Supporting Information

Collagen I weakly interacts with the β-sheets of β_2-microglobulin and enhances conformational exchange to induce amyloid formation

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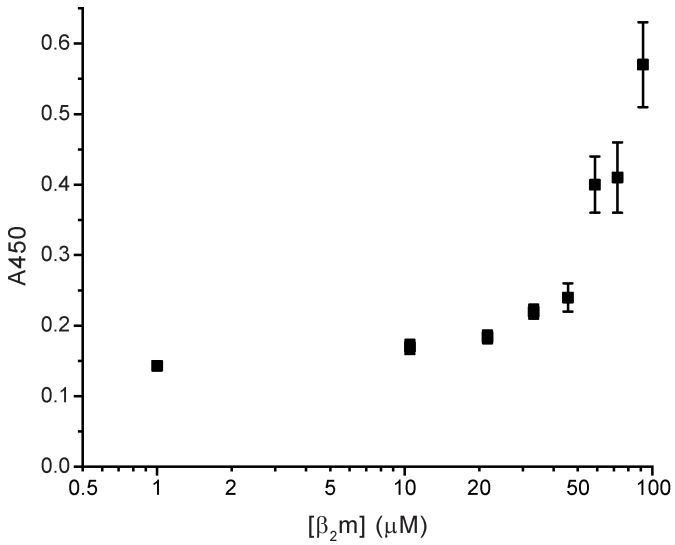

Figure S1. Weak β_2m–collagen I binding as assessed by ELISA. Dose-dependent binding of varying concentrations of β_2m (1–100 μM) to immobilized collagen I at pH 7.4. The x-axis is plotted on a logarithmic scale. Each point is the average absorbance at 450 nm of triplicates within the same plate. The error bars represent the standard deviation of the triplicates.

