SUPPLEMENTAL ITEMS

FIGURE S1, related to FIGURE 3

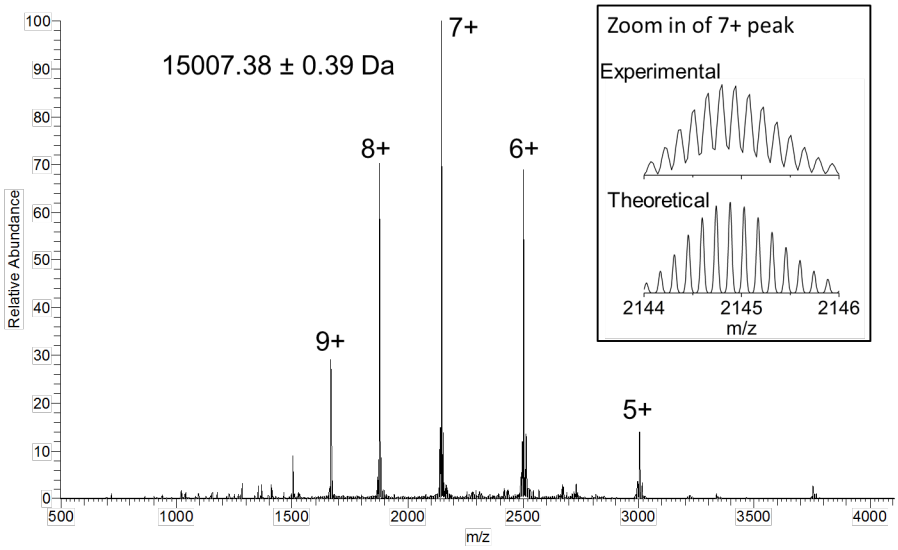

Figure S1, related to Figure 3. Mass spectrum of 95 uM Bsc4 EC1118 in 100 mM ammonium acetate and 1 mM DTT acquired on a Thermo Exactive Plus EMR Orbitrap mass spectrometer with 100 V HCD voltage in order to produce monomer. Inset: the zoomed in 7+ peak matches well with the theoretical isotopic distributions calculated from the sequence of the Bsc4 subunit with a processed N-terminal methionine.

FIGURE S2, related to FIGURE 3

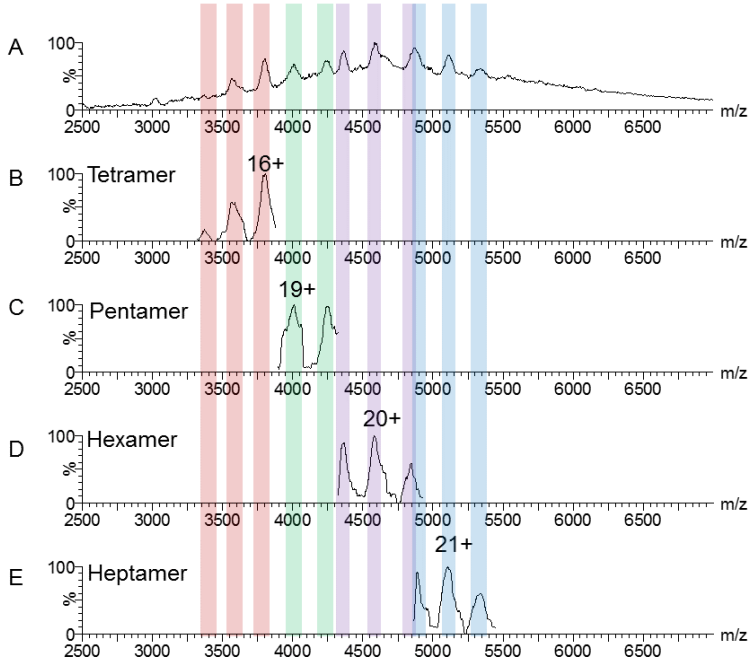

Figure S2, related to Figure 3. (A) Mass spectrum corresponding to the IM-MS spectrum in Figure 3 with (B) tetramer, (C) pentamer, (D) hexamer and (E) heptamer spectra extracted.

