ANALYTICAL ULTRACENTRIFUGATION

METHODS:

All sedimentation equilibrium experiments were performed with a Beckman Optima XL-I at the Center for Analytical Ultracentrifugation of Macromolecular Assemblies (CAUMA, the University of Texas Health Science Center at San Antonio, Dept. of Biochemistry). Equilibrium and Monte Carlo analyses were performed with UltraScan version 6.2 ([1]). Hydrodynamic corrections for buffer conditions were made according to data published by Laue et al[2], and as implemented in UltraScan. The partial specific volume of the DrM peptide was estimated according to the method by Cohn and Edsall[3], and as implemented in UltraScan and was found to be 0.72734 ccm/g. Data were fitted to multiple models. The most appropriate model was chosen based on visual inspection of the residual run patterns, and based on the best statistics. 95% confidence intervals were determined by Monte Carlo analysis. Monte Carlo analyses were calculated on a 40-processor Linux Beowulf cluster running Slackware Linux version 9.1. All samples were analyzed in a buffer containing 25 mM sodium phosphate buffer, pH 7.8 and 150 mM DTT. Sedimentation equilibrium experiments were performed at 4° C and speeds ranging between 18,000 – 40,000 rpm. Absorbance samples were spun in six-channel epon/charcoal centerpieces in the AN-50-TI rotor. Scans were collected at equilibrium at 230 nm and at 280 nm in radial step mode with 0.001 cm step size setting and 20-point averages. Multiple loading concentrations ranging between 0.3 - 0.7 OD were measured at the given wavelength, data exceeding 0.9 OD were excluded from the fit. Data in the concentration range between 0 and 34 micromolar were examined. Wavelength scans of were measured between 220 to 340 nm for each concentration and simultaneously fitted to a global extinction profile using UltraScan (Figure 1). The extinction profile was normalized with the extinction coefficient at 280 nm by estimation from the protein sequence according to the method by Gill and von Hippel[4] and as implemented in UltraScan. The extinction values determined in this fashion were used to correct the extinction at the measured wavelengths.

RESULTS:

Sedimentation Equilibrium: In order to establish the association properties of the processed and unprocessed form of the DrM protein we conducted sedimentation equilibrium experiments. To assure a good signal from both the monomer and dimer species, it is important to span a large concentration range with the experimental data. This can be accomplished by varying the loading concentration and the wavelength of the measurement, which exploits the various absorption properties of the protein. By globally

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1B. Demeler. UltraScan 6.2 - An integrated data analysis software package for sedimentation experiments. (2004) University of Texas Health Science Center at San Antonio, Dept. of Biochemistry. http://www.ultrascan.uthscsa.edu

2Laue, T. M., Shah B. D., Ridgeway, T.M., Pelletier, S. L. Computer-aided interpretation of analytical sedimentation data for proteins. *Analytical Ultracentrifugation in Biochemistry and Polymer Science.* 90-125 Edt. S. E. Harding, A. J. Rowe, and J. C. Horton. Cambridge, Royal Society of Chemistry, 1992

3Cohn, E. J., Edsall, J. T. *Proteins, Amino Acids and Peptides as Ions and Dipola Ions.* New York, Reinhold. 1943

4Gill, S. C. and von Hippel, P. H. Calculation of protein extinction coefficients from amino acid sequence data. *Analytical Biochemistry* **182**:319-326 (1989)

fitting data observed under multiple conditions, such as multiple rotor speeds and multiple loading concentrations, it is possible to enhance the confidence in each fitted parameter value (Johnson et al., 1981[5]). In such a fit, parameters such as monomer molecular weight and association constants are considered global parameters and forced to be the same for all included datasets. In order to compensate for the different absorption properties at different wavelengths, we measured wavelength scans between 220 nm and 350 nm with 1 nm intervals in triplicate, with 20 repetitions for each data point, using the analytical ultracentrifuge. The wavelength scans were globally fitted to a sum of Gaussian terms, whose width, amplitude and offset was allowed to float but considered global for all scans. Individual concentrations were adjusted by floating the amplitude of the sum for each scan (Figure 1). The resulting extinction profile was normalized by using an estimated extinction coefficient at 280 nm from the sequence of the denatured protein by the method of Gill and von Hippel [4]. The extinction coefficient for DrM protein was found to be 292,872 OD/mol at 230 nm and 27,284 OD/mol at 280 nm.

