## Probing motions and structural rearrangements by RDCs

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The antibiotic squalamine, originally isolated from dogfish shark, forms a lyotropic liquid crystal at very low concentrations in water (0.3-3.5% w/v), which remains stable over a wide range of temperature (1-40 °C), pH (4-8), and pressure (1-2500 bar). Squalamine is positively charged, and comparison of the alignment of ubiquitin in this medium relative to 36 previously reported alignment conditions shows that it falls closest to liquid crystalline cetyl pyridinium bromide. High precision $^{1}H$-$^{15}N$, $^{15}N$-$^{13}C'$, and $^{13}C'$-$^{13}C^{\alpha}$ and $^{13}C^{\alpha}$-$^{13}C^{\beta}$ residual dipolar couplings (RDCs) in squalamine medium fit well to the static structural model previously derived from NMR data. Inclusion of the new RDCs into the structure refinement procedure results in improved agreement between alignment-induced changes in $^{13}C'$ chemical shift (RCSA) and experimental values, thereby validating the high quality of the static structural model. Our result indicates that fitting of a single model to experimental data can provide a better description of the time- or ensemble-averaged conformation than do ensemble representations, whereas the latter potentially can capture dynamic aspects of a protein, thus making them valuable complements to one another. An accurate average structural model is particularly important for identifying subtle structural changes that precede pressure-induced unfolding.