Supplementary Information

Biotechnological Production of the Mussel byssus derived collagen preColD

Communication

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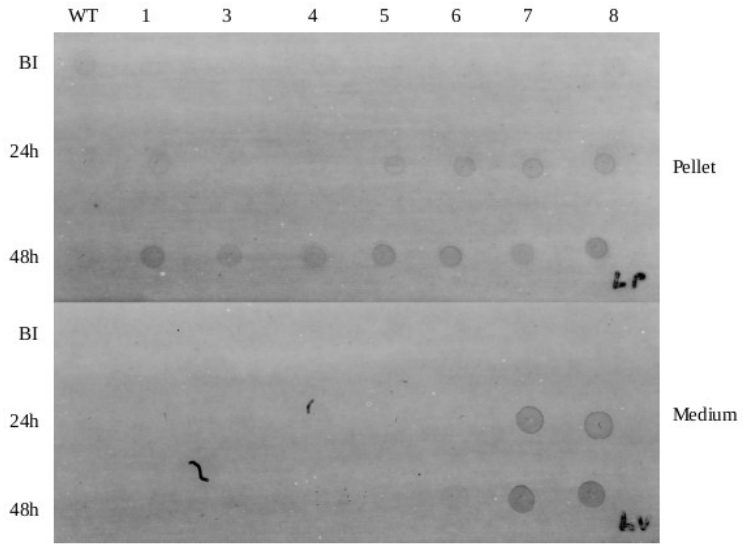
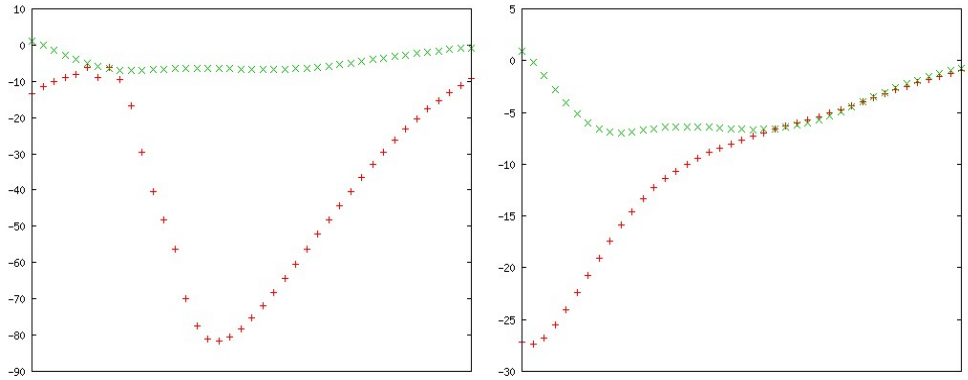
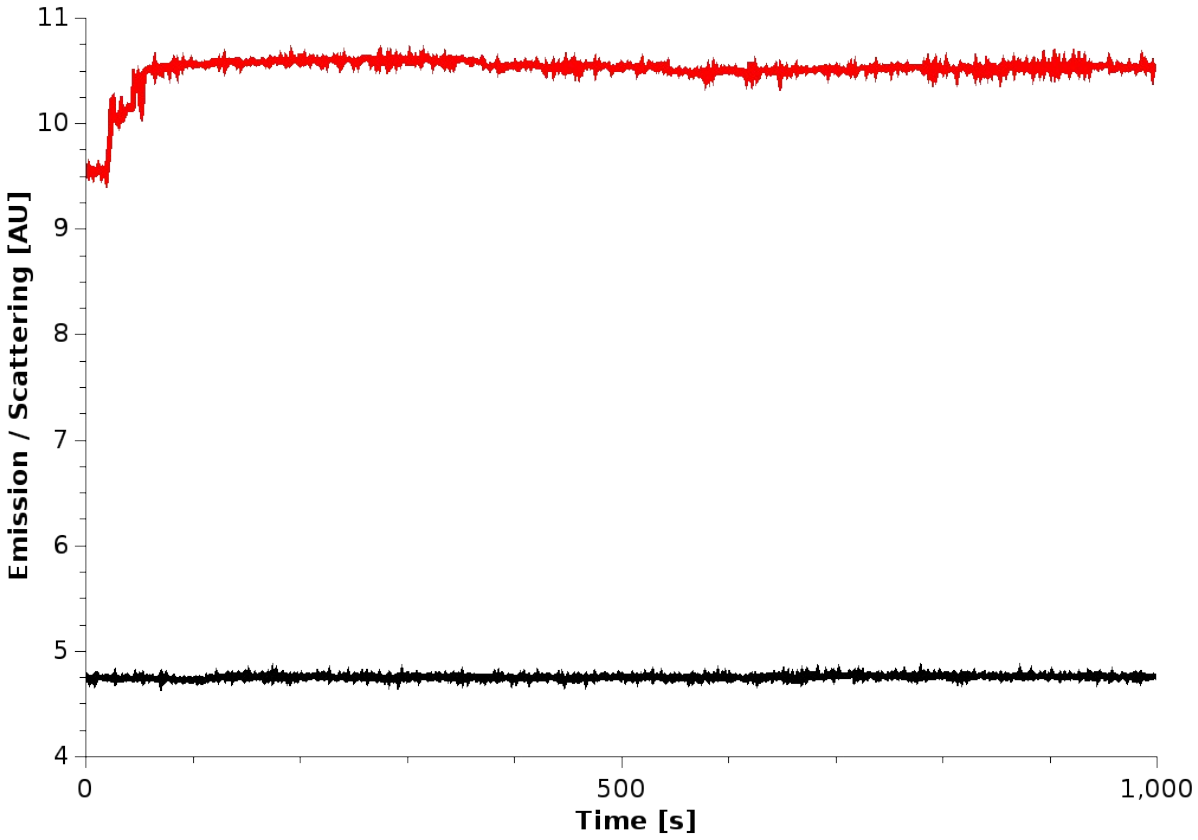