For the unprocessed sample, the experimental data could be well described by a single ideal species model with a molecular weight of 34.84 kD (+0.30/-0.28 kD). This finding is in good agreement with the theoretical molecular weight of 36.6 kD based on amino acid sequence. A plot of the residuals and overlays for this fit is shown in Figure 2. Data from the processed sample could best be described by a monomer-dimer model, with a monomer molecular weight of 31.56 kD (+0.71/-0.86 kD) and a dissociation constant of 272 nM (+129/-154 nM). A plot of the residuals and overlays for this fit is shown in Figure 3. A plot of the relative concentration distribution of monomer and dimer at different total concentrations is shown in Figure 4. A fit to a single ideal species model resulted in poor residuals and a higher variance (data not shown).

5Johnson, M. L., Correia, J. J., Yphantis, D. A., and Halvorson, H. R. (1981) Analysis of Data from the Analytical Ultracentrifuge by Nonlinear Least Squares Techniques. Biophysical Journal **36**, 575-588

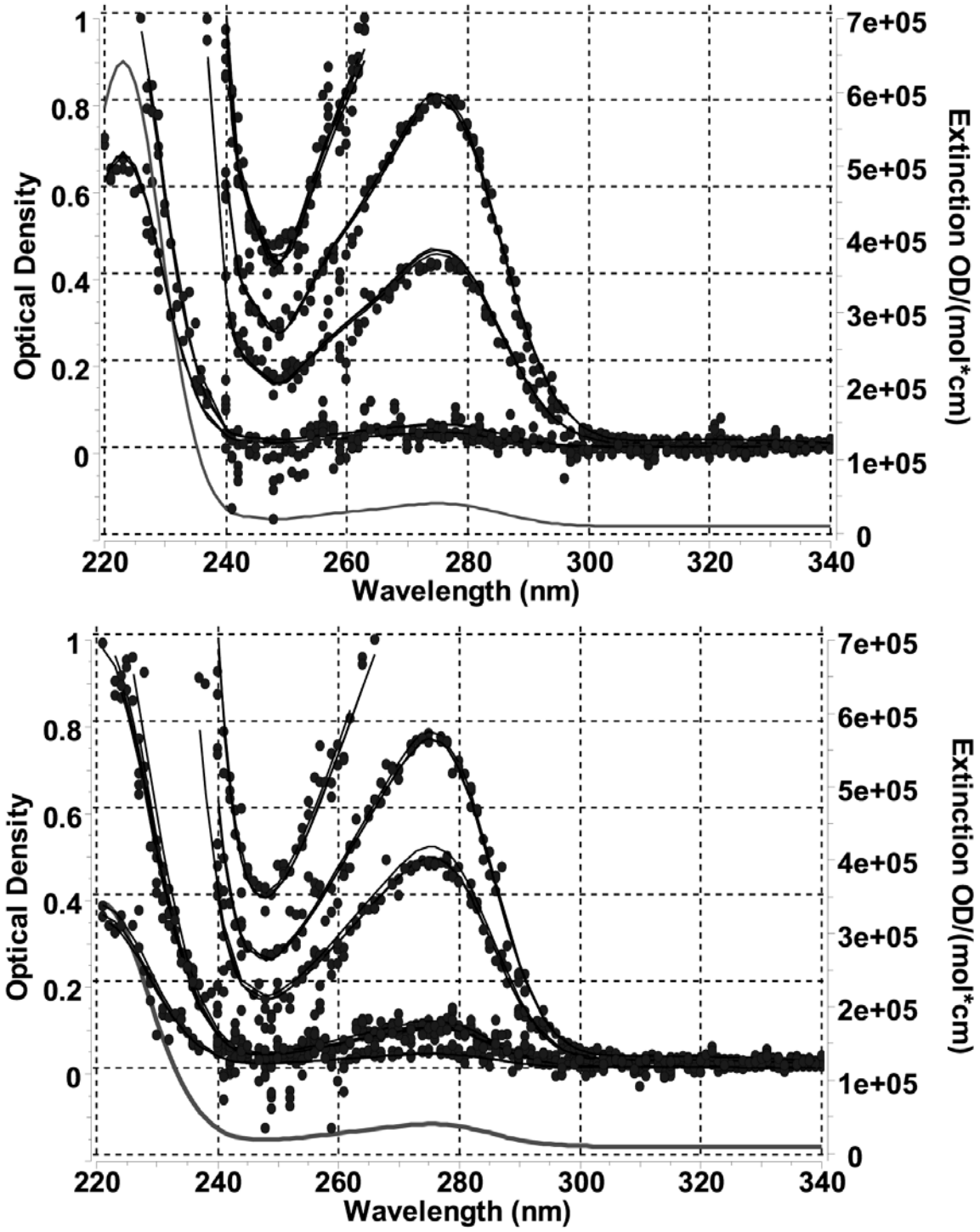

Figure 1: Globally fitted extinction profile for DrM. The processed sample is shown on the top, on the bottom the unprocessed sample is shown. The red line indicates the globally fitted extinction profile corrected for molar extinction, the blue dots identify absorbance measurements of samples at different concentrations, and the black lines indicate the individual fits of the measured absorbances. Concentration points above one OD are excluded from the analysis.