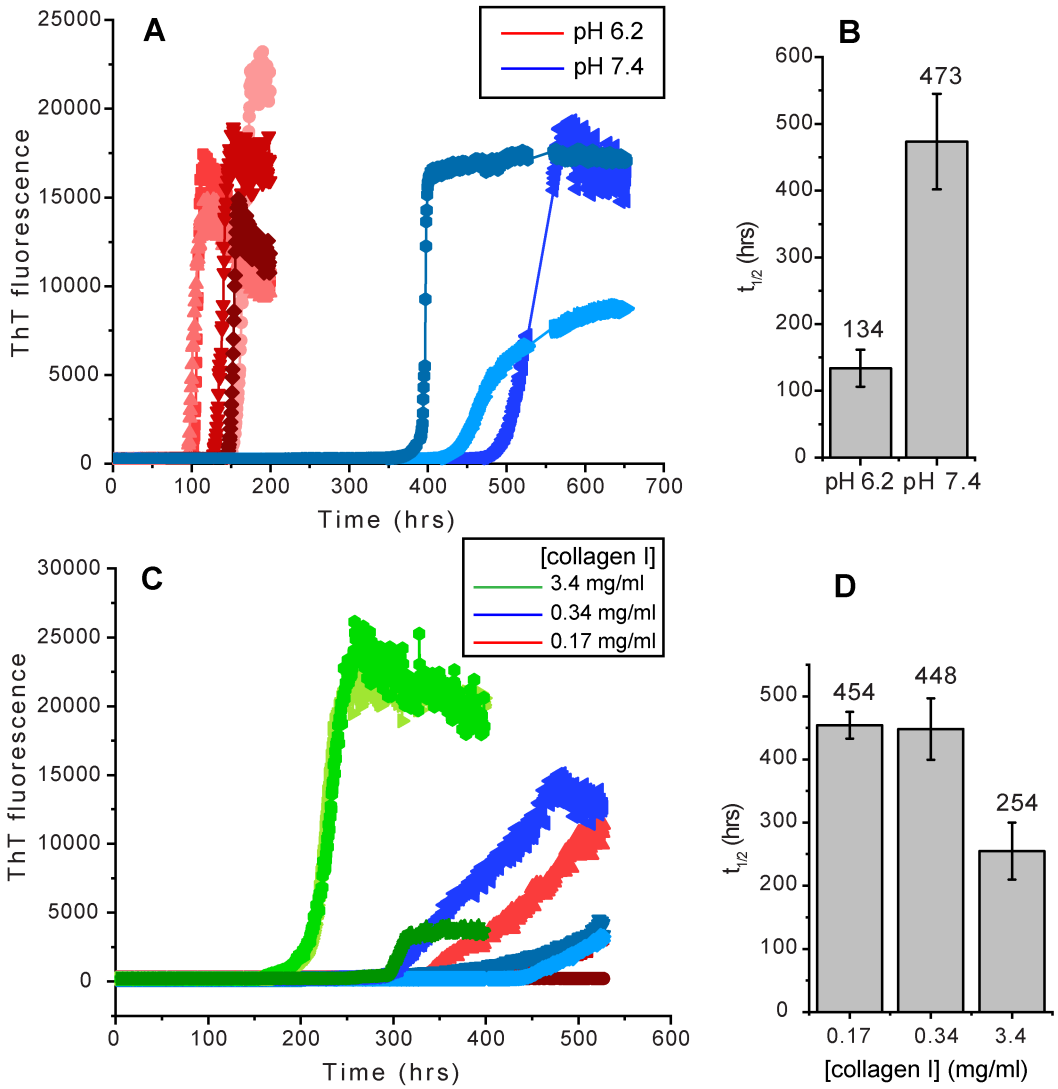

Figure S2. Collagen I-induced amyloid formation of β_2m observed by ThT fluorescence. A) ThT fluorescence curves of 85 μM β_2m with 3.4 mg/ml collagen I in 10 mM sodium phosphate buffer pH 6.2 (red shades) or pH 7.4 (blue shades). The pH 7.4 data are the same data presented in Figure 1 in the main text. Data were acquired at 37 °C with shaking (600 rpm). B) Average half-times (t_{50} values) of β_2m amyloid formation in the presence of 3.4 mg/ml collagen I at pH 6.2 or pH 7.4 calculated from the ThT fluorescence curves in panel A. Error bars represent the standard deviation of t_{50} values calculated from the multiple curves in the same condition. The mean t_{50} value (hrs) is given above each bar. C) ThT fluorescence curves of 85 μM β_2m in the presence of collagen I at different concentrations (green shades- 3.4 mg/ml, blue shades- 0.34 mg/ml, red shades- 0.17 mg/ml) in 10 mM sodium phosphate buffer, pH 6.2. D) Average t_{50} value of β_2m amyloid formation in the presence of different concentrations of collagen I at pH 6.2 calculated from the ThT fluorescence curves in panel C. Error bars represent the standard deviation of t_{50} value calculated from the multiple curves in the same condition. The mean t_{50} value (hrs) is given above each bar.

