**Biophysical Journal**

**Supporting Material**

# Variable-Field Analytical Ultracentrifugation: I. Time-Optimized Sedimentation Equilibrium

Jia Ma,[1] Michael Metrick,[1] Rodolfo Ghirlando,[2] Huaying Zhao,[1] and Peter Schuck[1,*]

[1]Dynamics of Macromolecular Assembly Section, Laboratory of Cellular Imaging and Macromolecular Biophysics, National Institute of Biomedical Imaging and Bioengineering and [2]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland

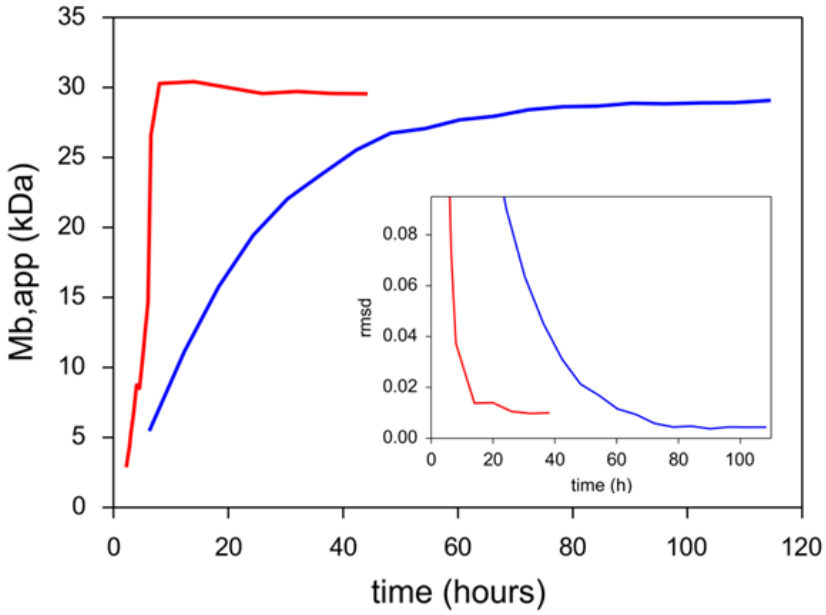


**Figure S1:** Approach to equilibrium for a 162bp DNA in a 4.7 mm solution column at 7,000 rpm in a conventional constant speed SE (blue) and toSE experiment (red), optimized with an over-concentration factor 1.5. Buoyant molar mass values were calculated with a single species model fitted to consecutive scans, using a fixed common baseline. The inset shows the rmsd of consecutive scans relative to the last scan for conventional SE (blue) and toSE (red).