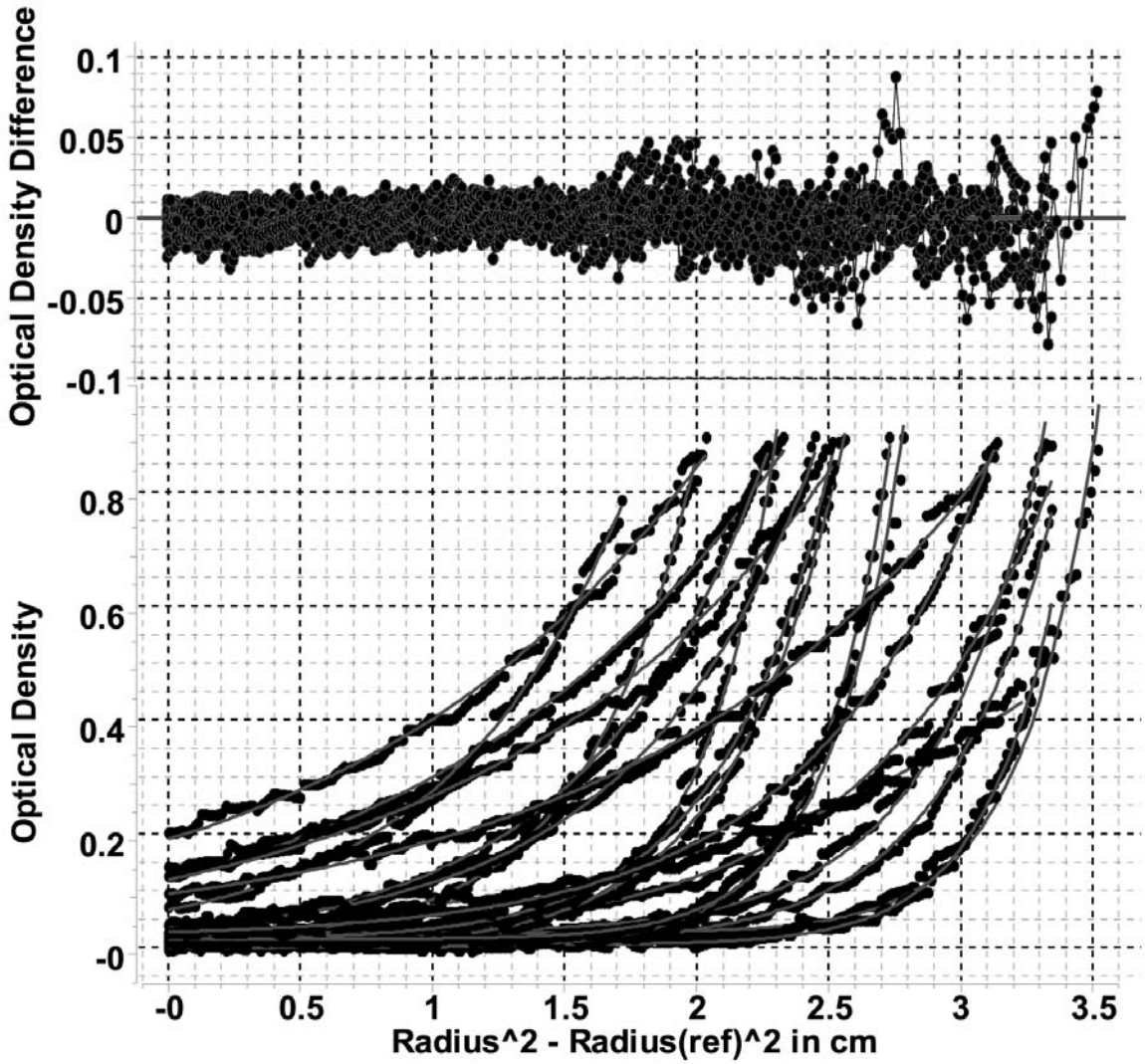

Figure 2: DrM (unprocessed) fitted to a global single, ideal species model. Residuals of the fit are shown on the top, overlays on the bottom. Black points represent experimental data, red lines represent the fitted model. 20 scans of different loading concentrations and speeds were measured.

