Northeast Structural Genomics Consortium
Using NMR to Determine Oligomerization States of Proteins

A priori knowledge of the oligomerization state of a protein is critical to accurate protein structure determination by solution NMR techniques. The principle approaches employed in the NESG for elucidation of the oligomerization state of targets selected for NMR structure determination include: i) analytical gel filtration chromatography, ii) static light scattering, and (iii) ^{15}N NMR relaxation. Here we discuss the latter. The rotational correlation time of a protein in solution is the time for a protein to rotate one radian. For an approximately spherical globular protein, the rotational correlation time, τ_c, is related to its effective hydrodynamic radius (*a*), and thus its oligomerization state, according to the Stokes-Einstein equation (Eq. 1), where η is viscosity and *T* is temperature.

$$\tau_c \approx \frac{4\pi\eta a^3}{3kT} \qquad (1)$$

$$\tau_c \approx \left(\sqrt{\frac{6T_1}{T_2} - 7}\right) / 4\pi\nu_N \qquad (2)$$

In the limit of slow molecular motion (τ_c >> 0.5 ns), the correlation time of a protein is related to the ratio of the longitudinal (T_1) and transverse (T_2) ^{15}N relaxation times, and nuclear frequency (ν_N) according to Eq. 2, which is derived from Eq. 8 in Kay *et al*. (1989, Biochemistry *28*, 8972) by considering only $J(0)$ and $J(\omega)$ spectral densities and neglecting higher frequency terms. In practice, global ^{15}N T_1 and T_2 relaxation times for an unknown protein target can be obtained quickly (ca. 1 h) on a 1.7-mm microcyroprobe using 1D ^{15}N-edited relaxation experiments (Farrow *et al*., 1994, Biochemistry *33*, 5984), by fitting integrated signal in the backbone amide ^{1}H region of the spectrum as a function of delay time to an exponential decay (Figure 1). One then computes the correlation time using Eq. 2, and compares it to a standard curve of τ_c *vs*. protein molecular weight (MW) obtained at the same temperature on a series of known monomeric proteins of varying size (Figure 1). As a general rule of thumb, the τ_c of a monomeric protein in solution in nanoseconds is approximately 0.6 times its molecular weight in kiloDaltons. This approach is reliable up to MW ≈ 25 kDa, where accurate measurement of the diminishing ^{15}N T_2 becomes problematic. For larger systems we are exploring direct measurement of τ_c using the ^{15}N,^{1}H -TRACT NMR approach (Lee *et al*., 2006, J. Magn. Reson. *178*, 72).

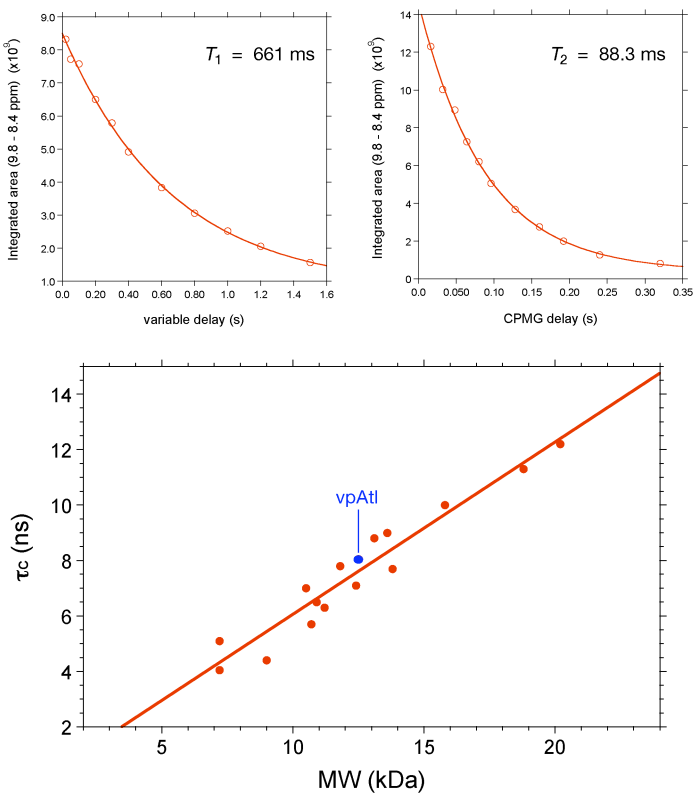

Fig. 1. ^{15}N T_1 and T_2 relaxation data for *U*-5%-^{13}C, *U*-^{15}N vpAtl (NESG ID, VpR247). The data were acquired on a Bruker AVANCE 600 MHz spectrometer with 1.7-mm microcryoprobe at 298 K using pseudo-2D ^{15}N T_1 and T_2 gradient experiments. T_1 spectra were acquired with delays, *T* = 20, 50, 100, 200, 300, 400, 600, 800, 1000, 1200 and 1500 ms, and a relaxation delay of 3s. T_2 spectra were acquired with CPMG delays, *T* = 16, 32, 48, 64, 80, 96, 128, 160, 192, 240 and 320 ms, and with a relaxation delay of 1.5s. (Top): ^{15}N T_1 and T_2 values were extracted by plotting the decay of integrated $^1H^N$ intensity between $\delta \approx 8.4$ to 9.8 ppm and fitting the curves with standard exponential equations using the program 't1guide' within Topspin2.0 (Bruker BioSpin). (Bottom): Plot of rotational correlation time, τ_c (ns), versus protein molecular weight (kDa) for known monomeric NESG targets of ranging size (taking into account isotope enrichment as well as affinity tags in the sequence). ^{15}N T_1/T_2 data for all monomeric proteins used for the τ_c vs. MW plot (red) were obtained on the same Bruker 600 MHz spectrometer at 298 K, and analyzed as described above. Using this approach, we obtain a τ_c of 8.0 ns for vpAtl (blue), which is consistent with a monomer.