BSGC NMR Screening Protocol Overview

For proteins that have not been enriched in 15N, samples of relatively small proteins (ca. 150 amino acids) can be screened for "foldedness" and non-aggregation. Samples with ~100 micromolar concentration will have 1D 1H NMR spectra measured. The protein may be His6- tagged or not, but should be without a fused protein domain. The full normal spectral range (12 ppm to -2 ppm) will be examined. Factors to be assessed include:

- dispersion of resonances, particularly amide protons (12 to 6 ppm) and methyls (2 to -2 ppm). Substantial dispersion of resonances is good evidence of a folded structure.

- linewidth of the majority of peaks. From the molecular weight, an estimate of the expected linewidth can be made; the presence of much broader lines indicates aggregation.

- uniformity of linewidths. Unfolded proteins often have sharp resonances near random coil chemical shifts; aggregated proteins have broad lines. A few resonances from the His tag or termini is acceptable, but the majority should have relatively uniform and appropriate width.

- Further assessment can be done by collecting a 1H-1H NOESY spectrum, particularly looking at regions corresponding to NH to NH NOEs (indicative of helix), and aromatic to methyl NOEs (indicative of a hydrophobic core forming).

For proteins enriched in 15N the range that can be studied increases up to 250-300 amino acids (assuming monomeric state). Samples may be His6- tagged but preferably should not have a fused protein domain. Samples with ~100 micromolar concentration will have 1H-15N HSQC spectra measured. Factors to be assessed include:

- dispersion of 1H and 15N chemical shifts, a narrow range in 1H particularly is indicative of an unfolded protein.

- count HSQC correlation peaks, there should be one for each amino acid except proline, plus two for each glutamine or asparagine sidechain. A significant number of missing peaks is indicative of either aggregation or slow timescale conformational fluctuations in the protein.

- linewidths of the correlation peaks should be appropriate for the molecular weight, and should be relatively uniform in width.

For proteins with good HSQC properties a final check for NMR structure determination feasibility can be done with doubly 13C/15N labeled protein. Two dimensional projections of 3D HNCA and HNCO spectra can be collected.

- Count cross peaks; the projections onto the HN plane should have peaks equivalent to the 1H-15N HSQC.