Amyloid formation of fish β-parvalbumin involves primary nucleation triggered by disulfide-bridged protein dimers

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Supporting information

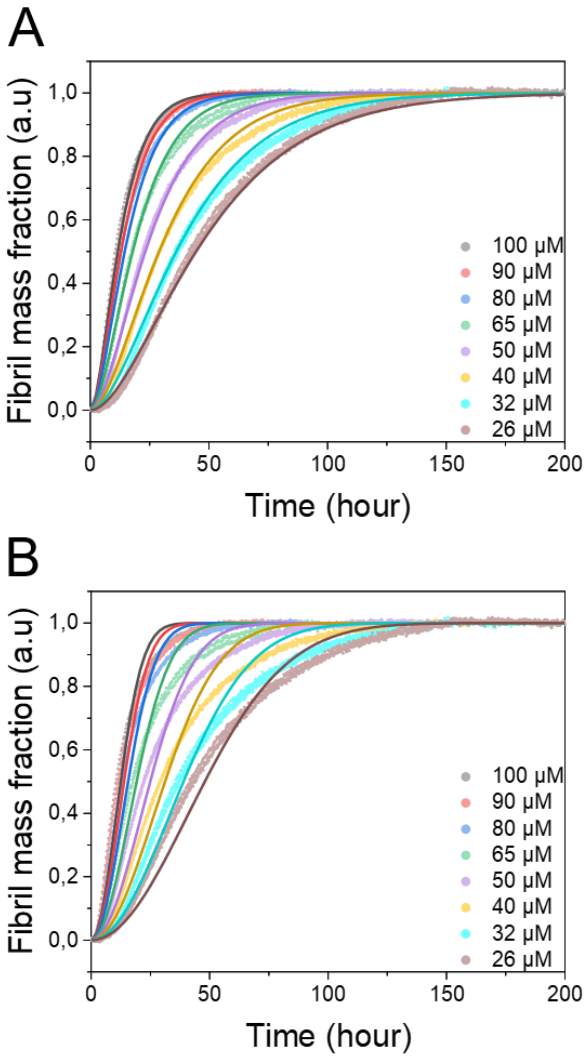

Figure S1: Normalized kinetic ThT aggregation of different apo-β-PV concentrations (triplicates) profiles at 37˚C under quiescent conditions in 150 mM NaCl, 1 mM $CaCl_2$, 5 mM EDTA and 25 mM Tris-HCl, pH 7.4. The ranges represent monomeric starting material, fit with either a secondary nucleation (**A**) or fragmentation (**B**) dominated mechanism by Amylofit. The secondary nucleation fit does not improve the fit compared to the simple nucleation elongation model (shown in **Figure 1**), whereas fragmentation worsens the fit.

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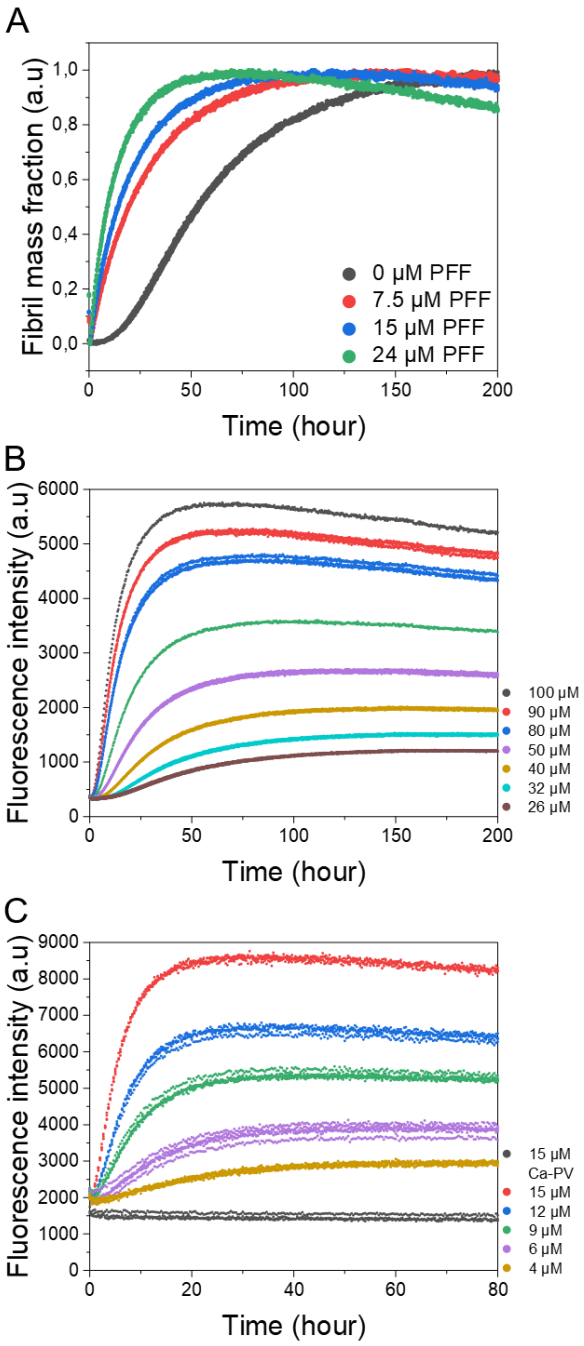

