Fully Reduced Granulin-B is Intrinsically Disordered and Displays Concentration-dependent Dynamics.

Gaurav Ghag[1], Lauren M. Wolf[2], Randi G. Reed[1], Nicholas P. van der Munnik[3], Claudius Mundoma[4], Melissa A. Moss[2,3] and Vijayaraghavan Rangachari[1].

[1]Department of Chemistry and Biochemistry, University of Southern Mississippi, Hattiesburg, MS 39406

[2]Biomedical Engineering Program and [3]Department of Chemical Engineering, University of South Carolina, Columbia, SC 29208

[4]Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306

SUPPLEMENTAL INFORMATION

EXPERIMENTAL PROCEDURES

Mass spectrometry. The purity of protein preparation was analyzed using MALDI-ToF mass spectrometry (Bruker Daltonics Inc). The sinnapinic acid (SA) matrix was prepared by resuspending 10 mg of SA (Sigma) in 1 mL of 1:1 acetonitrile:water with 0.1% TFA. One µL of protein sample was mixed with 1 µL of SA matrix such that the final protein concentration was in the range of 0.01-0.1 nM. The protein-matrix mixture was then spotted in duplicates (1 µL) on a MSP 96 microchip target (Bruker). The laser intensity and the detector gain were maintained between 50 and 70% laser and 3x detector gain. For alkylation of rGrnB with iodoacetamide, 40 mM iodoacetamide was added to a 20 µM rGrnB aliquot in the presence of 20 mM guanidine hydrochloride and incubated overnight in dark at 25 °C. The samples were subsequently spotted on MALDI-ToF MSP 96 microchip target as described above.

Fluorescence spectroscopy. Fluorescence measurements were performed on a Cary Eclipse spectrometer (Agilent Inc.). Spectral scans arising from intrinsic tryptophan fluorescence were measured by exciting the samples at 280 nm and monitoring emission from 320 to 400 nm. Excitation and emission slits were set at 10 or 20 nm. For ANS binding, the concentration of ANS was held constant at 500 µM as the protein was diluted from 25 µM to 1 µM. Scans were obtained by exciting the samples at 380 nm and monitoring emission from 410 to 600 nm, with excitation and emission slits set at 10 nm. For both tryptophan and ANS experiments, three scans were collected and averaged. The emission scans between 450 and 550 nm were integrated and the data were generated by plotting the integrated values as a function of protein concentration. The fluorescence vs. concentration data obtained was fit to the following equation for a monomer-dimer model using Origin 8.5 software.

$$F = \frac{2}{M} * \left\{F_0 - \left(F_0 - F_f\right) * \left(\frac{(4M + K_d) \pm \sqrt{(4M + K_d)^2 - 16M^2}}{8}\right)\right\} \qquad \textit{Eq 1}$$

Here, F is the fraction of dimer, M is the total rGrnB concentration, F_0 is initial fluorescence value, F_f is the final fluorescence value, and K_d is the dissociation apparent equilibrium constant.

Circular dichroism (CD). Far-UV CD spectra were obtained on a Jasco J-815 CD spectrometer. For protein samples with concentrations ≤100 µM, a 1 mm path length quartz cuvette (Hellma) was used, whereas for concentrations >200 µM, a 0.1 mm path length quartz cuvette (Precision cell) was used. To examine the effect of TMAO on rGrnB structure, a 0.1 mm path length quartz cuvette (Precision Cell) was used to minimize the scattering. The samples were monitored in a continuous scan mode from 260 to 198 nm with a scanning speed of 50 nm min^{-1} with a data integration time of 8 seconds, 1 nm bandwidth, and a data pitch of 0.1 nm. Each data set represents an average of 3 scans with appropriate blank subtraction as indicated in the results sections. The corrected scans were subjected to smoothening via the Savitzky-Golay algorithm (provided by the manufacturer) with the convolution width of 15 using the Jasco spectrum analysis program.

Analytical ultracentrifugation (AUC). AUC experiments were performed in a Beckman XL-I centrifuge (Beckman Coulter, Inc.) using absorbance optics by measuring intensity at 280 nm. The experiments were performed at 20 °C in two-channel Epon centerpieces with an AN60 Ti rotor at 55,000 rpm. The protein was solubilized in 20 mM Tris, pH 6.5. Data were analyzed using the UltraScan III version 3.3 software suite (1). Data were first analyzed via application of the two-dimensional spectrum analysis with simultaneous time invariant noise subtraction according to the method of Schuck and Demeler (2). After noise subtraction, the data were examined for heterogeneity with the enhanced van Holde−Weischet analysis (3). The partial specific volume at 20 °C of the rGrnB protein (0.7021cm^3 g^{-1}) was estimated from the peptide sequence, as described by Durchschlag (4). All the raw data were processed using SEDFIT program (3) to generate $c(s)$ distributions. The frictional coefficient (f/f_0) value of 1.4 was used for data processing and calculations.

