Superfast Gelation of Spider Silk-Based Artificial Silk Protein

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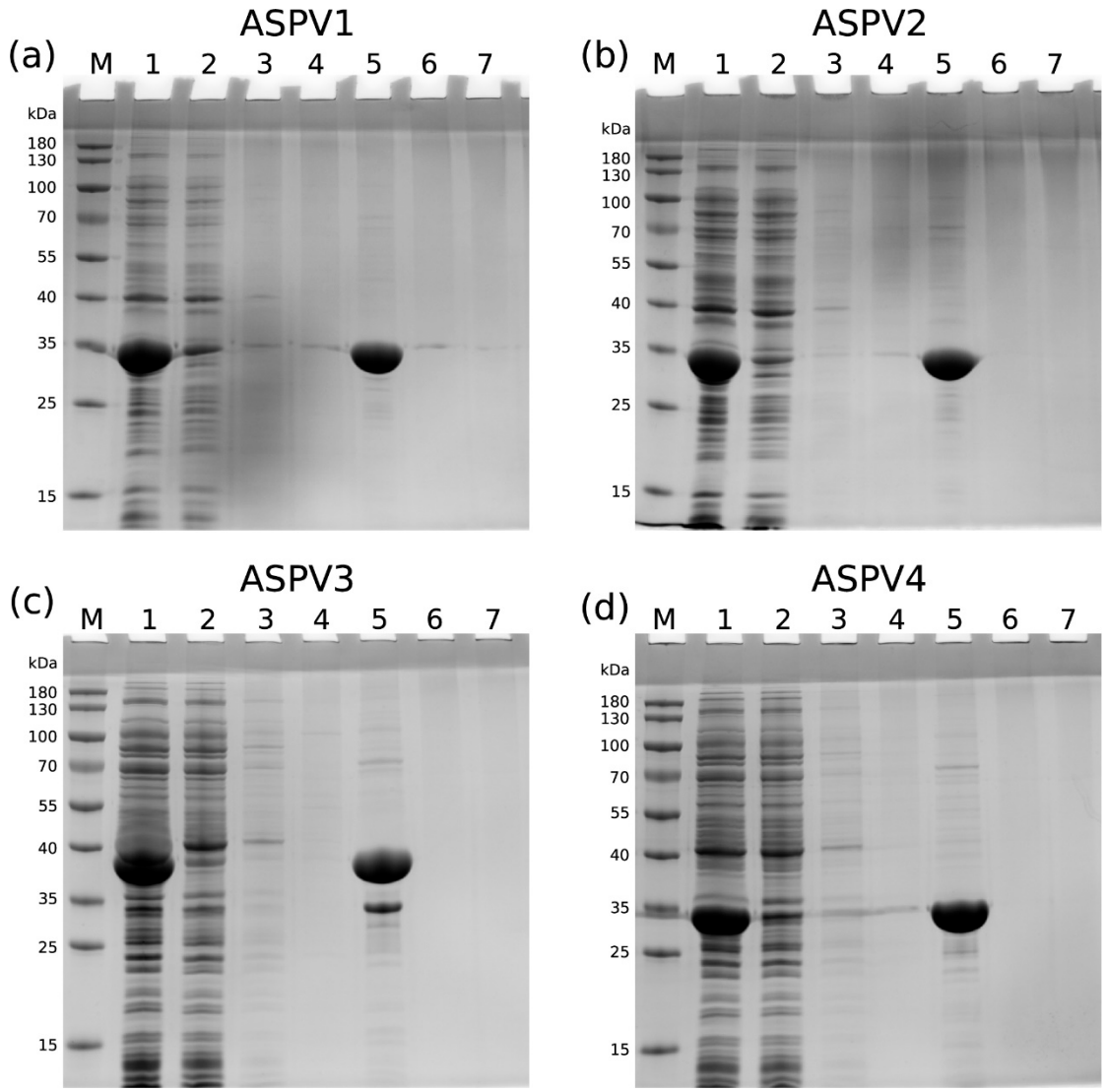

Figure S1. Analysis of protein purification stages for ASPV1, ASPV2, ASPV3, and ASPV4. SDS-PAGE was performed to assess the purification process of four different proteins designated as ASPV1 (a), ASPV2 (b), ASPV3 (c), and ASPV4 (d). Each panel represents a distinct protein purification profile. Lane M contains molecular weight markers with sizes indicated in kilodaltons (kDa). Lane 1 shows the initial cell lysate. Lane 2 contains the flow-through fraction from the binding buffer. Lanes 3 display the flow-through from the wash buffer. Lanes 4 to 7 represent successive elution fractions (Elute 1-4) with each elute volume being 5 ml, illustrating the proteins eluted during the purification process.

