***Supporting Information*: Early stages of misfolding and association of β_2-microglobulin – insights from infrared spectroscopy and dynamic light scattering**

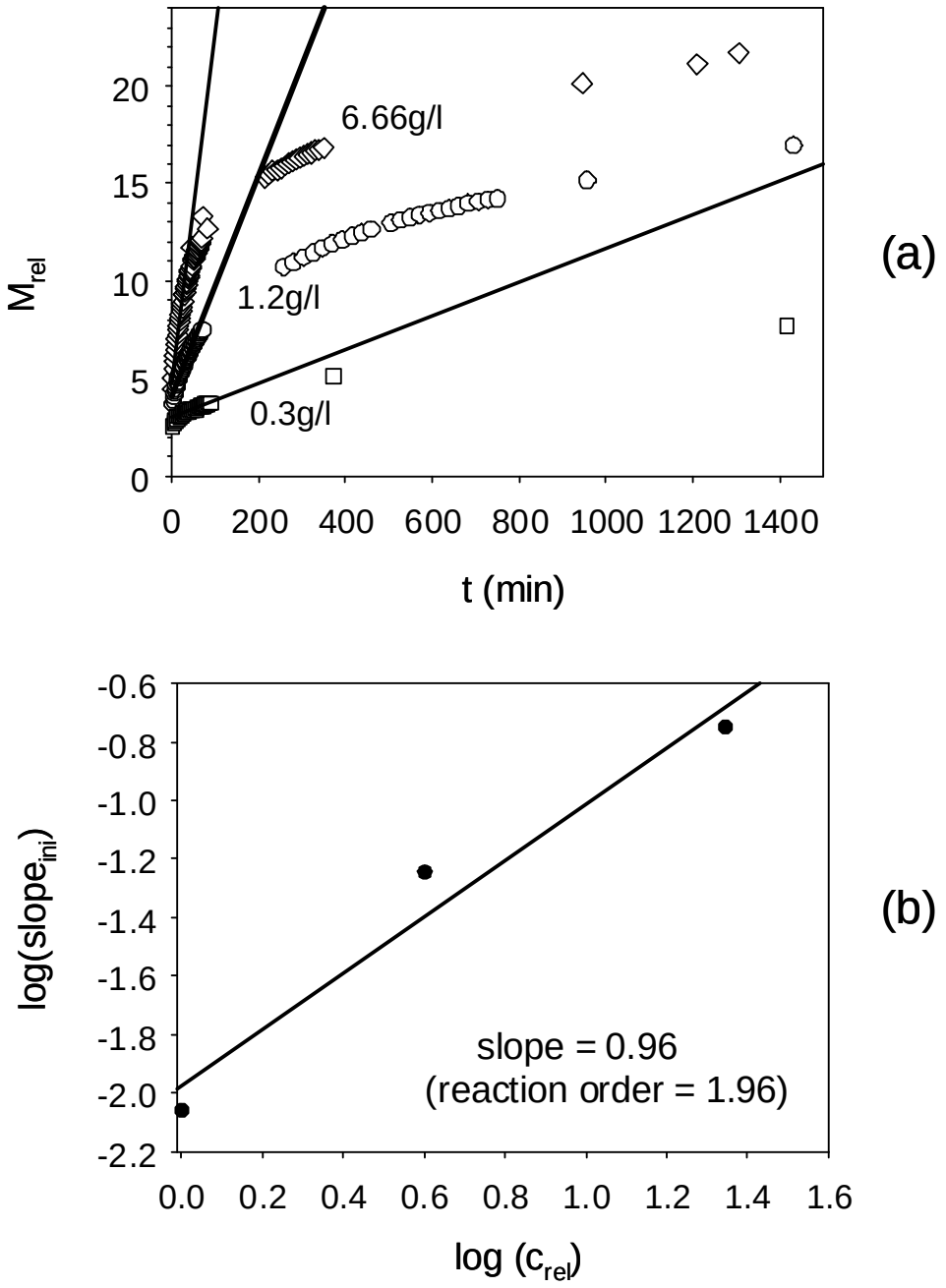
Heinz Fabian, Klaus Gast, Michael Laue, Rolf Misselwitz, Barbara Uchanska-Ziegler, Andreas Ziegler, and Dieter Naumann