FIGURE S3, related to FIGURE 3

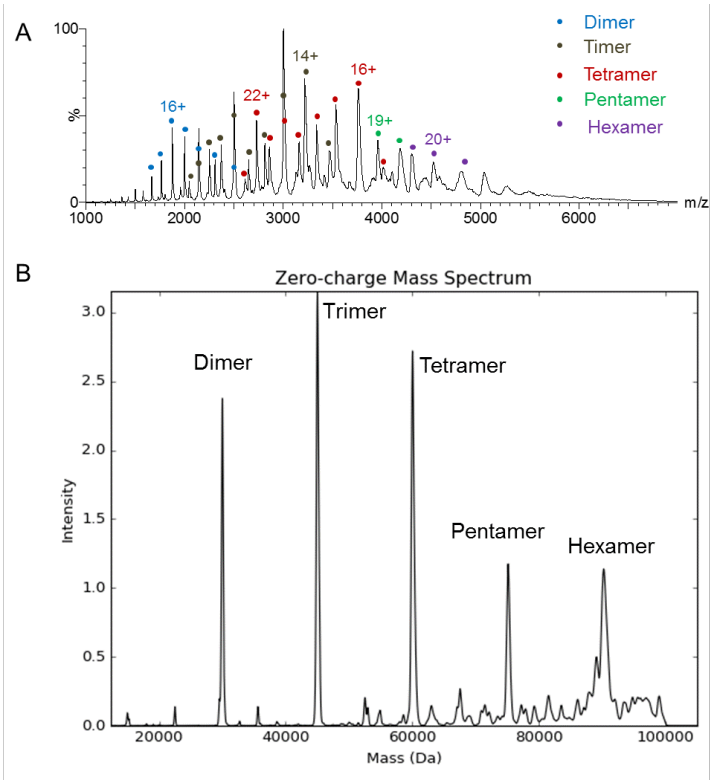

Figure S3, related to Figure 3. (A) Mass spectrum acquired from 255 µM Bsc4 in 100 mM ammonium acetate and 1 mM DTT indicates a continuous distribution of oligomers from dimer to hexamer in this sample. A 100 V cone voltage was used. (B) Deconvoluted 100 V spectrum shows that a distribution of oligomeric states from dimer to hexamer can be observed. The Unidec software was used for deconvolution (Marty et al., 2015). As noted in the main text, the source sample for this run had an apparent oligomer size of tetramer as judged by size exclusion chromatography.