Figure S2: (**A**) Normalized ThT aggregation profiles of 30 µM monomeric apo-β-PV at no or different concentrations of sonicated pre-formed fibrils (PFF) (*i.e.,* fiber-seeded reactions). (**B**, **C)** ThT aggregation profiles (a.u) of monomeric (triplicates) (**B**) and dimeric (quintuplicates) (**C**) starting material throughout a concentration range at 37˚C under quiescent conditions in 150 mM NaCl, 1 mM $CaCl_2$, 25 mM Tris-HCl, pH 7.4, with or without 5 mM EDTA. For dimer experiments (**C**), the protein concentration is given in monomer-equivalents. One set of data in B is without added EDTA, *i.e.,* the Ca^{2+}-bound form.

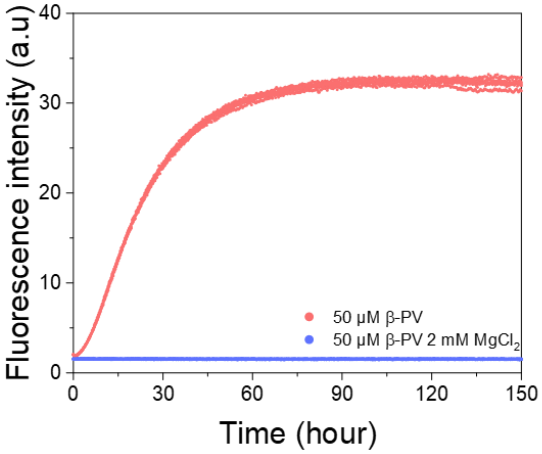

Figure S3: ThT aggregation profiles (a.u) of apo- and Mg-β-PV at 37°C under quiescent conditions in 150 mM NaCl, 2 mM $MgCl_2$, 25 mM Tris-HCl, pH 7.4, with or without 10 mM EDTA, show that Mg^{2+} prevents amyloid formation of β-PV.

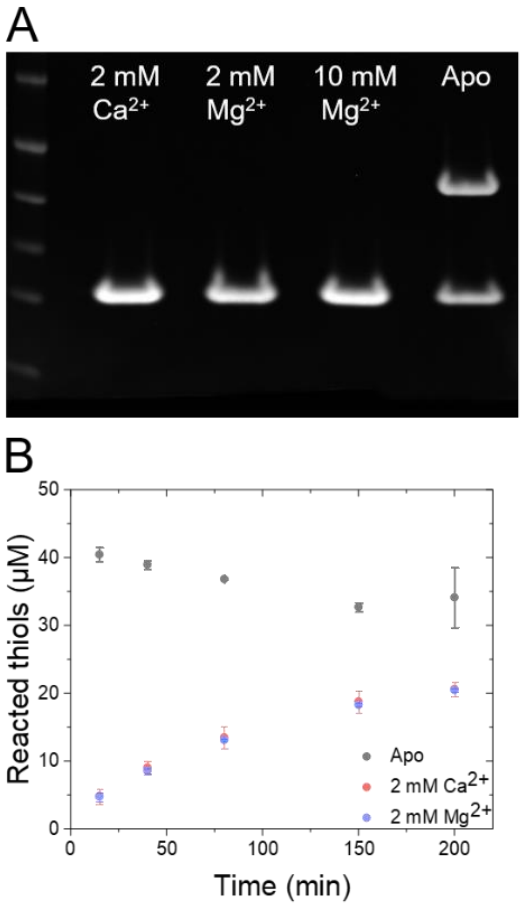

Figure S4: (**A**) SDS-PAGE of 50 μM apo-, Ca^{2+}- or Mg^{2+}-β-PV incubated at room temperature for 20 hours in 25 mM Tris-HCl (pH 7.8) and 50 μM H_2O_2. (**B**) Ellman's assay data revealing accessible cysteine sulfurs of 35 μM β-PV with 5 mM EDTA (apo), 2 mM Ca^{2+} or 2 mM Mg^{2+} at room temperature in 25 mM Tris-HCl, pH 7.8 as a function of incubation time (duplicates).