1. Dynamic Light Scattering (DLS)

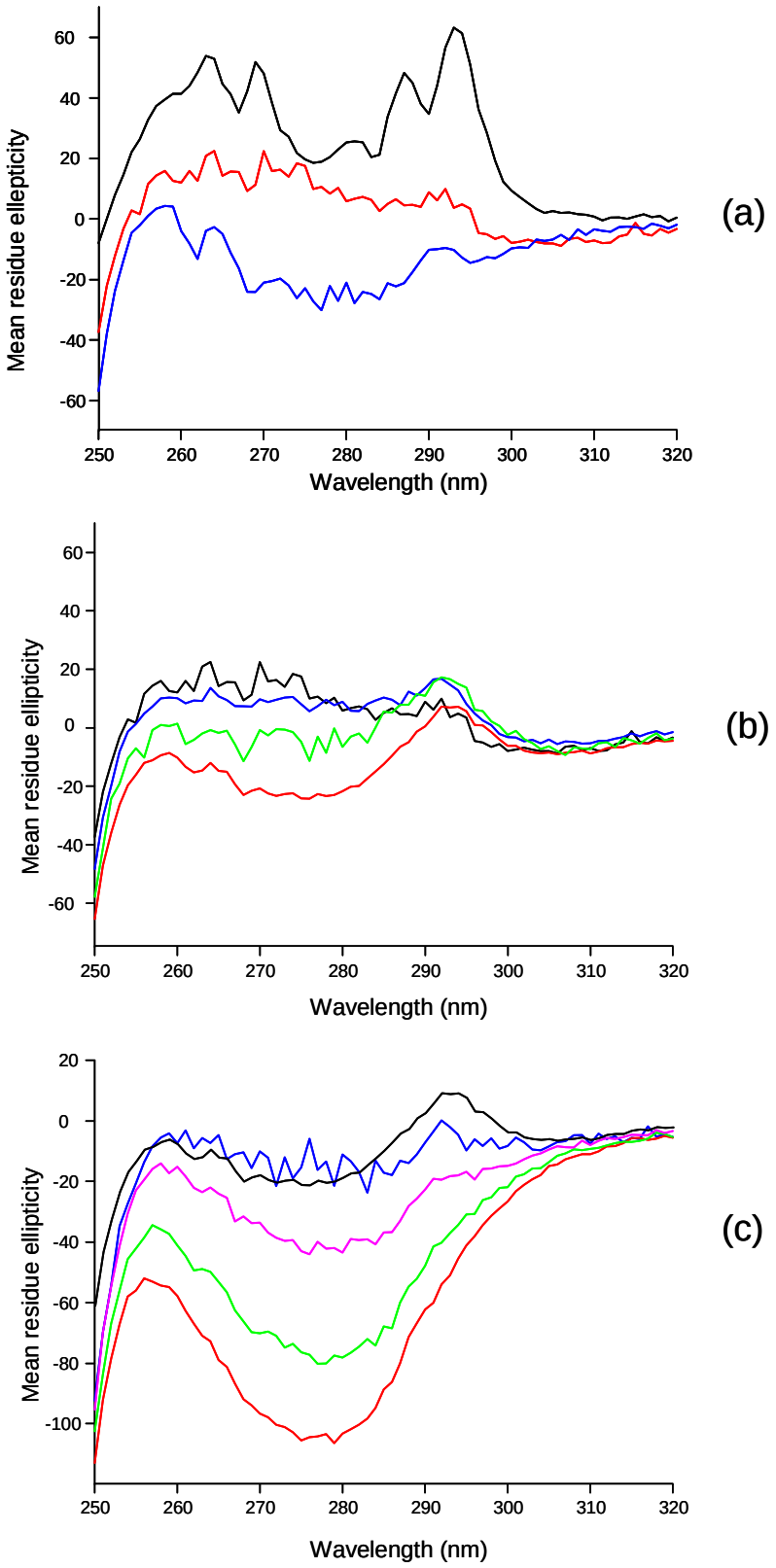
DLS data were measured at a scattering angle of 90° with a laboratory-built apparatus specifically developed for kinetic experiments as described elsewhere (1). $M_{rel} = M_{app}/M_{aa}$ was used as a measure of the state of aggregation of the protein. Apparent molecular masses (M_{app}) were estimated from the relative scattering intensities using toluene as a reference sample, while M_{aa} was the molecular mass calculated from the amino acid composition (11.86 kDa).

