be calibrated by factory-standard loads without need for additional correction for connector artifacts or losses in the probe, and the reflection coefficient of the sample is a precisely known and sensitive function of its dielectric properties. Finally, the load reflection coefficient is relatively small for a sample of appropriate

thickness (e.g., 0.1–0.2 vs. >0.7 for the probe) which, as shown below, leads to substantially smaller errors in measurements of the reflection coefficient.

RESULTS

We consider the power attenuation coefficient, α_{power}, defined as

$$\alpha_{power} = (4\pi/\lambda)(Im(\sqrt{\epsilon^*})), \qquad (4)$$

(where λ is the wavelength in free space), which was the parameter used by Edwards et al. to report the DNA resonances. For one sample of supercoiled DNA (0.53 mg/ml), the resonances reported by Edwards et al. corresponded to variations of α_{power} from 0.2 to 0.7 cm^{-1} at 2.5, 4, 6.5, and 8.5 GHz. The half-widths of the resonances varied from 0.5 to 1 GHz.

We were unable to confirm these observations. The few differences between the DNA and buffer solutions that could be reliably detected were smoothly varying functions of frequency that were associated with differences in the ionic conductivity of the solutions.

Fig. 1 a shows the power attenuation coefficient of a sample of supercoiled DNA (1.5 mg/ml) and buffer, measured with the probe technique. (Measurements were

also performed on this sample with the transmission line method but the data would be indistinguishable from those shown in the figure.) The difference in attenuation coefficient ($\Delta\alpha_{power}$) between the DNA solution and buffer are shown, on a greatly expanded scale, in Fig. 1 b, with the data from the transmission line method now included. Oscillations do appear in the probe measurements, when displayed on this expanded scale. However, their magnitude is smaller than for the supercoiled DNA reported by Edwards (reference 1, Fig. 5), even though the present sample has three times the DNA concentration.

Most of the data obtained with the probe technique showed similar effects. Typically they corresponded to variations of 0.2 cm^{-1} or less, but sometimes were as large as 0.5 cm^{-1} in $\Delta\alpha_{power}$. They showed no obvious dependence on the concentration or nature of the DNA, and appeared even when comparing separate measurements on the same buffer solution. Moreover, they varied with the length of the probe and with the gating parameters of the ANA. We argue below that they arise from imperfect cancellation of system errors.

DISCUSSION

The measurement problems are best illustrated by comparing the change in the measured quantity (the reflection coefficient from the probe) with the capabilities of the instrumentation as reflected in the manufacturers' specifications (Fig. 2).

For the ANA, the largest errors are source match errors (from stray reflections in the system) and directivity errors (from imperfections in the directional couplers). For the
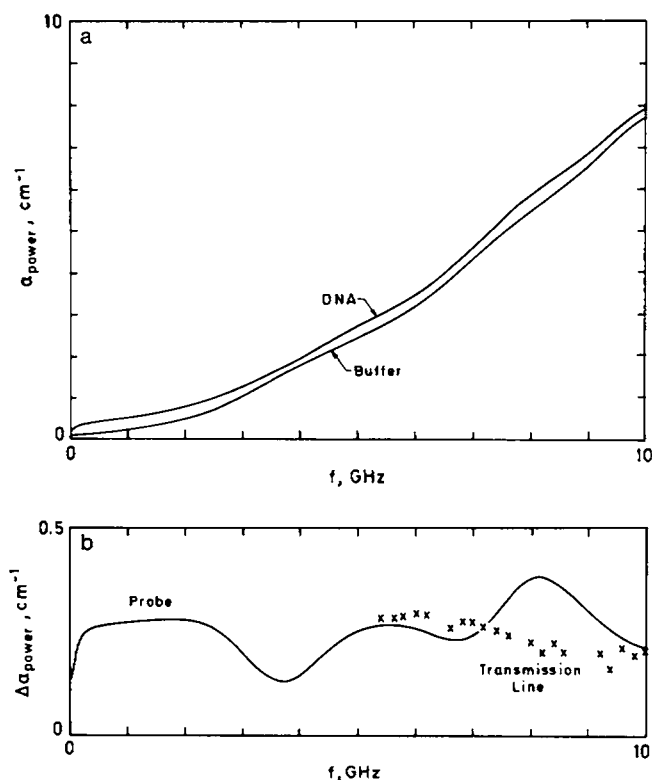