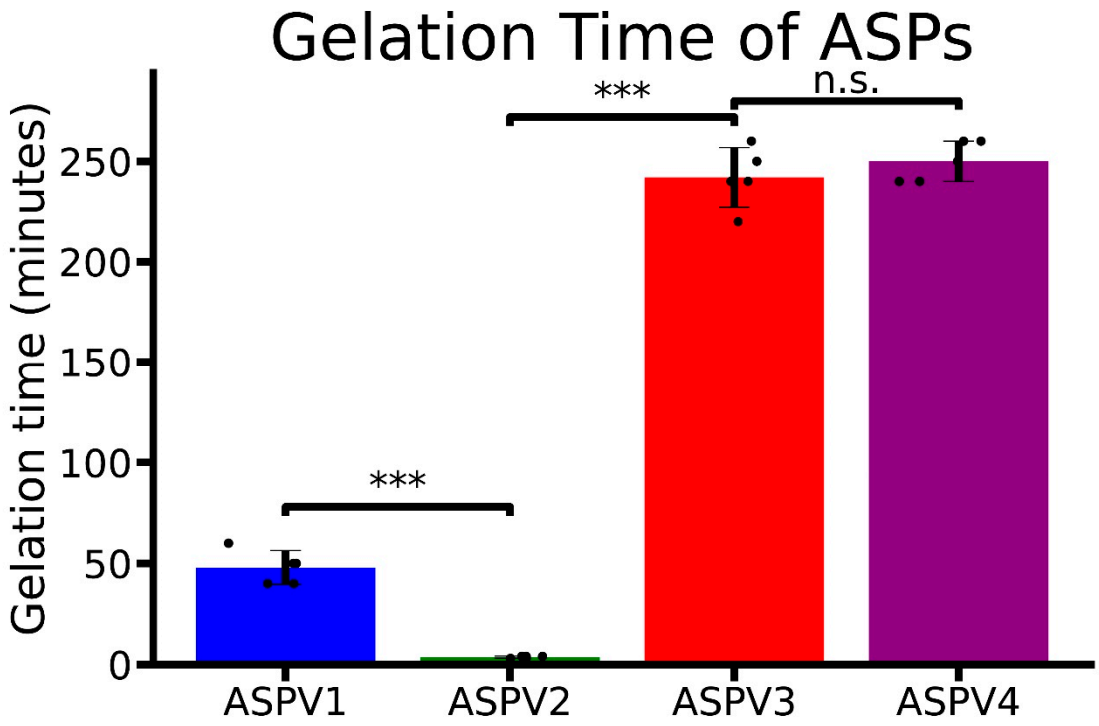

Figure S2. Gelation Time of ASP Proteins at 20 mg/ml Concentration. ASPV1 shows a significantly longer gelation time compared to ASPV2, indicated by the triple asterisks, denoting a p-value < 0.001. ASPV3 and ASPV4 have markedly increased gelation time compared to ASPV2, also indicated by triple asterisks. No significant difference (n.s.) in gelation time is observed between ASPV3 and ASPV4. Error bars represent standard deviation.