FIGURE S4, related to FIGURE 2

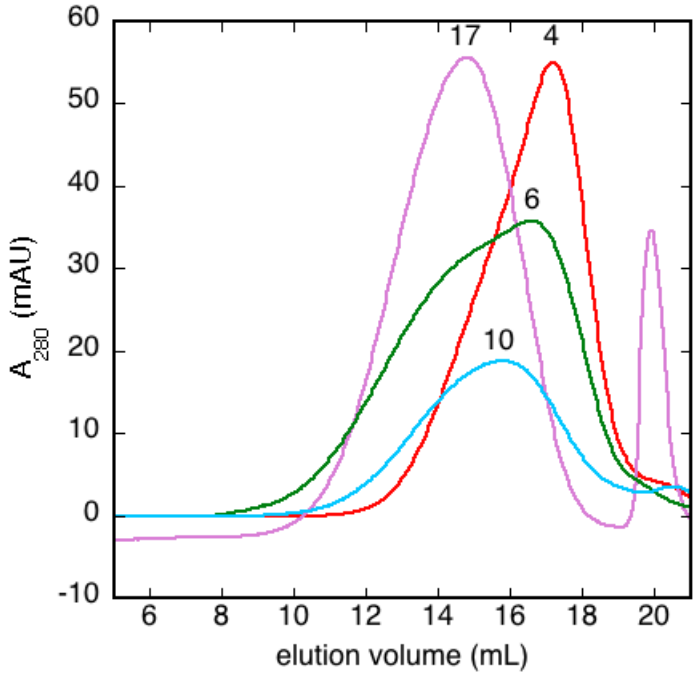

Figure S4, related to Figure 2. Size exclusion chromatograms of affinity-purified Bsc4 S288C with N-terminal affinity tag, refolded by dialysis from 6 M guanidine into 50 mM MES (pH 5.5), 100 mM KCl (red); 50 mM MES (pH 5.5), 250 mM KCl (purple); 50 mM Tris (pH 7.5), 100 mM KCl (green); or 50 mM Tris (pH 7.5), 250 mM KCl (cyan). All solutions contained 0.2 mM EDTA, plus 1 mM TCEP as a reducing agent. To show estimated oligomer sizes, peaks are annotated with nearest integral number of Bsc4 subunits, based the molecular weight calculated from a five-protein calibration curve (see Methods). Initial concentration for refolding was 280 μM. Comparison to Figure 2 suggests that placement of the affinity tag at the C-terminus may somewhat modulate the effect of the hydrophobic C-terminal tail in S288C on oligomerization and aggregation.