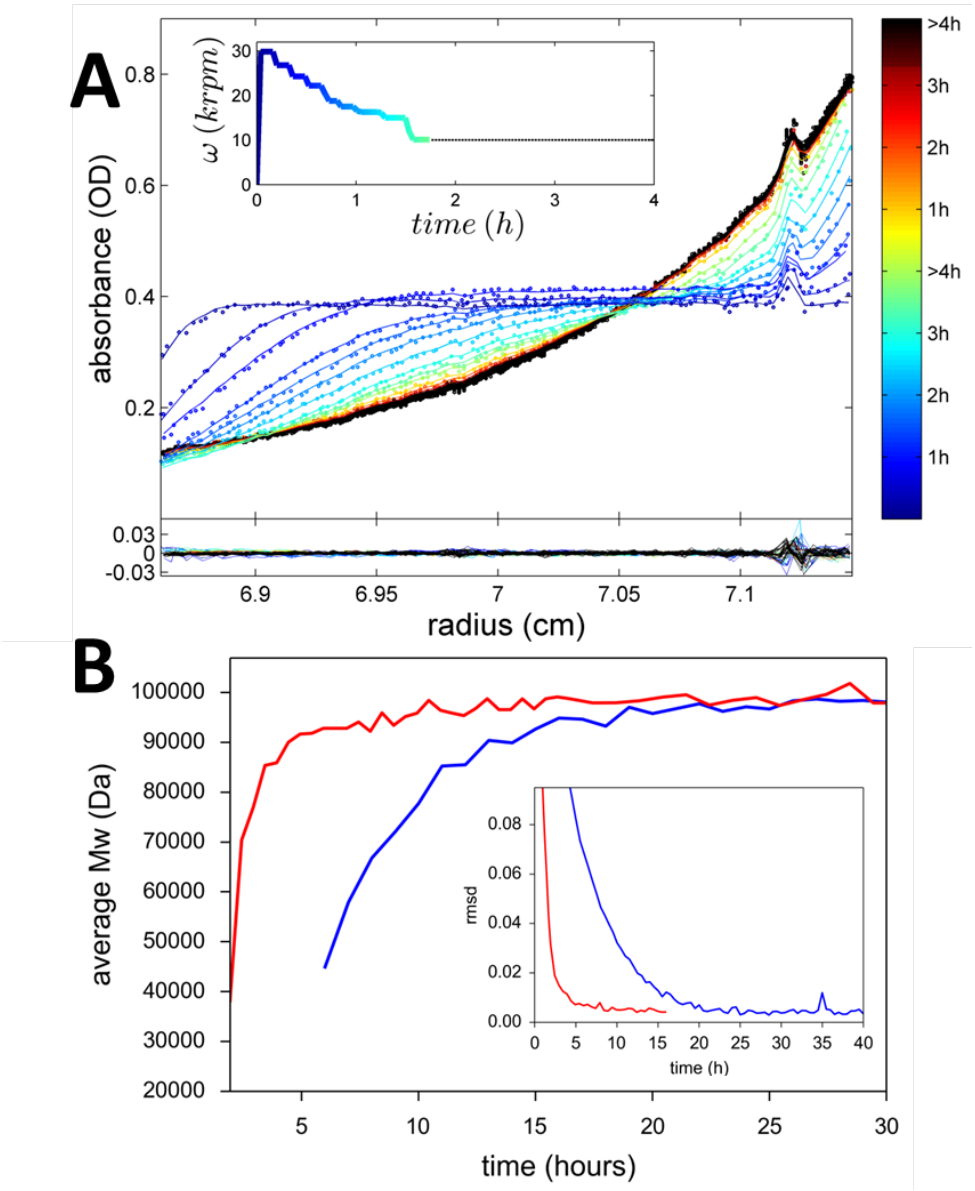


**Figure S2:** Application of toSE to shorter solution columns: Shown are data from an equivalent experiment to that of **Figure 3** and **Figure S2**, but with a shorter solution column of 130 µL (3.5 mm) and a final rotor speed of 10,000 rpm. (A) Absorbance scans at 280 nm were acquired at each step in the rotor speed profile shown in the inset, and radially translated to compensate for differential rotor stretching. Experimental scans (dots) and best-fit with the *c*(*s*) model (lines) are colored as indicated in the sidebar to depict the passage of time. The residuals are shown in the lower plot, with rmsd of 0.0041 OD. (B) The approach to equilibrium is visualized by the apparent average molar mass obtained for data in the accessible radial range at different times (red). For comparison, also shown are the results from a conventional SE experiment under equivalent conditions with constant rotor speed (blue). The inset shows the time-course of the rmsd difference of individual scans to the last scan for the toSE experiment (red), and the conventional approach to SE at constant speed (blue).