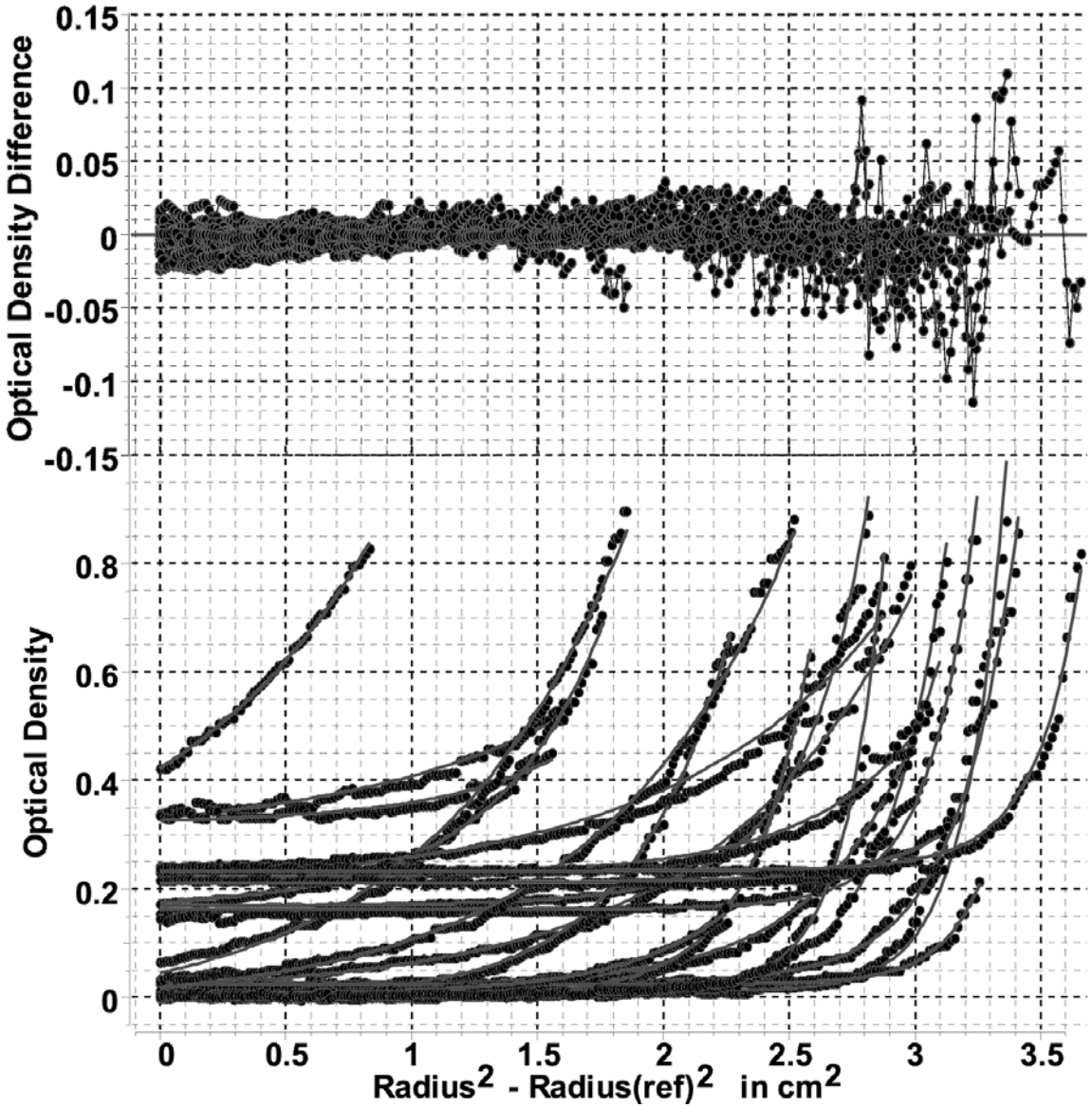

Figure 3: DrM (processed) fitted to a global monomer – dimer model. Residuals of the fit are shown on the top, overlays on the bottom. Black points represent experimental data, red lines represent the fitted model.

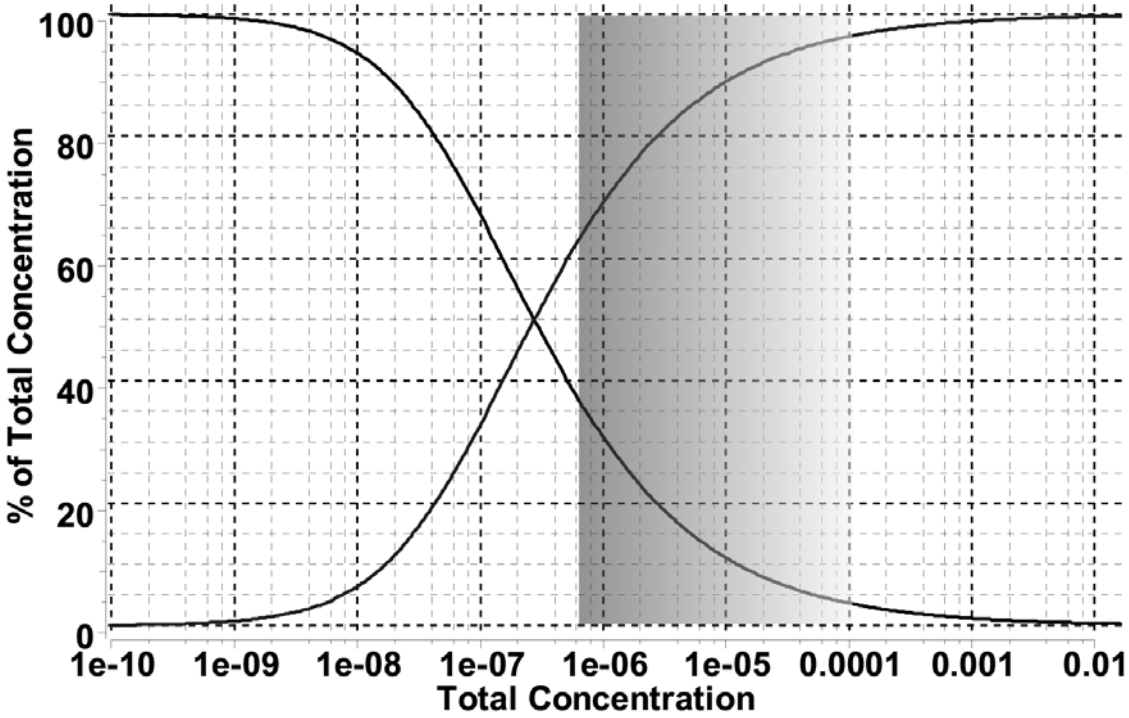

Figure 4: Relative distribution of monomer and dimer versus molar concentration. The blue line indicates the relative concentration of the dimer, the black line represents the relative concentration of the monomer. The grey shaded area indicates the region of concentration measurements in the analytical ultracentrifuge, the darkness of the grey indicates the relative number of datapoints available for fitting. The sensitivity of the UV absorbance optics is limited to a DrM concentration of approximately 300 nM. From this plot it is clear that most datapoints in this experiment provided a good signal for the dimer species, less so for the monomer species.

Acknowledgements: The development of the UltraScan software is supported by the National Science Foundation through grant DBI-9974819 to B. Demeler