FIGURE S5, related to FIGURE 2

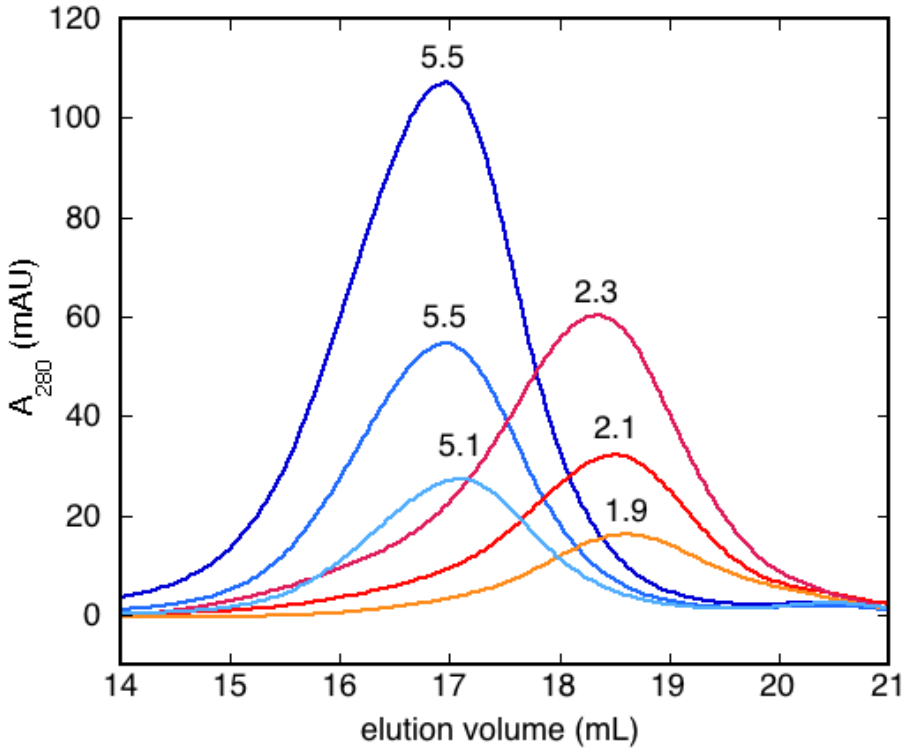

Figure S5, related to Figure 2. Superose 6 size exclusion traces of Bsc4 variants refolded in 50 mM MES (pH 5.5), 100 mM KCl, 1 mM TCEP, 0.2 mM EDTA at a range of initial protein concentrations: Bsc4 S288C (long form) at 40 μM (orange), 85 μM (red) and 170 μM (magenta); Bsc4 EC1118 (short form) at 70 μM (cyan), 140 μM (blue) and 280 μM (dark blue). Peaks are annotated with calibrated numbers of subunits corresponding to the elution volume of the peak maximum; i.e. the apparent predominant form of Bsc4 EC1118 in these experiments is pentamer to hexamer. The results show that even under conditions which disfavor large oligomers (low pH/low salt), Bsc4 is still predominantly oligomeric down to at least the 10^{-4}-10^{-5} M range of protein concentration. The fairly broad peak shape and slight increase in apparent size with refolding concentration are both consistent with a narrow distribution of oligomer sizes, as opposed to either a single oligomeric state or a complete lack of oligomerization specificity.