SUPPLEMENTAL FIGURES

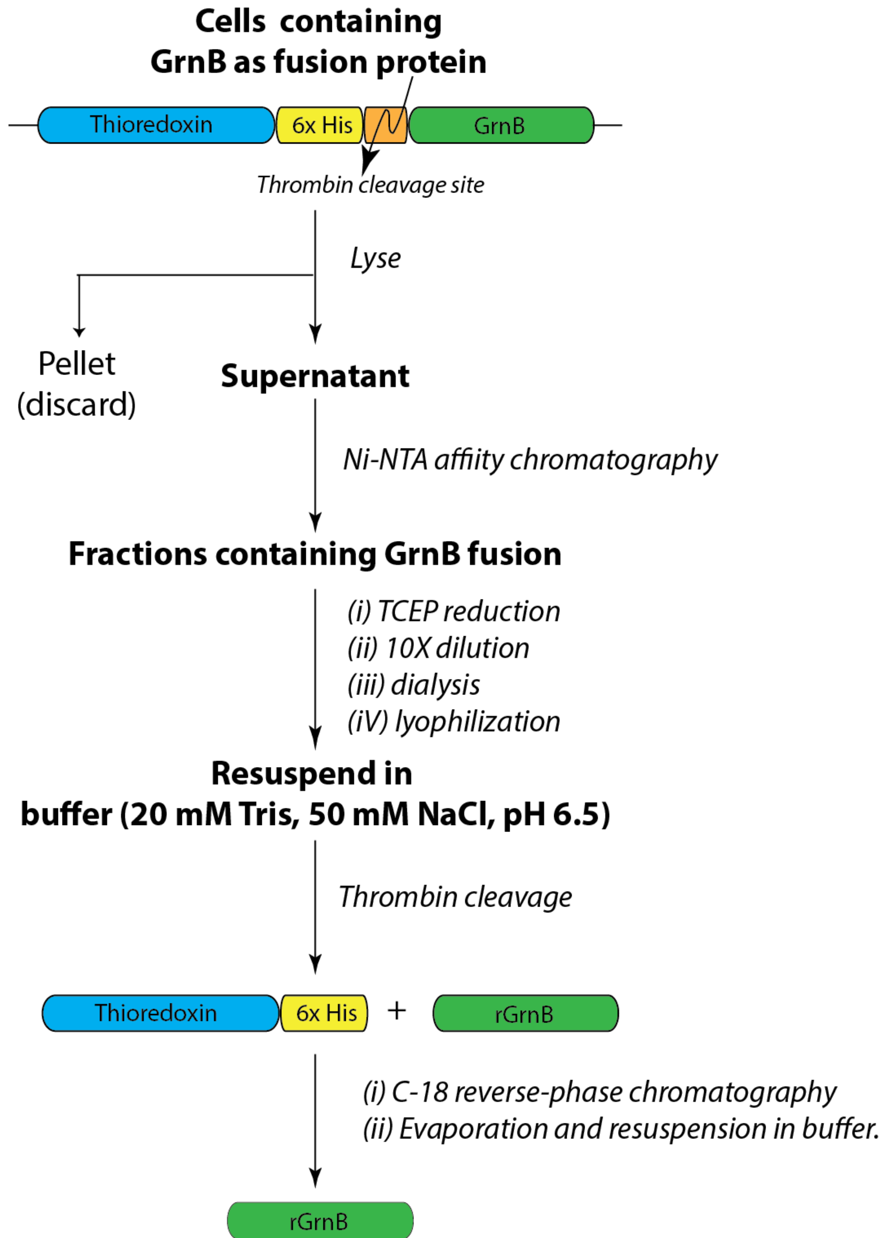

FIGURE S1: **Schematic of rGrnB purification.**

A

Ellman's Assay

Time	Blank (AU)	rGrnB (AU)	% free sulfhydryls
0 hr	0.1942	0.4797	95 %
6 hr	0.1677	0.4691	99 %