FIGURE 1 (a) Attenuation coefficient of buffer and DNA solution, as measured by the probe technique. (b) Same data, subtracted on an expanded scale, showing also measurements with the transmission line technique (×). The sample consisted of plasmid DNA at a concentration of 1.5 mg/ml.
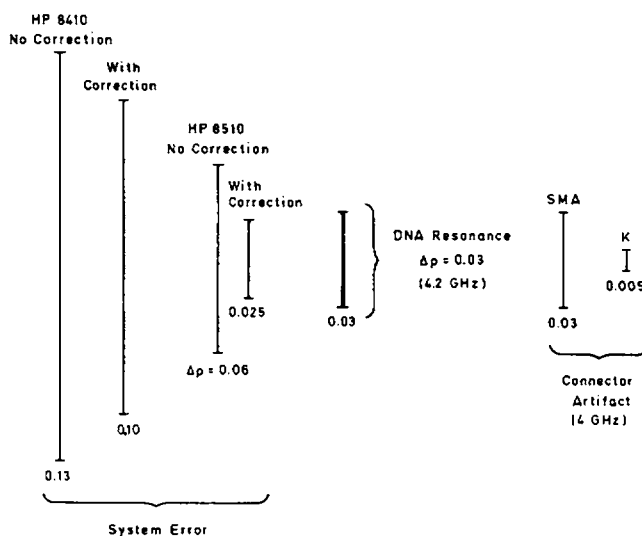

FIGURE 2 Comparison of change in the probe reflection coefficient expected from the resonance in supercoiled plasmid DNA of 0.22 mg/ml (reference 1) with errors in two different automatic network analyzers: the HP 8410 (used in references 1–3) and the HP 8510 system used in the present study. Also shown are manufacturers' specifications for two kinds of coaxial connectors: type SMA (previous study) and K (present study).

HP 8410 ANA between 2–8 GHz, the manufacturer quotes a total uncertainty ρ_u

$$\rho_u = 0.032 + 0.03\,\rho + 0.09\,\rho^2 \qquad (5)$$

in measuring a load of reflection coefficient ρ (the errors above 8 GHz are higher). The quadratic term arises from standing waves within the instrument that give rise to resonant-like artifacts in the measured reflection coefficients. Through a complex calibration process using factory-standard loads the manufacturer states that ρ_u can be reduced by 0.03, independent of the load reflection coefficient.

Edwards et al. reported only one set of data (1) from which the reflection coefficient from the probe can be calculated: a resonance in plasmid DNA (0.22 mg/ml) in which the power attenuation coefficient of the DNA solution differed from that of the buffer by 0.4 cm^{-1} at 4.2 GHz (1). Using the values for C_o and C_f quoted by Stuchly et al. (14) we find that the reflection coefficient of the probe varied by 0.03 about a baseline value of 0.85. Fig. 2 compares this change with the stated uncertainty ρ_u for each ANA. Also shown are the manufacturers' specifications for the reflection coefficient from the SMA and K connectors used with the probe in the former and present study. By this measure, the system errors are potentially comparable to (in the present study) or much larger than (Edwards' study) the effect to be measured. How large the system errors actually were in Edwards' study is an experimental question that was not discussed.

And these errors are resonant-like in appearance. Fig. 3 a shows the reflection coefficient of a probe with a precision K connector immersed in buffer solution, with and without time gating to remove the connector artifact. The values of α_{power} for the buffer that would be calculated from such data are shown in Fig. 3 b, with the resonances reported in a DNA sample (1) included for comparison.

Thus, the artifacts due to a single precision microwave connector are larger than the resonances attributed to the DNA, and they might arise in many places within the system. To detect the DNA resonances with the probe technique requires correction for system errors that are potentially much larger than the effect to be studied and lead to resonance-like artifacts. This is true even with the more precise instrumentation used in this study. Such data are easily misinterpreted, and we suggest this might have happened in the former study.

