

Functionalized biopolymer particles enhance performance of a tissue-protective peptide under proteolytic and thermal stress

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SUPPORTING INFORMATION

Oligonucleotides for Plasmid Construction

Sequence (5' → 3')

[VPGVG]5_F	CGTGGGTGTTCCGGCGTAGGTGTCCCAGGTGTGGCGTACCGGGCGTTGGTGTCC TGGTGTCGCGTGCCGGG
[VPGVG]5_F	CGGCACGCCGACACCAGGAACACCAACGCCCGTACGCCACACCTGGACACCTAC GCCCGGAACACCCACGCC
ARA290_F	CCAGGAACAGCTGGAACAGTGCCTGAACAGCAGCGG
ARA290_R	GCTGCTGTTCAGCGCACGTTCCAGCTGTTCTGGCC
Myc_F	CGAACAGAAACTGATTAGCGAAGAAGATCTGGG
Myc_R	CAGATCTTCTCGCTAATCAGTTCTGTTCGCC

ELP and ARA290-ELP Fusion Protein Primary Sequences

ARA290-ELP & ELP SDS-PAGE

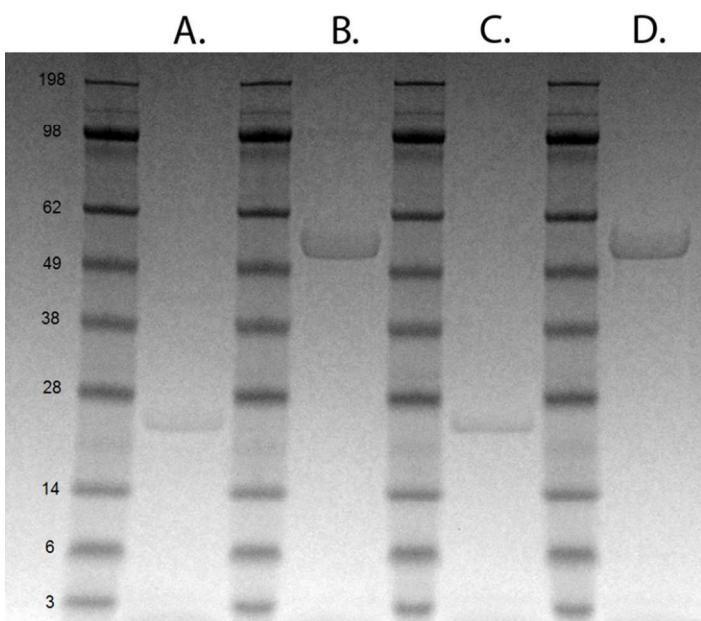


Figure S1. SDS-PAGE analysis for (A.) A-V40, (B.) A-V120, (C.) V40, (D.) V120. Approximately 5 µg of protein was loaded on a 4-12% Bis-Tris gel following 3 rounds of inverse transition cycling. Estimated molecular weights for each construct are 19.3, 52.1, 18.0, and 50.8 kDa, respectively. The protein ladder is given in kDa.