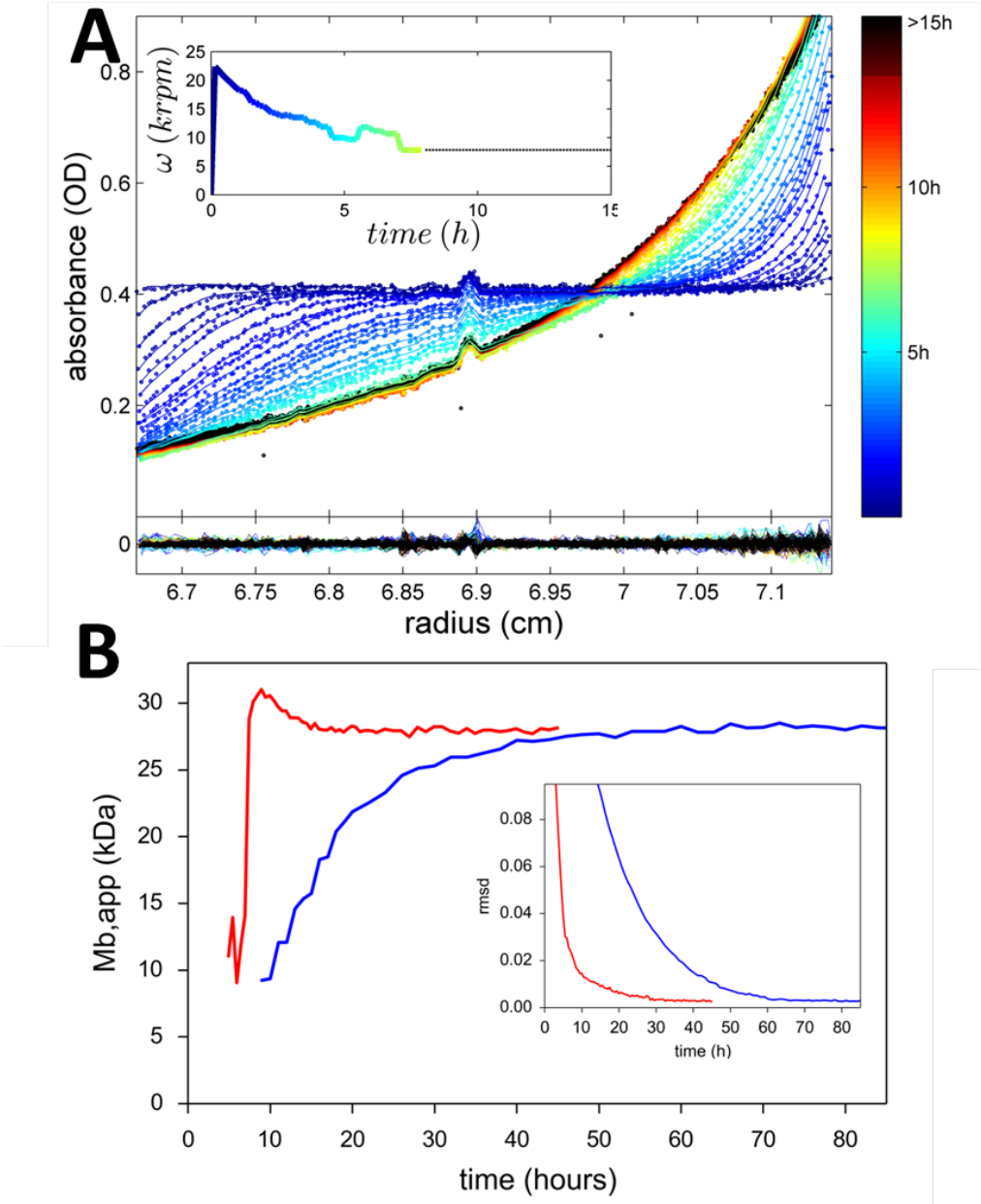


**Figure S3:** Long-column toSE experiment with BSA in a 200 µL, 7 mm solution column brought to equilibrium at 7,800 rpm at 20 °C. (A) Experimental scans (dots) and best-fit from the *c*(*s*) model (lines) using the color scheme in the sidebar to indicate passage of time. Scans were radially translated to compensate for differential rotor stretching. The inset depicts the time-dependent rotor speed obtained after toSE optimization with maximal calculated over-concentration of 70%. The residuals are shown in the lower plot, with rmsd of 0.0027 OD. (B) The approach to equilibrium is visualized by the apparent average buoyant molar mass obtained for data in the accessible radial range at different times (red). For comparison, also shown are the results from a conventional SE experiment under equivalent conditions with constant rotor speed (blue). The inset shows the time-course of the rmsd difference of individual scans to the last scan for the toSE experiment (red), and the conventional approach to SE at constant speed (blue).