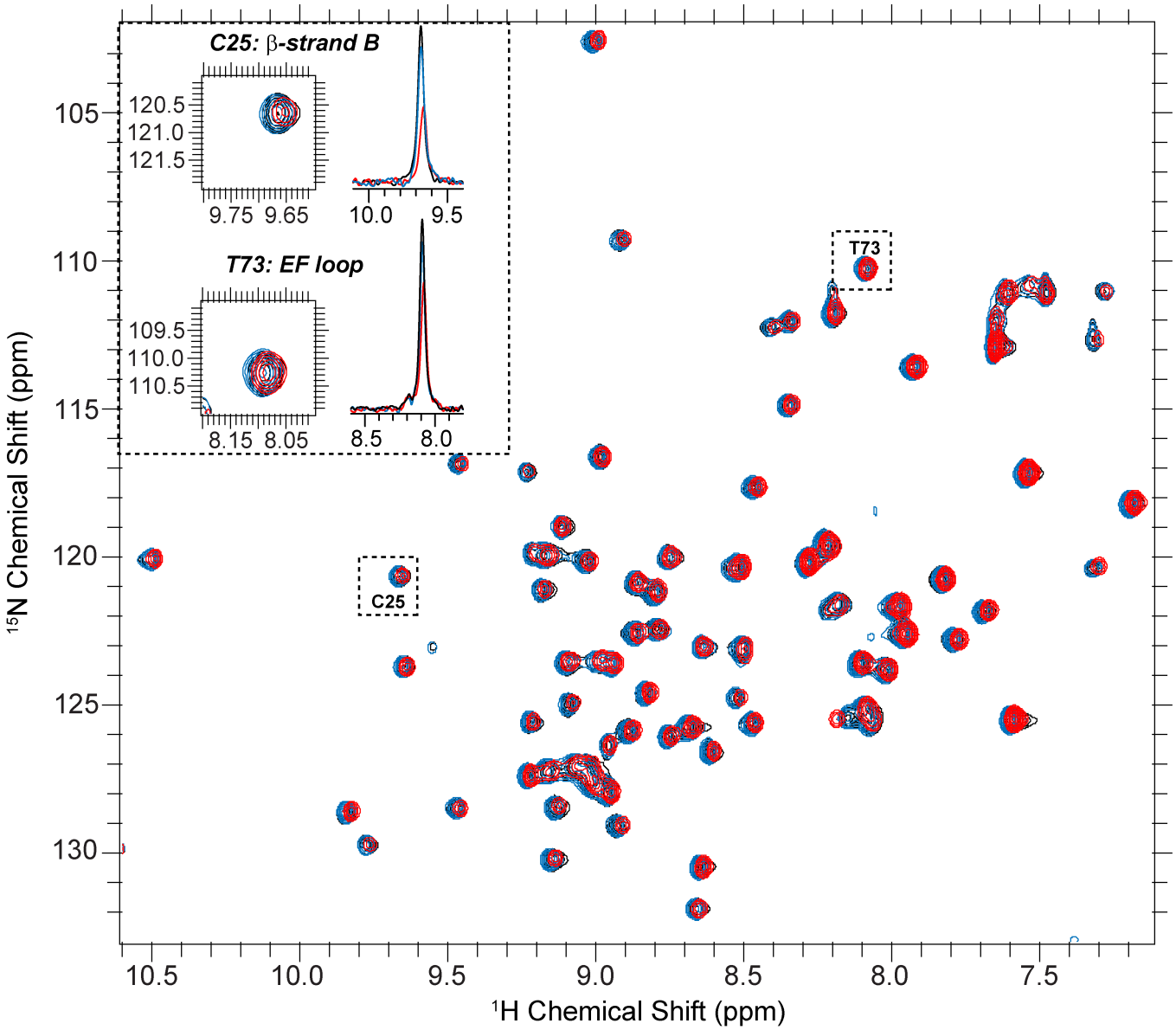

Figure S3. Minimal chemical shift perturbation with residue-specific intensity losses observed by titration of collagen I into β_2m. ^{1}H–^{15}N-HSQC spectra of 300 μM β_2m in TBS, pH 7.4 containing 0.5 mg/ml casein in the absence (black) or presence of different concentrations of collagen I (blue- 0.12 mg/ml collagen I and red- 1.2 mg/ml collagen I). The inset shows a zoom-in on the 2D contours and the extracted ^{1}H 1D projections of a residue that has a higher degree of peak intensity loss (Cys 25, $I/I_0 = 0.48$) and one that has a low level of intensity loss (Thr 73, $I/I_0 = 0.76$) upon addition of collagen I. Experiments were conducted in 10% D_2O at 700 MHz ^{1}H Larmor frequency and 10°C.