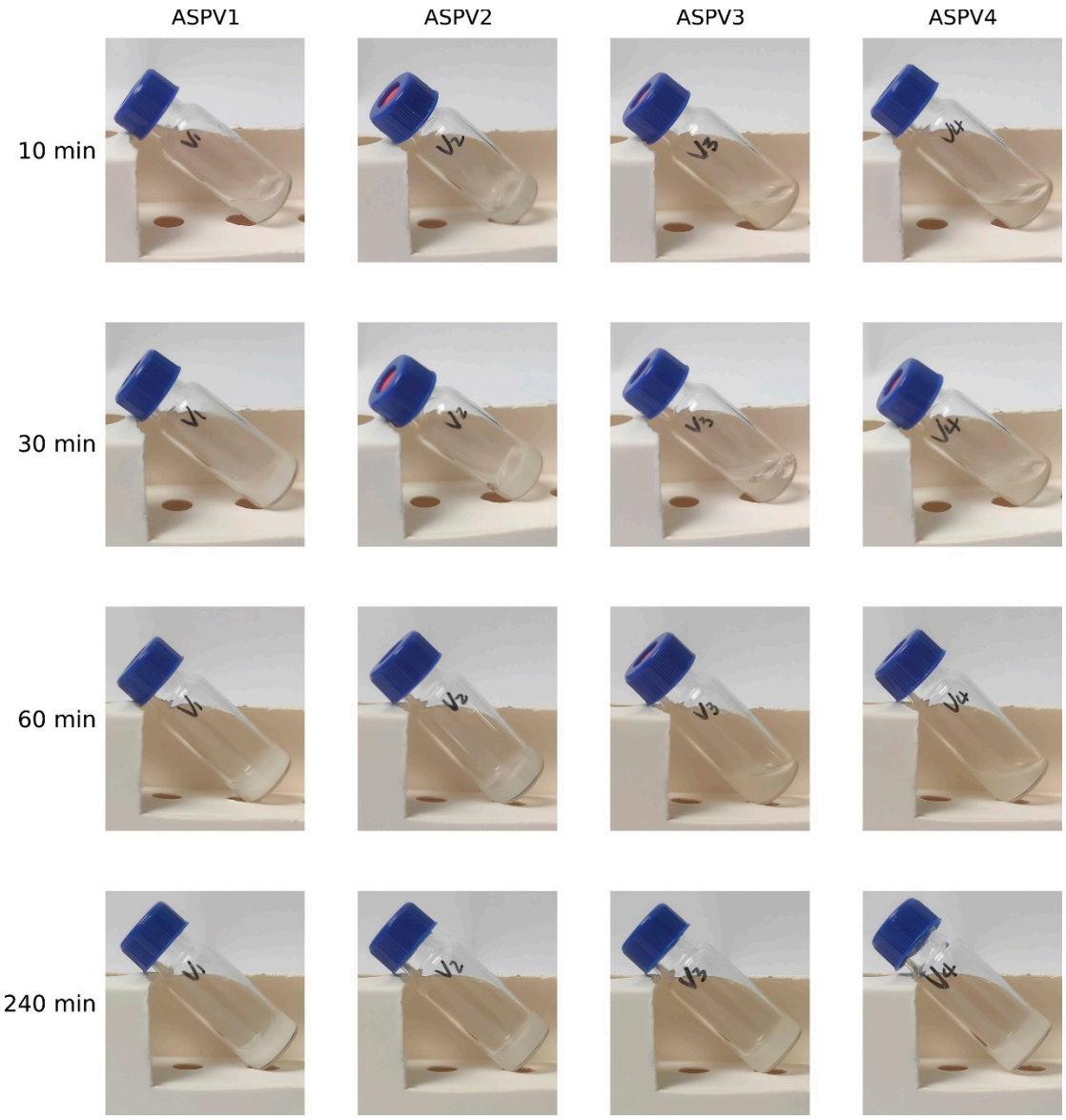

Figure S3. Visual Representation of Gelation Process for ASPs at Different Time Intervals. The series of images demonstrates the gelation of ASPV1, ASPV2, ASPV3, and ASPV4 proteins over time at a concentration of 20 mg/ml. Each row represents time points at 10, 30, 60, and 240 minutes post-initiation of the gelation process. The columns correspond to the different ASP proteins. At 10 minutes, solid hydrogel already formed for ASPV2. At 60 minutes, ASPV1 exhibits full gelation. At 240 minutes, complete gelation is observed for all ASPs.