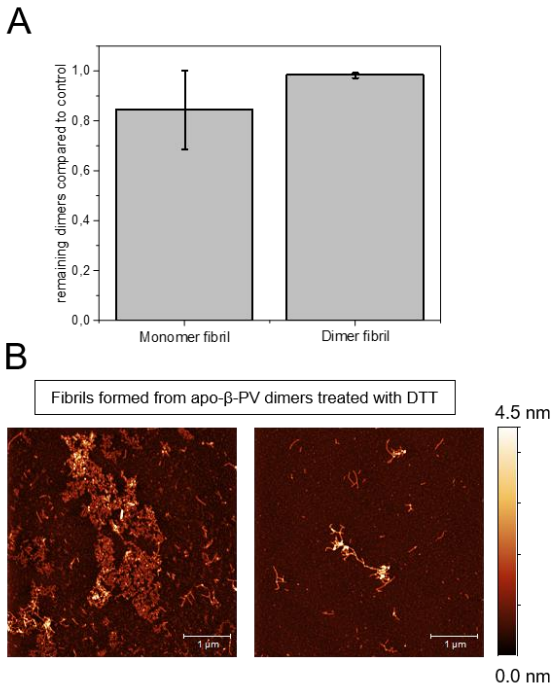

Figure S5: (**A**) SDS-PAGE densitometric analysis of dimer content upon treatment of apo-β-PV fibrils with 100x excess DTT at room temperature in 150 mM NaCl, 1 mM $CaCl_2$, 5 mM EDTA and 25 mM tris-HCl, pH 7.8 (duplicates). As the ratio (dimers in amyloids with DTT treatment/dimers in amyloids without DTT treatment) is close to 1, for both monomer and dimer starting material, there is no cleavage of disulfide bonds by DTT in the amyloid state. (**B**) AFM analysis of amyloid fibrils (made from dimeric starting material) after DTT treatment.

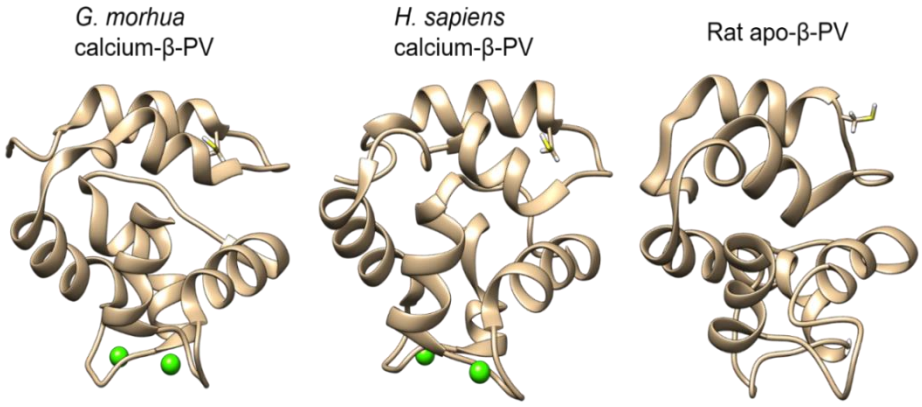

Figure S6: Apo- or Ca^{2+}-β-PV NMR solution structures of atlantic cod (*G. morhua* PDB: 2MBX), human (PDB:1TTX) and rat (PDB: 2NLN) proteins. The cysteine side chain is shown in in stick representation and the models show that it is buried in the core of the protein in the holo-forms, whereas it is exposed in the apo-form. Ca^{2+} ions are displayed as green spheres. Molecular graphics performed with UCSF Chimera (developed by the Resource for Biocomputing, Visualization and Informatics, at the University of California, San Francisco, with support from NIH P41-GM103311.)

