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, $\tau_c$, is related to its effective hydrodynamic radius ($a$), and thus its oligomerization state, according to the Stokes-Einstein equation (Eq. 1), where $\eta$ is viscosity and $T$ is temperature.

$$\tau_c = \frac{4 \pi \eta a^3}{3kT}$$  \hspace{1cm} (1)$$

In the limit of slow molecular motion ($\tau_c >>> 0.5$ 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 ($\nu_N$) 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 microcryoprobe 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 $\tau_c$ 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 $\tau_c$ 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 $\approx 25$ kDa, where accurate measurement of the diminishing $^{15}$N $T_2$ becomes problematic. For larger systems we are exploring direct measurement of $\tau_c$ 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 $^1$H intensity between $\delta = 8.4$ 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, $\tau_c$ (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 $\tau_c$ 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 $\tau_c$ of $8.0$ ns for vpAtl (blue), which is consistent with a monomer.