FIGURE S6

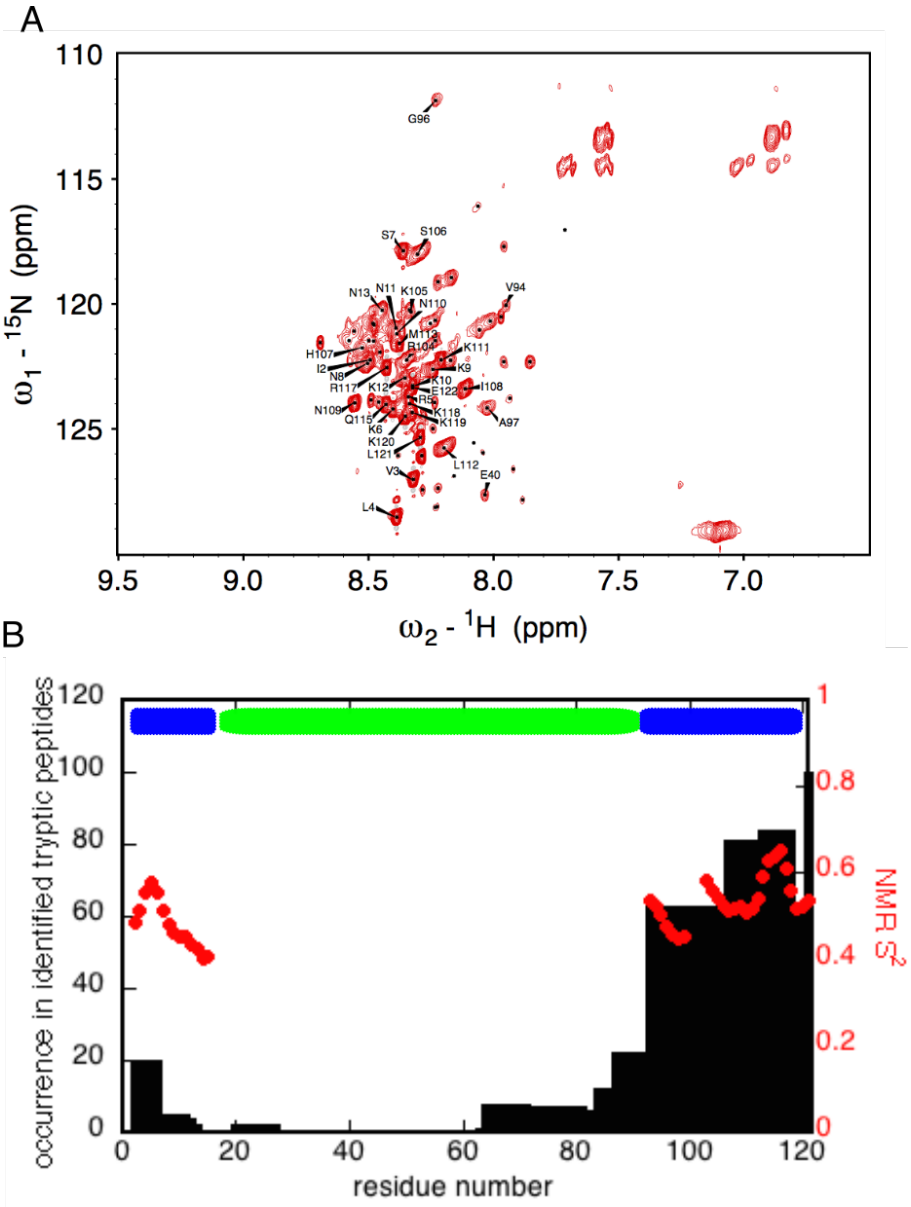

Figure S6, related to Figure 1. Putatively unfolded regions of Bsc4 EC1118. (A) ^{15}N-^{1}H correlation spectrum of 1 mM ^{13}C/^{15}N/^{2}H-labelled Bsc4 EC1118 in 50 mM MES (pH 5.5), 50 mM KCl, 1 mM TCEP, with strong, assignable resonances (from putatively unfolded regions) labeled. (B) Probable folded (green) and unfolded (blue) regions of Bsc4, based on NMR S^2 order parameters (red) and peptide identifications (black bars) in a limited trypsinolysis of Bsc4 EC1118. Black bars represent the number of times a residue occurred in tryptic peptides identified by MS-MS analysis of Bsc4 EC1118 incubated with Mag-Trypsin for 10 min (see Methods). S^2 order parameters were derived from partial backbone chemical shift assignments using TALOS-N. $S^2 < 0.7$ tend to indicate dynamic, disordered regions. Some residues with S^2 values shown in the bottom panel do not have assigned amide resonances in the top panel; these S^2 values were based on carbon shift assignments for proline or other resonances correlated to adjacent residues with assignable amides. This analysis agrees well with disorder prediction by JRONN (Figure 1).