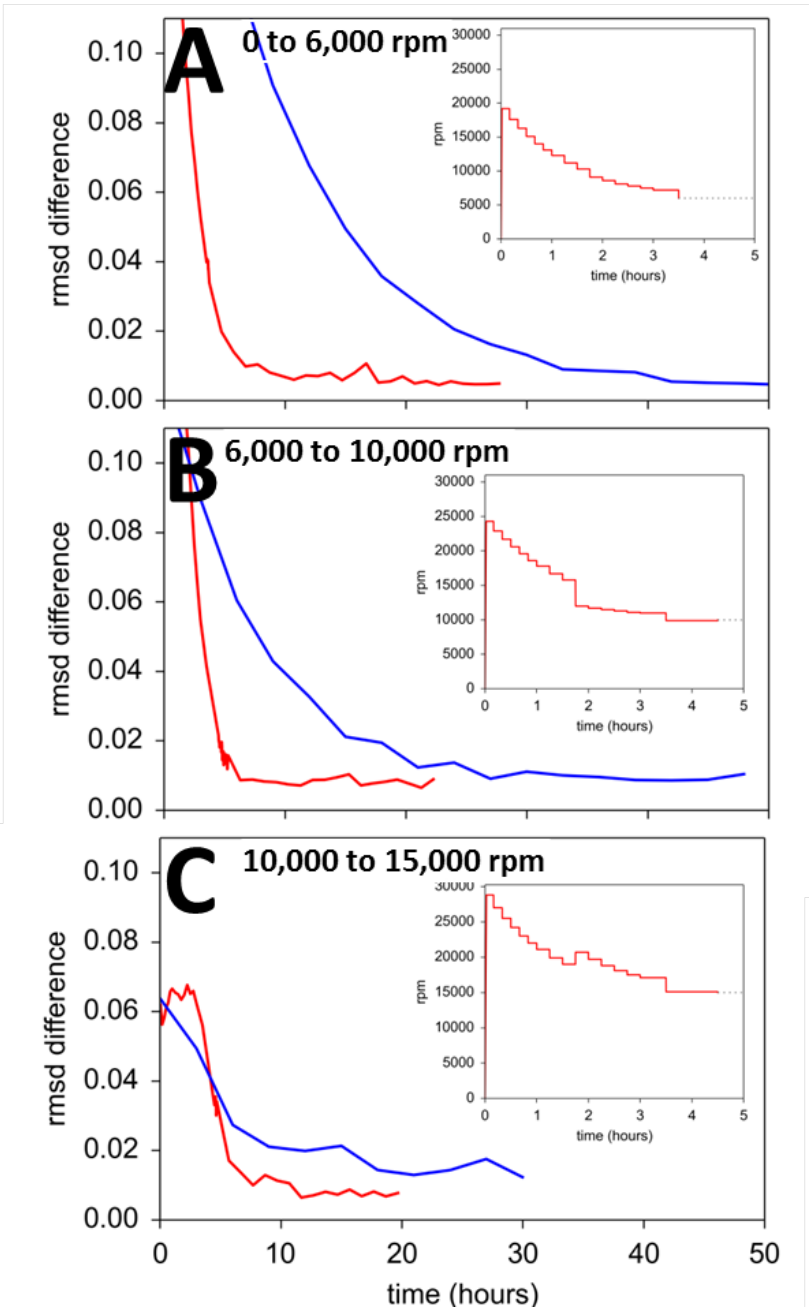


**Figure S4:** Sequential approach to multiple equilibria for BSA at rotor speeds of 6,000 rpm (A), 10,000 rpm (B), and 15,000 rpm (C). Experimental conditions were as in **Figure 3**, with the toSE optimized speed profiles as shown in the insets. Shown are the rmsd of scans at different points in time relative to the last scan for the toSE experiment (red) and the conventional constant speed experiment (blue).