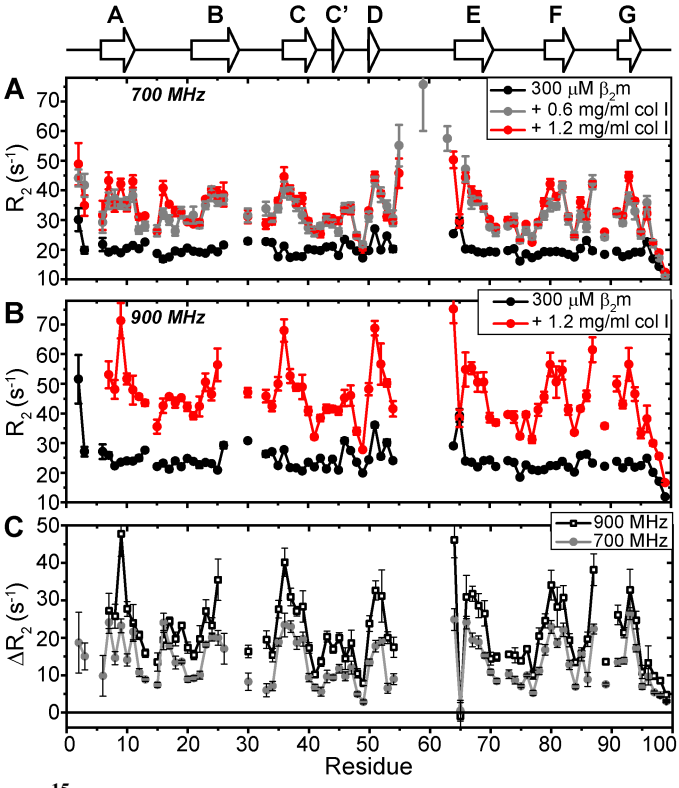

Figure S4. Perturbation of β_2m ^{15}N-R_2 values at different magnetic field strengths. A) Residue-specific ^{15}N-R_2 measurements at 700 MHz ^{1}H Larmor frequency of 300 μM β_2m in the absence (black) or presence of 0.6 mg/ml (gray) or 1.2 mg/ml (red) collagen I in TBS, pH 7.4 containing 0.5 mg/ml casein. ^{15}N-R_2 data in the absence (black) or presence of 1.2 mg/ml collagen I (red) are replotted from Figure 2B in the main text. B) ^{15}N-R_2 measurements at 900 MHz ^{1}H Larmor frequency of 300 μM β_2m in the absence (black) or presence (red) of 1.2 mg/ml collagen I in TBS, pH 7.4 containing 0.5 mg/ml casein. C) Residue-specific ^{15}N-ΔR_2s at 700 MHz (gray) or 900 MHz (black), taken as the difference in ^{15}N-R_2 of β_2m in the absence or presence of 1.2 mg/ml collagen I. All experiments were conducted in 10% D_2O and 10°C. All error bars are propagated from the fitting errors.

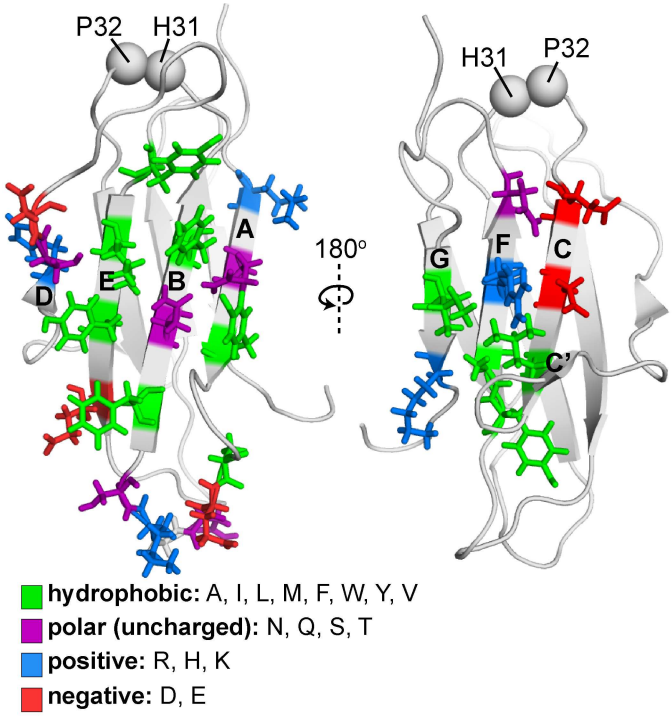

Figure S5. Amino acid composition of the β_2m interface for collagen I interactions. Amino acids determined to be at the β_2m–collagen I interface by ^{15}N-DEST and that have side-chains oriented toward the interaction surface are shown in stick representation and colored by amino acid type (hydrophobic= green; polar, uncharged= purple; positive charge= blue; negative charge= red). His 31 and Pro 32 are shown as spheres. Both β_2m β-sheets are composed of a mixture of hydrophobic and hydrophilic amino acids, with the ABED β-sheet displaying several aromatic rings. Structural models are based on PDB: 2XKS[1].

REFERENCES

1. Eichner, T.; Kalverda, A. P.; Thompson, G. S.; Homans, S. W.; Radford, S. E., Conformational conversion during amyloid formation at atomic resolution. *Mol Cell* **2011,** *41*, 161-72.