Supplementary Figure S1: Dot-Blot screening for a high preColD-expressing X33 strain. Selection was performed using a cMyc antibody. The selected strain used in this publication was #7. Samples were taken before induction (BI) and after 24 and 48 hours of induction.

Supplementary Figure S2: Silver stained SDS-PAGE gel from the purification of preColD. M: PrecisionPlus(TM) prestained marker (BioRad, Hercules, CA USA). X: Extract before loading onto column. L: Flowthrough after column loading. W: Column wash. E1/E2 Eluted protein fractions. All samples were precipitated from the 4M Guanidinium HCl buffer with a 10x Volume of cold ethanol and resuspended in 2x Laemmli-buffer. The target protein can be seen eluting between the 75 and 100 kDa marker band (marked by arrow). A contaminating band, most likely a fragment upon unspecific proteolysis, can be seen eluting at < 25 kDa. When dialyzing the protein with a 30 kDa cutoff membrane, this contaminant was removed.



Supplementary Figure S3. Fit of the CD spectra of preColD in 4M urea buffer (left) and 0M urea buffer (right) to reference spectra using the K2D3 database (29). Input in red, prediction in green. The structure prediction determines a secondary structure content of 63.89% alpha helix / 12.28% beta sheet for the protein in 4M urea and 62.92% alpha-helix / 11.67% beta sheet for 0M urea. Both fits show a significant distance to the closest spectrum in the database, suggesting that the error of the structure prediction is large.



Supplementary Figure S4. Thioflavin T fluorescence (black; excitation: 450 nm, emission: 482 nm) and 90° light scattering (red; excitation: 350 nm, emission: 350 nm) of a preColD-solution diluted to a final concentration of 0.1 mg/ml from formic acid into PBS containing 50 µM Thioflavin T. While the increase of scattered light (red curve) suggests a 1st order formation of aggregates within 100 seconds of dilution, no increase in Thioflavin T fluorescence (black curve) can be seen. This indicates that the aggregates show no amyloid-like structure.