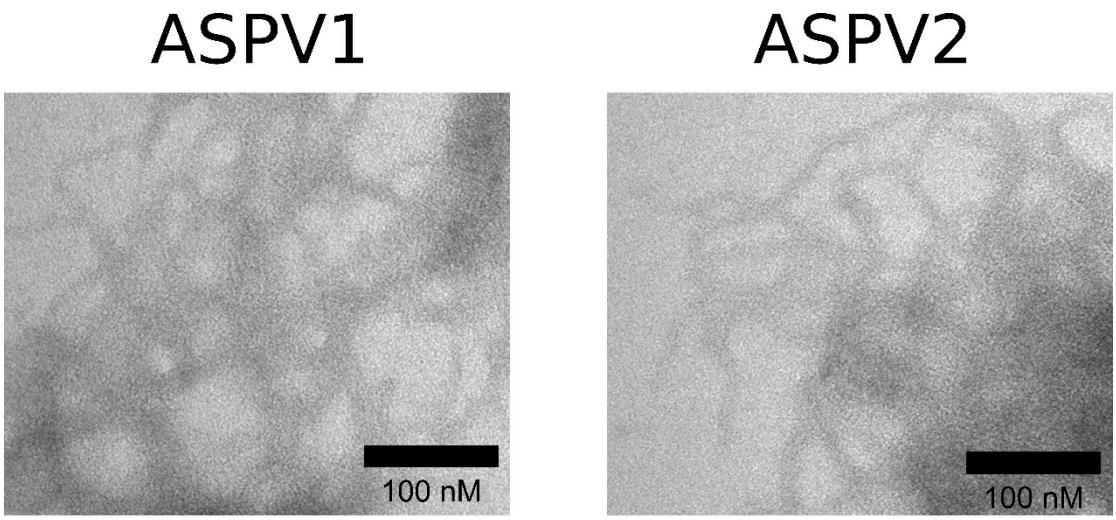

Figure S4. Transmission electron microscopy (TEM) images displaying the nanofibrillar structures of hydrogels formed by ASPV1 and ASPV2 (Samples were prepared without sonication).