B

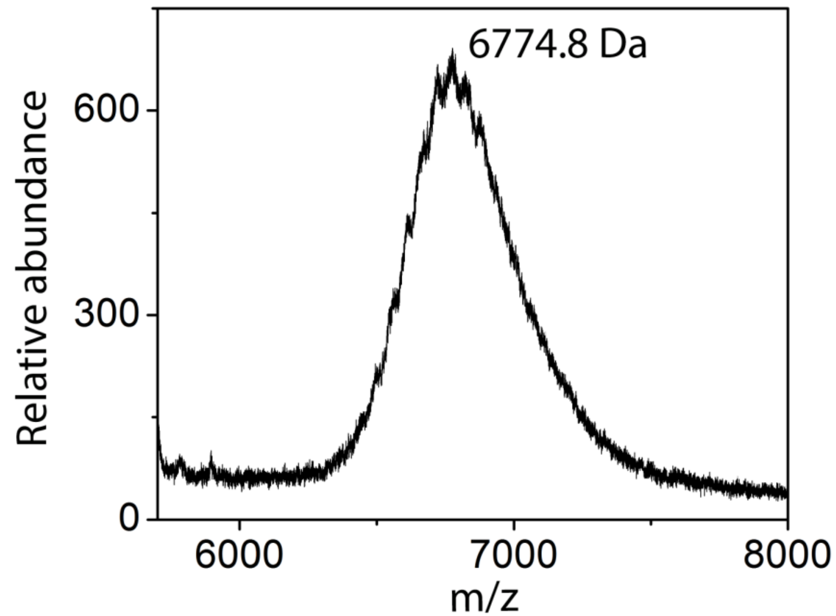

FIGURE S2: **Re-oxidation of rGrnB.** A) Ellmans assay results on potential re-oxidation of rGrnB within the experimental window. B) rGrnB was alkylated using iodoacetamide after incubation at 4 °C for 20 days, showing a signal corresponding to 6774.8 Da indicating presence of seven free sulfhydryls.

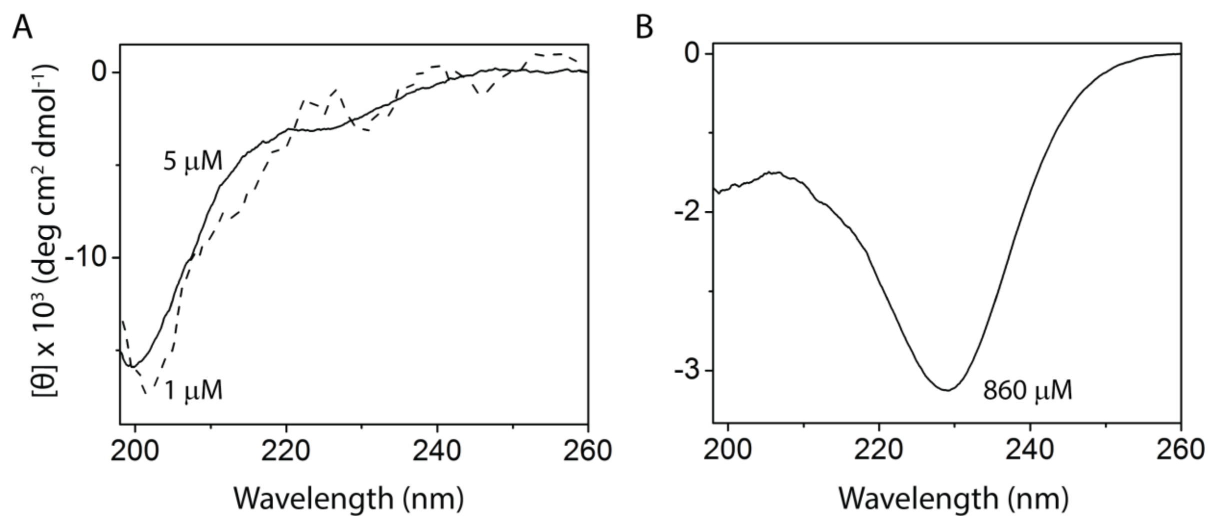

FIGURE S3: **Far-UV CD spectra of rGrnB at low and high concentrations in 20 mM Tris, pH 6.5.** *A,* 1 μM (dashed line) and 5 μM (continuous line), and *B,* 860 μM.

REFERENCES

1. Demeler, B. (2005) UltraScan: a comprehensive data analysis software package for analytical ultracentrifugation experiments. *Modern analytical ultracentrifugation: techniques and methods*, 210-229
2. Schuck, P., and Demeler, B. (1999) Direct Sedimentation Analysis of Interference Optical Data in Analytical Ultracentrifugation. *Biophysical Journal* **76**, 2288-2296
3. Van Holde, K., and Weischet, W. O. (1978) Boundary analysis of sedimentation-velocity experiments with monodisperse and paucidisperse solutes. *Biopolymers* **17**, 1387-1403
4. Durchschlag, H. (1986) Specific Volumes of Biological Macromolecules and Some Other Molecules of Biological Interest. in *Thermodynamic Data for Biochemistry and Biotechnology* (Hinz, H.-J. ed.), Springer Berlin Heidelberg. pp 45-128