FIGURE S7, related to FIGURES 6 and 7

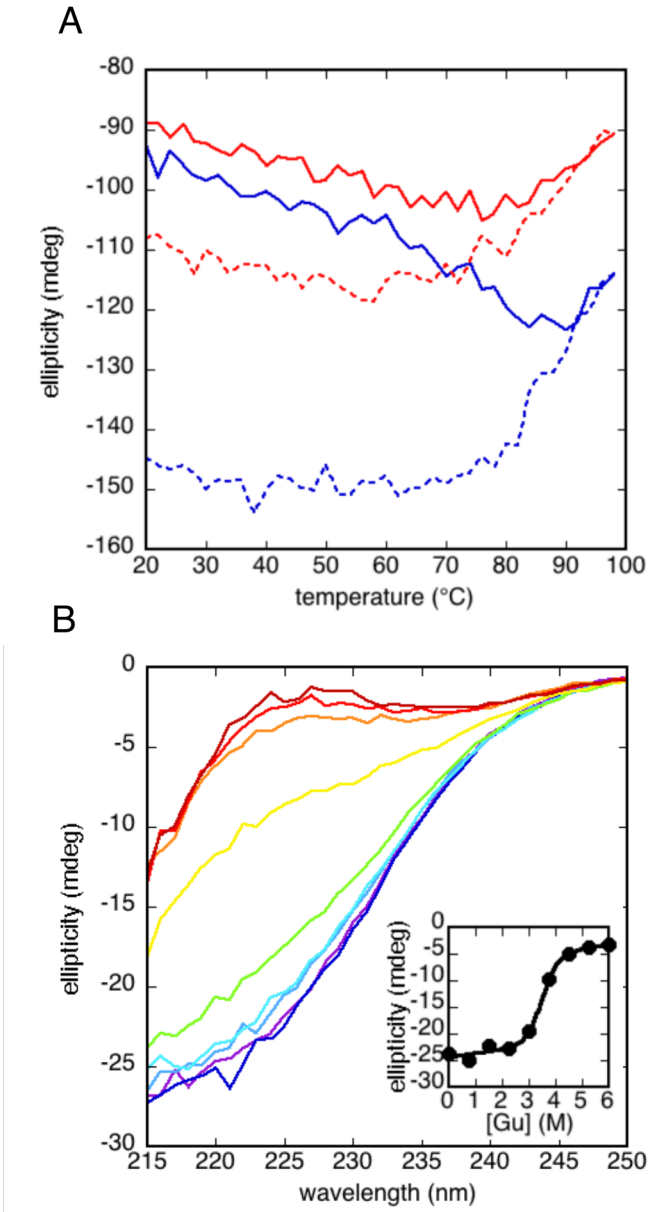

Figure S7, related to Figures 6 and 7. (A) Comparison of thermal denaturation of Bsc4 S288C with C-terminal (blue) and N-terminal (red) hexahistidine affinity tag, both forward (solid) and reverse (dashed) melts, at 100 μM protein concentration. (B) Cooperative guanidine denaturation of small oligomers of N-terminally histidine-tagged Bsc4 S288C in 50 MES (pH 5.5), 100 mM KCl, 1 mM TCEP, at 60 μM protein concentration in a 0.5 mm pathlength cell at 20 °C, monitored by circular dichroism from 250 nm to 215 nm. Guanidine concentrations range from 0 M (purple) to 6 M (maroon) in 0.75 M increments. Insets show fitting of the ellipticity at 222 nm to a standard two-state chemical denaturation model (see Methods). The guanidine denaturation properties are similar to those of C-terminally tagged Bsc4 S288C (Figure 7).