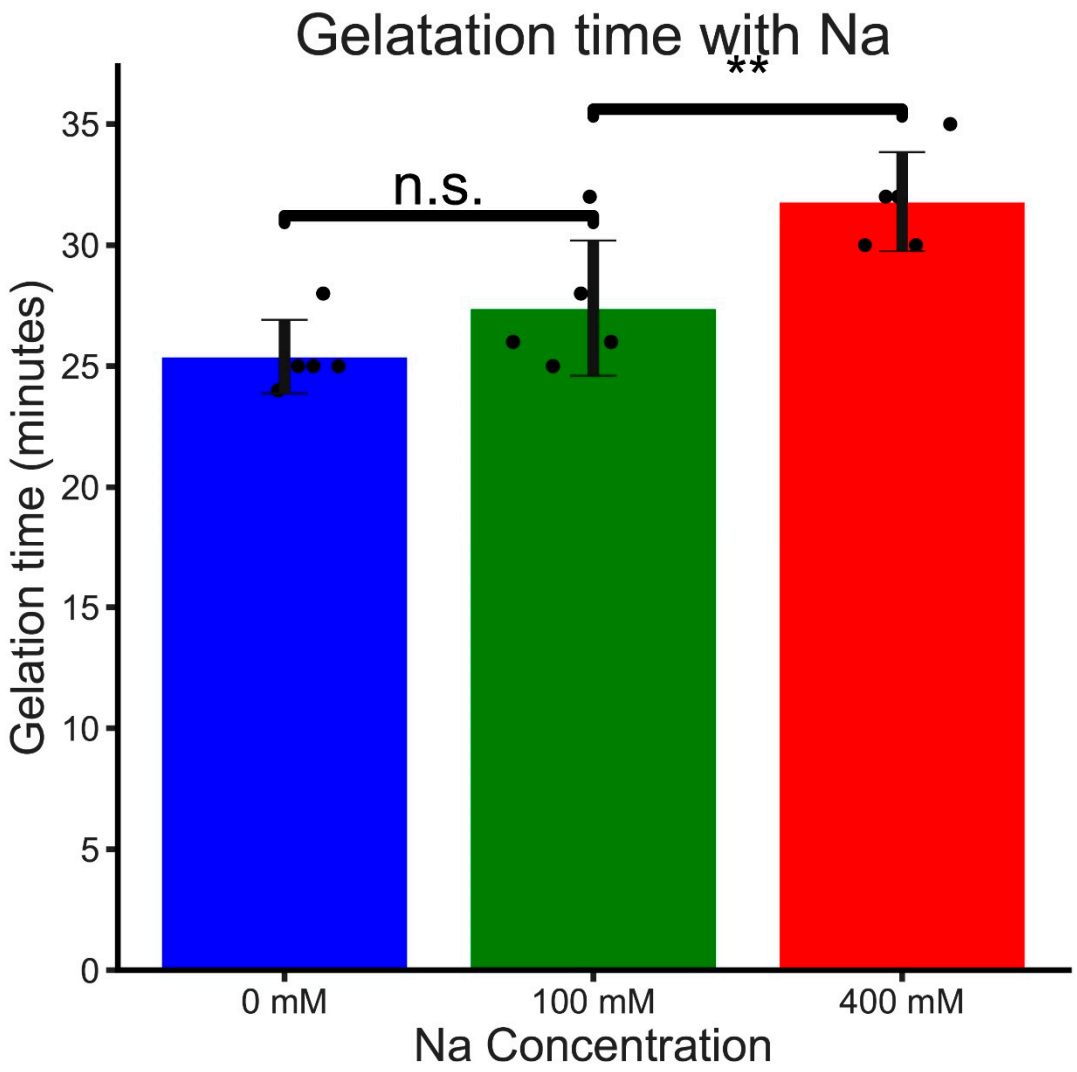

Figure S5. Impact of Sodium Concentration on Gelation Time. The bar for 0 mM Na shows the shortest gelation time. A non-significant difference (n.s.) is observed between 0 mM and 100 mM Na concentrations. However, a significant increase in gelation time is noted at 400 mM Na concentration, as indicated by double asterisks (**), corresponding to a p-value < 0.05. Error bars indicate standard deviation.