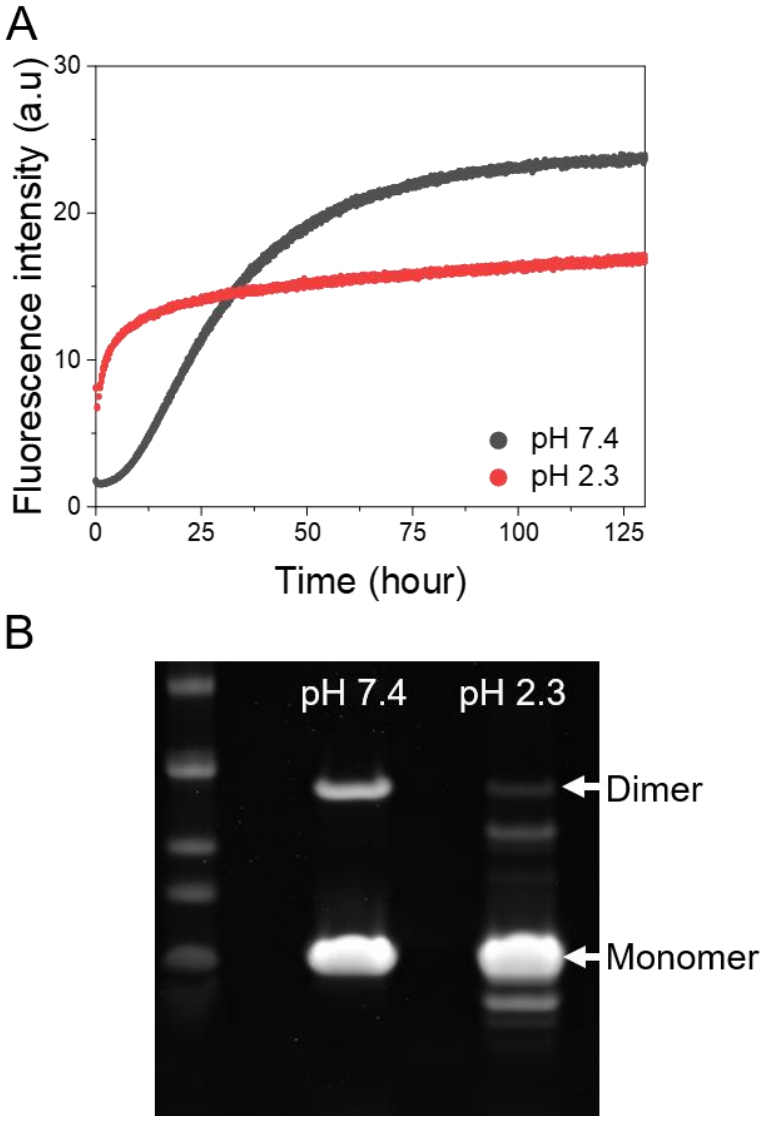

Figure S7: (**A**) ThT aggregation profiles of 50 μM monomeric apo-β-PV at 37˚C under quiescent conditions in 150 mM NaCl, 1 mM $CaCl_2$ and either 25 mM Tris-HCl with 5 mM EDTA, pH 7.4, or 50 mM Glycine-HCl buffer without EDTA, pH 2.3. In the case of the glycine-HCl buffer, holo-β-PV stored in 10 mM Tris-HCl (pH 7.4) was diluted to 3.3 mM Tris-HCl, yielding the final pH value of 2.3. (**B**) SDS-PAGE of apo-β-PV fibrils obtained from the samples in (**A**) after the experiment demonstrating the presence of disulfide-bridged dimers also at low pH (although lesser amount and with some smaller fragments detected, likely hydrolysis products, at pH 2.3).

Table SI

Convoluted rate constant ($k_n k_+$), scaling exponent (γ) and primary nucleation process reaction order (n_c; obtained from global fit) of three independent experiments of monomeric and dimeric apo-β-PV starting material. The convoluted rate constant denotes the rate of nucleation (k_n) and fibril elongation (k_+) and the scaling exponent narrows down possible aggregation mechanisms. (In the case of the nucleation-elongation model, the scaling exponent mathematically relates to n_c as $\gamma = -n_c/2$, see *G. Meisl et al., Molecular mechanisms of protein aggregation from global fitting of kinetic models. Nat. Protoc. 11, 252-272, 2016*).

Monomer starting material (n_c = 2.18)	
γ	**$k_n k_+$ ($M^{-2}h^{-2}$)**
-1.03	**1.99×10^6**
-1.09	**3.91×10^6**
-1.23	**2.12×10^6**
Dimer starting material (n_c=1.91)	
γ	**$k_n k_+$ ($M^{-2}h^{-2}$)**
-0.96	**3.89×10^7**
-0.97	**5.79×10^7**
-1.09	**7.33×10^7**