FIGURE S8, related to FIGURE 6

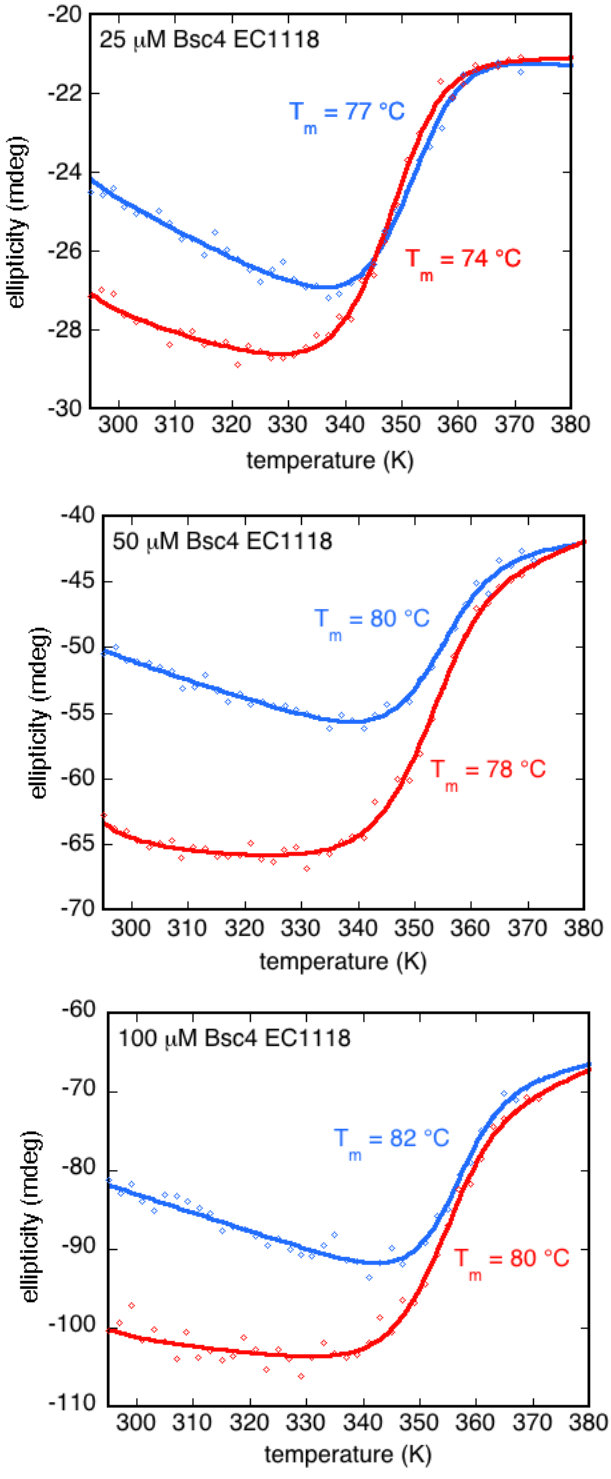

Figure S8, related to Figure 6. Forward (blue) and reverse (red) thermal denaturation curves of Bsc4 EC1118 at three different concentrations (same solution conditions as Figure 6). The fitted midpoint value increases with protein concentration, consistent with folded oligomers denaturing to unfolded monomers.

FIGURE S9, related to FIGURE 6

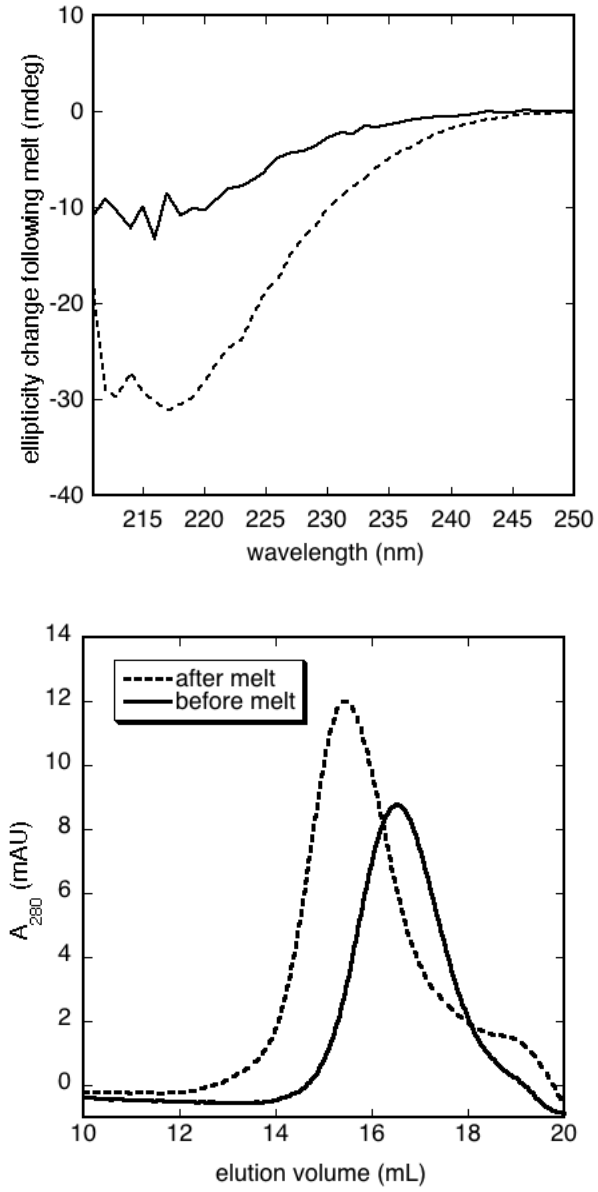

Figure S9, related to Figure 6. Circular dichroism difference spectra (top panel) of Bsc4 S288C (dashed) and Bsc4 EC1118 (solid) from before and after thermal denaturation, showing a probable gain in β-strand secondary structure; Superose 6 size exclusion traces (bottom panel) for Bsc4 EC1118 before and after thermal denaturation, showing an apparent increase in oligomer size. Size exclusion traces for Bsc4 S288C (not shown) showed large losses in apparent protein concentration after melting, potentially due to formation of large aggregates that did not enter the column. N-terminally tagged Bsc4 S288C, however, showed very similar behavior to the C-terminally tagged Bsc4 EC1118, with a similar gain in ellipticity, a similar shift toward higher oligomers and no apparent loss of protein (not shown).