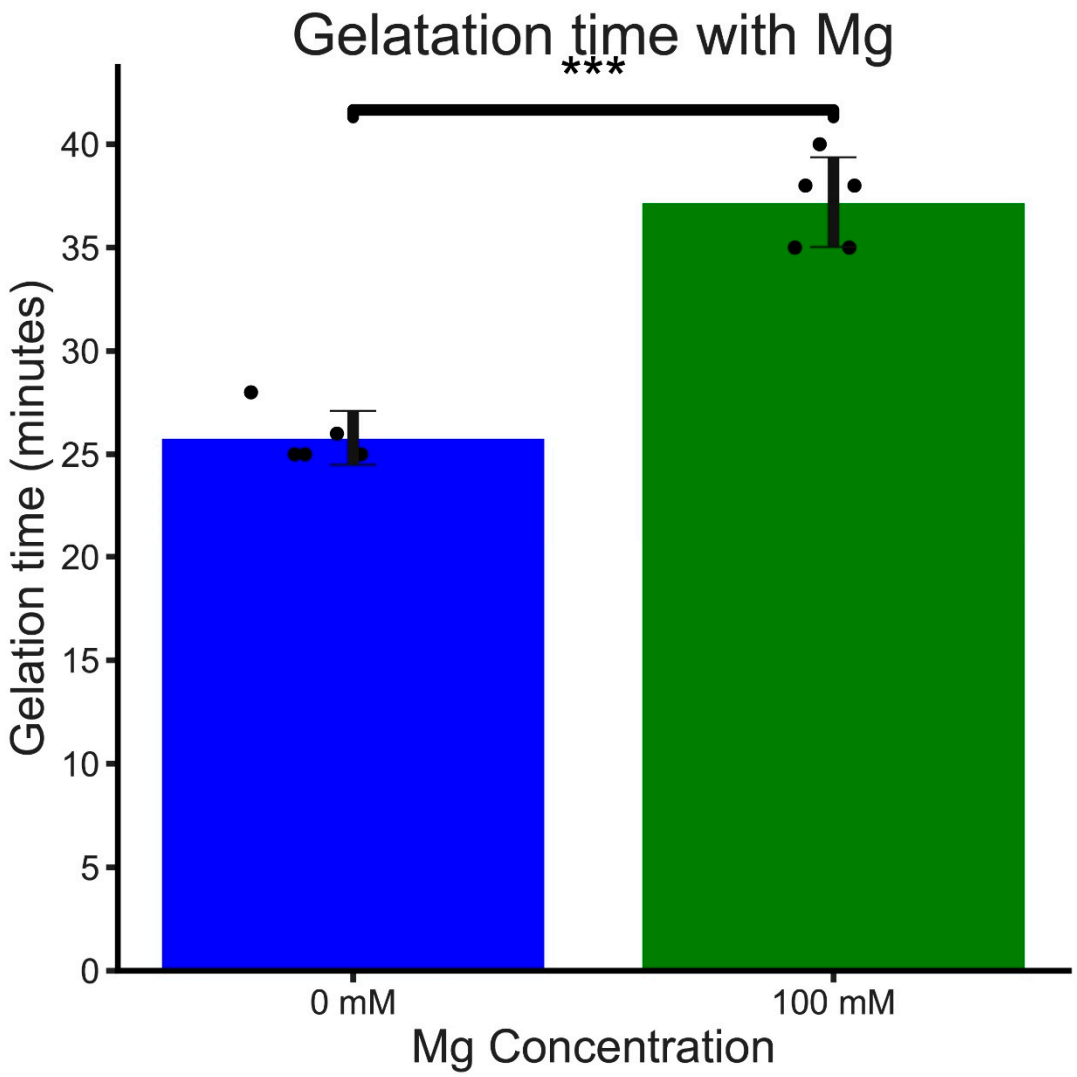

Figure S6. Influence of Magnesium Concentration on Gelation Time. The presence of 100 mM Mg significantly increases the gelation time compared to the absence of Mg, as shown by the green bar and denoted by the triple asterisks (***), indicating a p-value < 0.001. Error bars represent the standard deviation.