2. Circular Dichroism (CD)

CD experiments were performed on a Jasco J715 CD spectrometer at a bandwidth of 1 nm. CD signals were recorded in quartz cells with an optical pathlength of 0.5 cm. The sample temperature was stabilized by means of a Peltier temperature controller. Prior to the measurements of the changes of the CD spectra during association, the CD spectrum of acid-unfolded β_2m was first recorded at a protein concentration of 1.5 mg/mL. Then, a stock solution of 1 M NaCl in 10 mM DCl was rapidly mixed with the sample in the cell to yield a final concentration of 0.2 M NaCl, and a protein concentration of 1.2 mg/mL. The CD spectra of the solution were subsequently recorded after addition of sodium chloride using the interval scan mode of the Jasco software. The temperature jump from 25 to 50°C was established by inserting the sample cell into the pre-heated sample holder, and the temporal changes of the CD spectrum were recorded as described. Since higher time-resolution is needed right after changes in salt concentration or temperature, the early spectra have a lower signal-to-noise ratio than the final spectra.



Supplementary Figure S1. (a) Particle growth of β_2m in the presence of 0.2 M NaCl at high (6.6 mg/mL), medium (1.24 mg/ml), and low (0.3 mg/mL) protein concentrations as monitored by DLS over 24 hours. The straight lines indicate the result of a fit to estimate the relative mass increase during the initial growth phase at the corresponding conditions. (b) Plot of the initial slopes of the relative mass increase versus the corresponding protein concentrations.

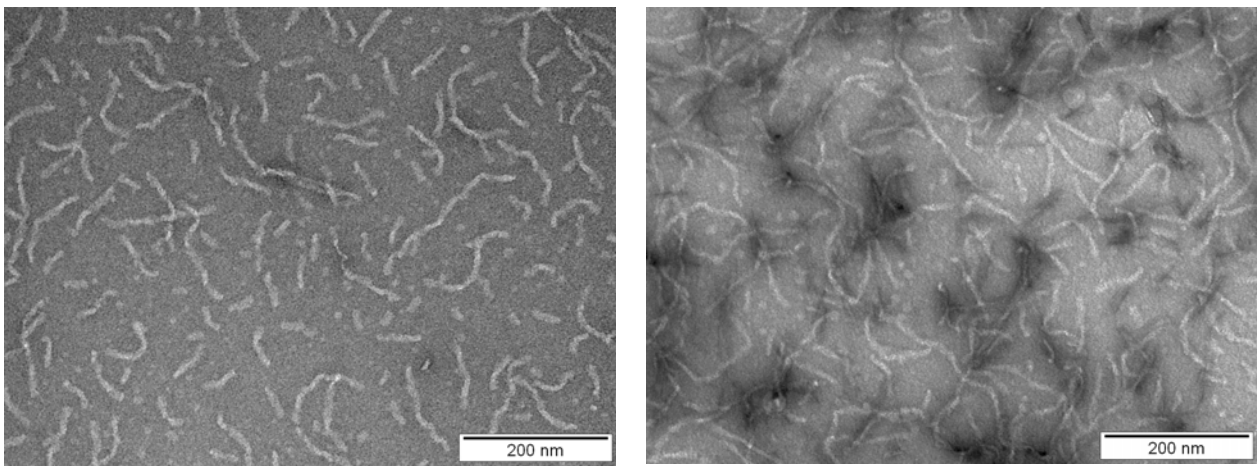


Supplementary Figure S2. (a) Near-UV CD spectra of different conformational states of β_2m: (a) CD spectra at native β_2m pH 7.5 (black), acid-unfolded β_2m in 2 mM DCl (blue),