ELP Characterization

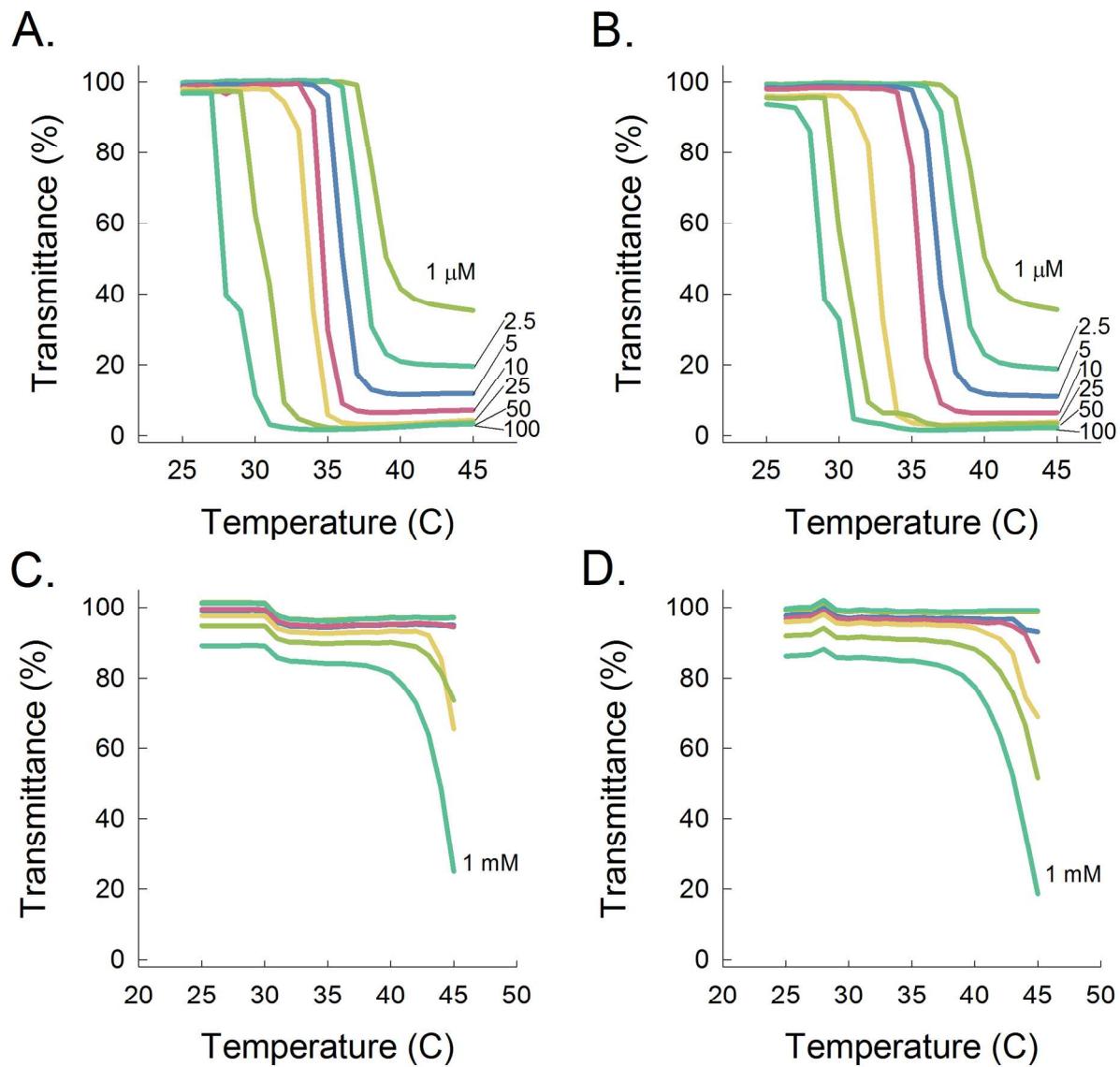


Figure S2. Turbidimetry analysis for (A.) A-V120, (B.) V120, (C.) A-V40, (D.) V40. Percent transmittance readings at 350 nm were taken in 1 minute intervals as the temperature was raised from 25 °C to 45 °C over 20 minutes. A-V120 and V120 underwent phase separation at concentrations as low as 1 μ M in the temperature range investigated. The shorter biopolymers, A-V40 and V40, did not plateau under these assay conditions, even at substantially higher concentrations (1 mM). All experiments were performed in triplicate and the lines represent the average values.

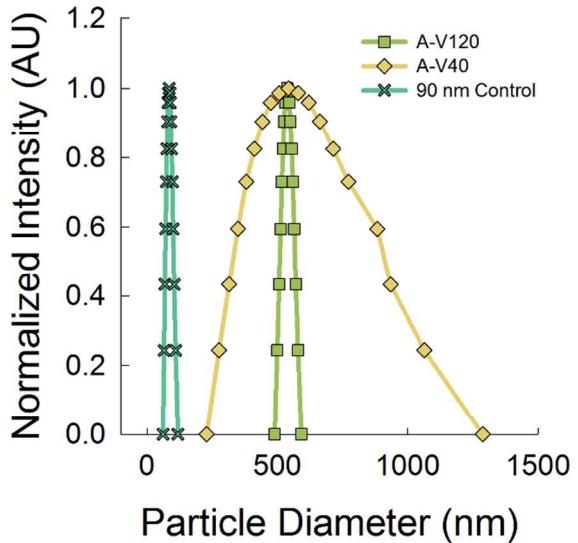


Figure S3. Particle sizing analysis for A-V120 and A-V40. Lognormal particle size distributions are given at 25 μM and a 90 nm size standard. ELP fusions were incubated at 60 $^{\circ}\text{C}$ for 10 minutes prior to analysis to induce aggregation. Similar trends were observed for V120 and V40 given in Figure 2.

Degradation Assay Sample

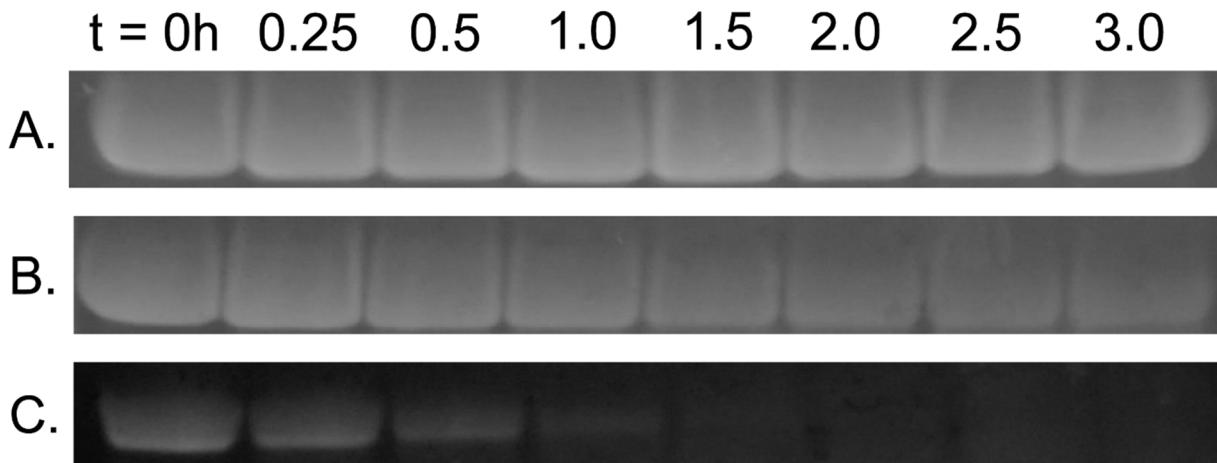


Figure S4. ~5 μg of FITC labeled V40 was incubated with lysozyme (A.), collagenase (B.), and elastase (C.) for 3 hours in PBS at 37 $^{\circ}\text{C}$. Fractions were run on a 4-12% Bis-Tris gel and imaged on a UVP transilluminator. Cleaved ELP chains appeared as smears below the main band. A blank lane loaded with enzyme only was used to normalize the background intensity. Each band was quantified using pixel intensity processing in ImageJ (Bethesda, MD). A clear drop in band intensity is observed over the 3-hour incubation for both collagenase and elastase.