APPENDIX

Radiation Conductance of the Coaxial Probe and Its Effect on System Calibration

In most applications of the probe technique, the ANA is calibrated using three standard loads consisting of the probe when open-ended, short-circuited, and immersed in a standard electrolyte solution. We consider two sources of error in the third of these standards, both of which are
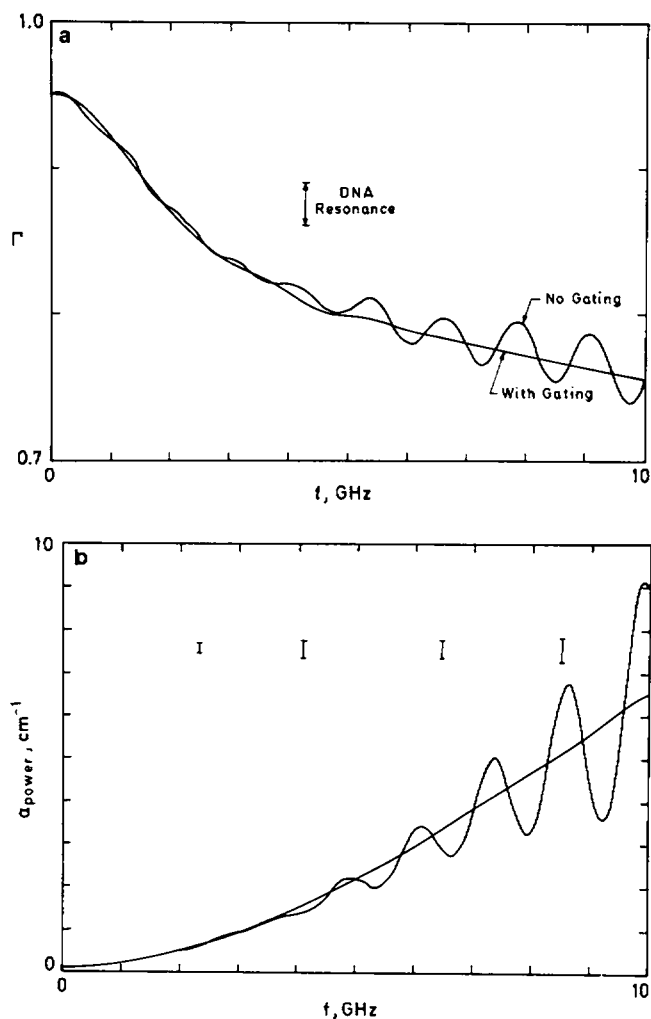
FIGURE 3 (a) Magnitude of the reflection coefficient of the probe immersed in buffer, with and without time-domain gating to remove connector artifact. The oscillations arise from small reflections from the connector used to attach the probe to the ANA test set. The arrow indicates the calculated change in the reflection coefficient from the probe corresponding to a resonance in the DNA as reported by Edwards in reference 1. (b) The calculated attenuation coefficient of the buffer, from the data in a. Shown for comparison are several DNA resonances from the previous study.

larger than the changes in reflection coefficient corresponding to the DNA resonances.

Uncertainty Due to Errors in Choosing C_o and C_f. To obtain the reflection coefficient of the calibration load, Edwards et al. used Eq. 2a, with values for C_o and C_f calculated by Stuchly et al. for the 3.6-mm semirigid coaxial line. These calculations assume ideal geometry, and nominal dimensions for this type of line as adopted by the industry. As noted above, they also neglect radiation effects in the probe, and thus are valid only at low frequencies.

To study the actual properties of such probes, we calibrated two probes constructed from 3.6-mm line, using water-dioxane solutions of known permittivity at a low frequency (0.5 GHz) at which radiation effects are negligible. The resulting values for C_o varied by 10% from Stuchly's values, presumably due to slight deviations from ideal geometry. The corresponding error is 0.15 in the reflection coefficient at 4 GHz when the
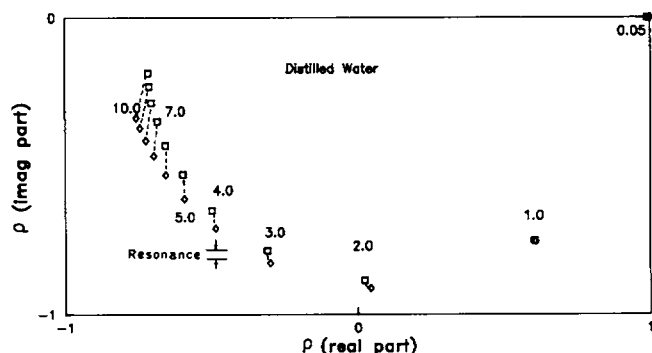
FIGURE 4 Polar plot showing the measured reflection coefficient (□) of a probe, consisting of an open-ended length of 3.6-mm semirigid coaxial transmission line, in distilled water. Also shown (◊) are the respective values calculated from Eq. 2a. The frequency ranges from 0.05–10 GHz, with several values indicated on the figure for reference. The dashed lines connect the measured and calculated reflection coefficients at each frequency. Also shown is the amplitude of the DNA resonance previously reported at 4.2 GHz.

probe is immersed in water. This is five times larger than the DNA resonance to be measured.

Error Due to Neglect of Radiation Conductance. The second term in Eq. 2b contributes significantly to the conductance of the probe above 1 GHz. Fig. 4 shows the measured reflection coefficient of a probe constructed from 3.6-mm coaxial transmission line, immersed in distilled water. Also shown are the "quasistatic" reflection coefficients, calculated from Eq. 2a with empirically determined values of C_o and C_f. Neglect of radiation effects leads to an error of 0.08 in magnitude at 4 GHz, which is two to three times larger than the DNA resonance.

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Note Added in Proof. A report has recently appeared of unsuccessful attempts by two different groups to confirm the resonances in dielectric absorption in puC8.c2 plasmid DNA (Gabriel, C., E. H. Grant, R. Tata, P. R. Brown, B. Gestblom, and E. Noreland, 1987, *Nature (Lond.)*, 328:145–146. See also the editorial by Frank-Kamenetskii on p. 108 of the same issue).

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