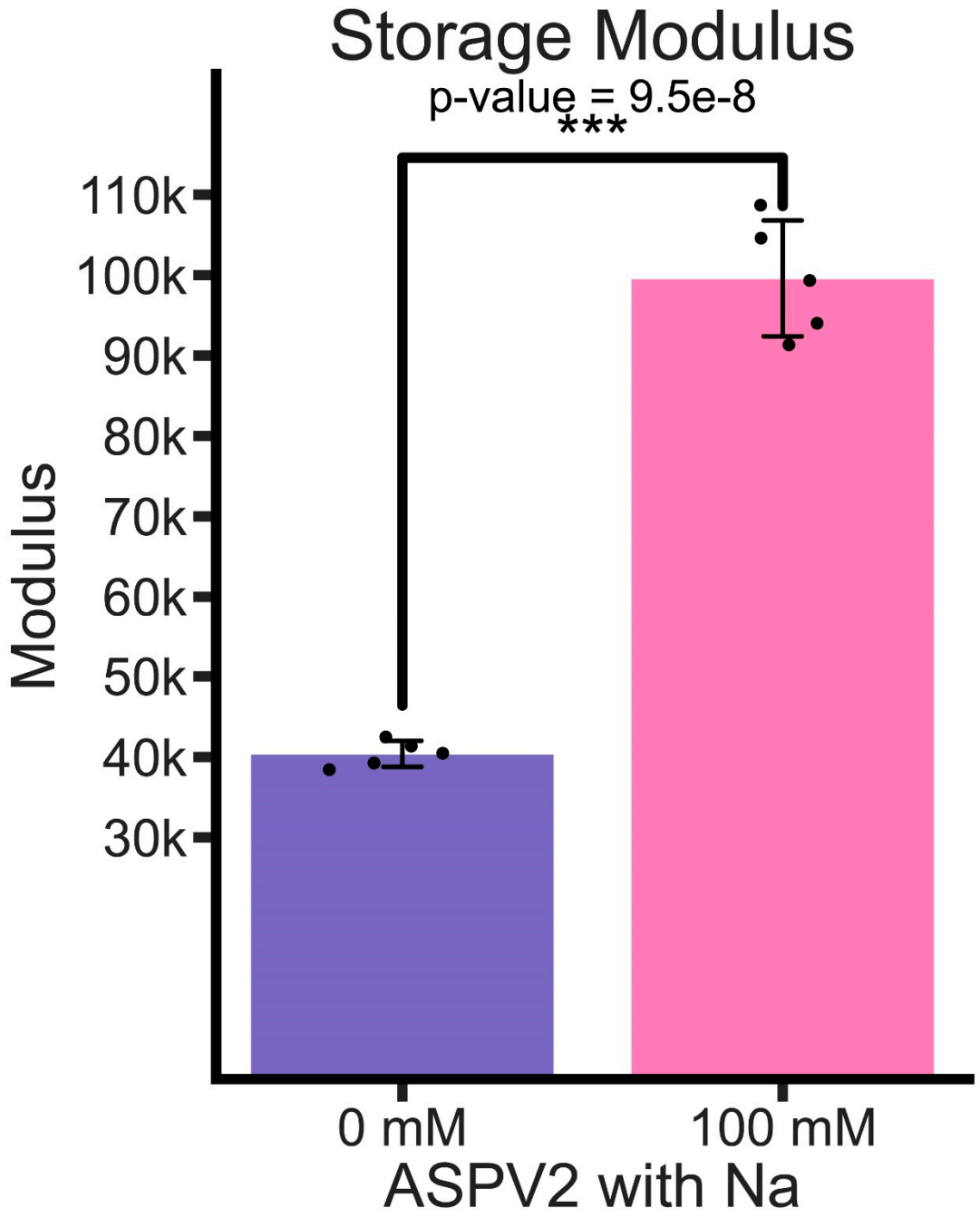

Figure S7. Effect of Sodium on the Storage Modulus of ASPV2. The left bar (purple) shows the storage modulus of ASPV2 without sodium (0 mM Na), and the right bar (pink) indicates the storage modulus with 100 mM sodium. Error bars depict the standard deviation, the statistical significance between the two conditions is denoted by asterisks (***), with a p-value of 9.5e-8.

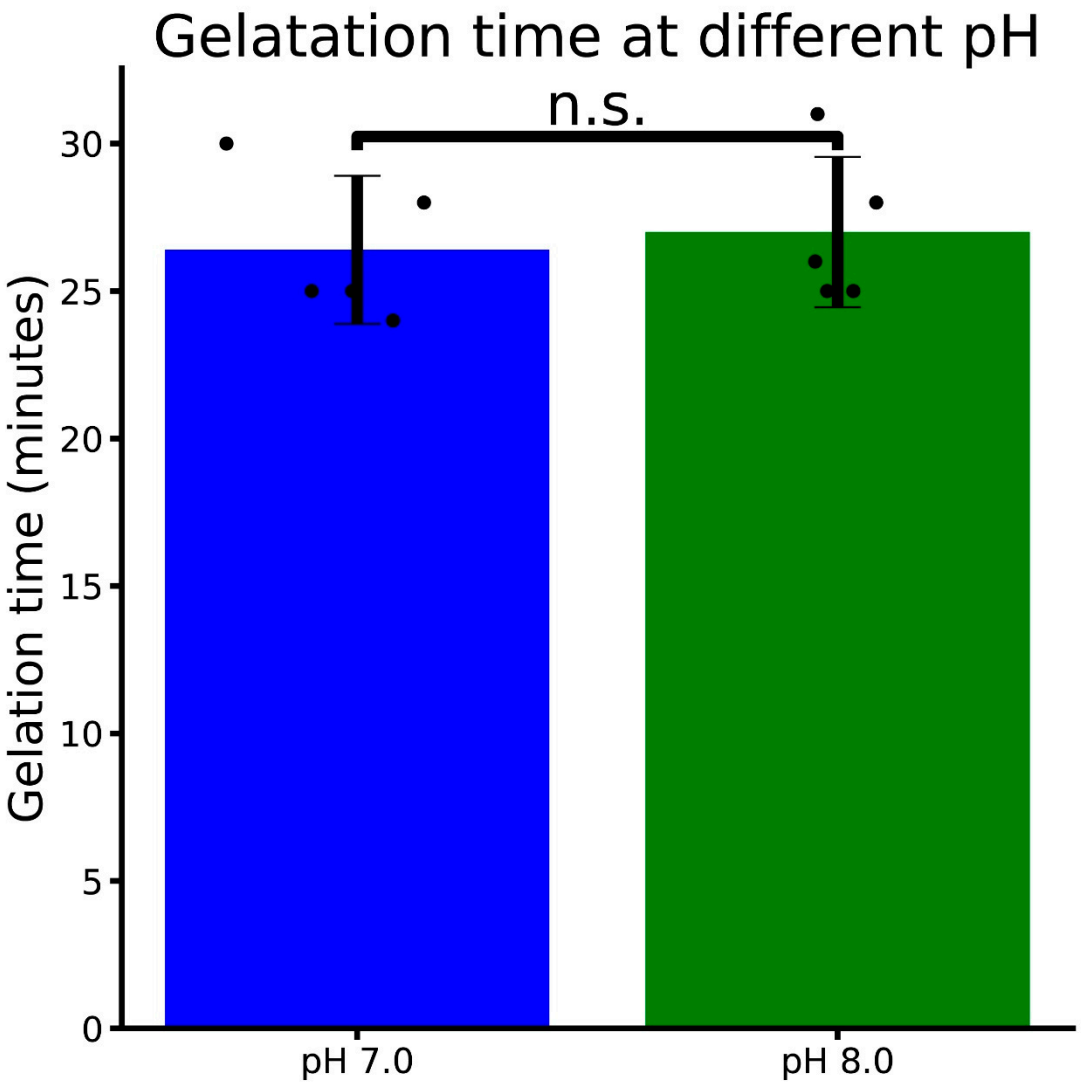

Figure S8. Gelation Time of Protein Solution at Different pH Levels. The chart reveals that the gelation time does not significantly change (n.s., not significant) with an increase in pH from 7.0 to 8.0. The error bars represent standard deviation. Error bars depict the standard deviation.