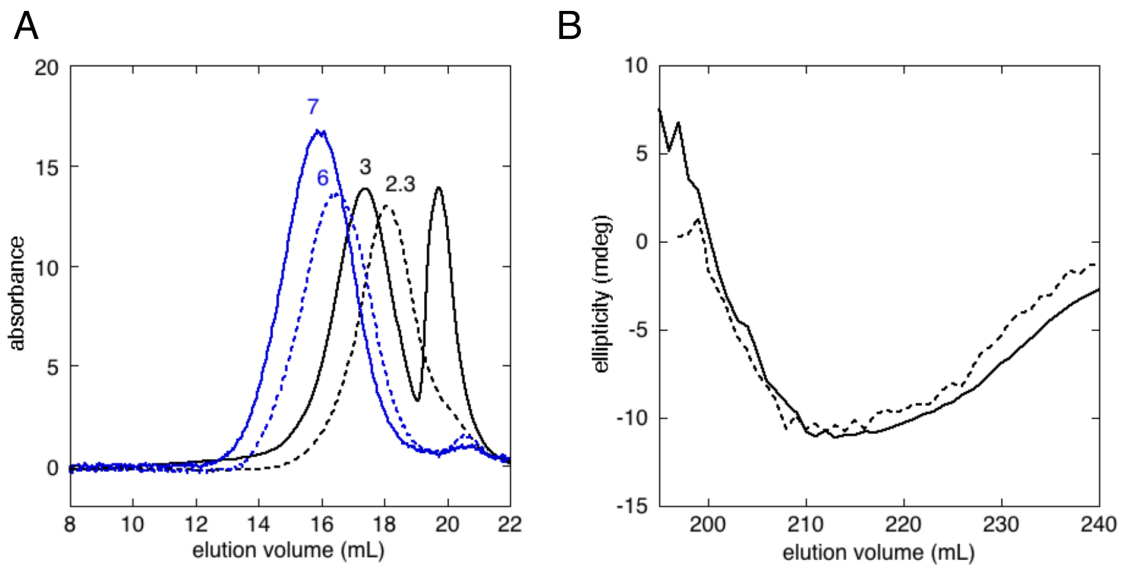

Figure S10, related to Figure 2 and Figure 4. Bsc4 S288C still refolds to β-sheet rich oligomers when an N-terminal histidine affinity tag is removed: **(A)** Size exclusion chromatograms of N-terminally histidine-tagged Bsc4 S288C (50-60 μM) with the tag either left intact (solid lines) or cleaved by thrombin (dashed lines), followed by purification on a HiTrap CM FF cation exchange column and dialysis refolding in 50 mM MES (pH 5.5) containing either 100 mM KCl (black) or 250 mM KCl (blue). (B) Far ultraviolet circular dichroism spectra of intact (solid) or cleaved (dashed) N-terminally histidine-tagged Bsc4 S288C (100 μM) in 50 mM MES (pH 5.5),100 mM KCl in a 0.1 mm cuvette at 20 °C. Cleavage of the tag was verified to be >90% complete by mass spectrometry. Size exclusion peaks in panel A are annotated with apparent oligomer size (in numbers of subunits) based on a calibration curve. The small changes in apparent oligomer size suggest that cleavage of the tag may have slight effects on oligomer size distribution (as is the case for changing the tag position; compare Figures S4 and 2). However, the peak width and dependence of elution volume on salt concentration are unchanged, strongly suggesting that the presence of the histidine tag is not responsible for the oligomerization behavior of Bsc4 described in the text. The peak at ~20 mL, prominent in the low salt refolding of uncleaved protein, appears with variable intensity in most of our refolding experiments (see Figure S4 and Figure 2). It may represent Bsc4 monomers, but its intensity shows poor reproducibility and attempts to isolate it have failed thus far.