and β_2m 3 minutes after addition of NaCl (red). (b) Near-UV CD spectra of β_2m recorded in the presence of 0.2 M NaCl 3 minutes (black), 1h (blue), 4 hours (green), and 48 hours (red) after addition of salt. (c) Near-UV CD spectrum of the salt-induced particles of β_2m (here termed ("A-type" structures) at 25°C (black), together with the near-UV CD spectra obtained at 7 minutes (blue), 2 hours (pink), and 6 hours (green) after a temperature jump from 25 to 50°C. The red trace is the near-UV CD spectrum of the temperature-induced new conformational state of β_2m (here termed "B-type" structure) recorded after 20 hours at 50°C. The CD spectra reveal major differences between the tertiary structures of the "A-type" and "B-type" particles (compare the black and red traces in Figure S2c).

3. Electron Microscopy (EM)

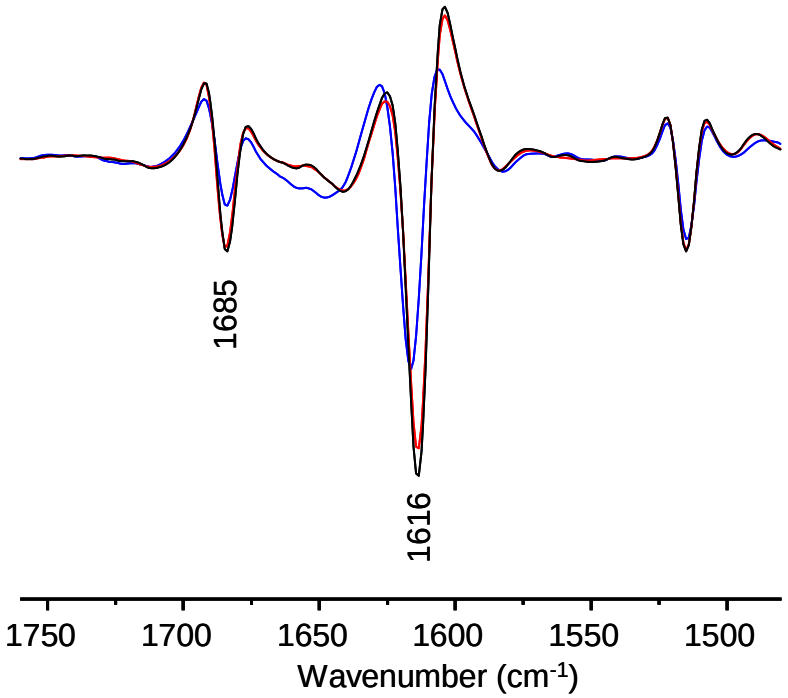
Samples were spread on carbon-coated grids, negatively stained with 1% uranyl acetate, and examined under a transmission electron microscope (Tecnai Spirit, FEI Co., USA) with an acceleration voltage of 120 kV.



Supplementary Figure S3. EM micrograph of "B-type" particles obtained after 20 hours at 50°C (left side) and after keeping a corresponding sample for eleven months at room temperature (right side), revealing practically no changes in morphology over the time.

4. Infrared Spectra (IR)

The protein samples were placed into demountable CaF_2 IR-cells with an optical pathlength of 50µm and the IR spectra were recorded with a Bruker IFS-66 Fourier transform infraredspectrometer equipped with deuterated triglycine sulphate detector and continuously purged with dry air. For each sample, 128 interferograms were co-added and Fourier-transformed to yield spectra with a nominal resolution of 4 cm^{-1}. The sample temperature was controlled by means of a thermostated cell jacket. Solvent spectra were recorded under identical conditions and subtracted from the spectra of the protein at the relevant temperature.



Supplementary Figure S4. Second derivatives of the IR spectra of the "B-type" particles before heating at 25°C (black), at 90°C (blue), and after cooling from 100°C back to 25°C (red), indicating that the structure of the "B-type" species obtained after mild heat treatment of the "A-type" form was much more stable than that of the initial short assemblies, and not amenable to further structural changes.

REFERENCES

1. Gast, K., Nöppert, A., Müller-Frohne, M., Zirwer, D., and Damaschun, G. (1997) Stopped-flow dynamic light scattering as a method to monitor compaction during protein folding, *Eur. Biophys. J. 25*, 211-219.