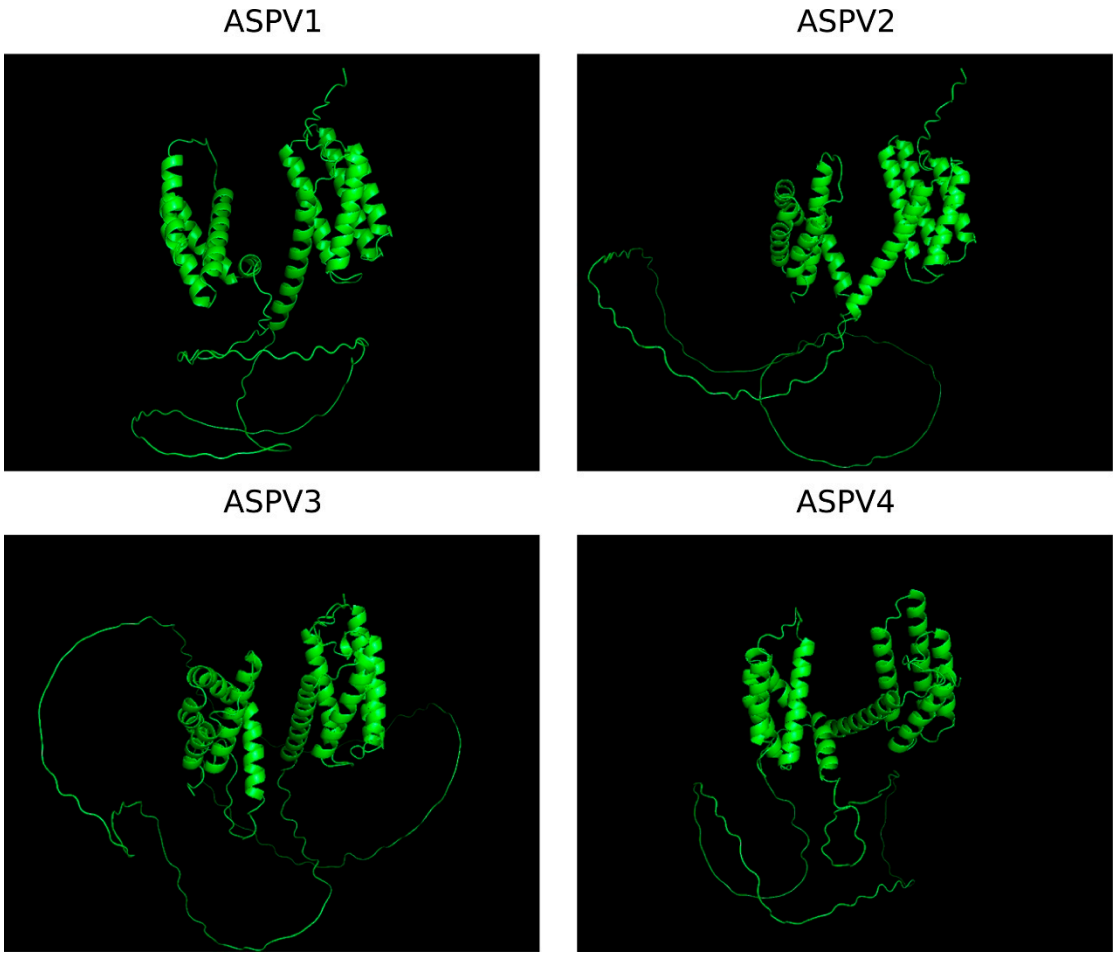

Figure S9. Predicted 3D Structures of ASPs by AlphaFold2. Each panel corresponds to a ASP, showing the intricate folds and coils characteristic of protein tertiary structures.

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