Supporting Information

for

Amyloid-based albumin hydrogels

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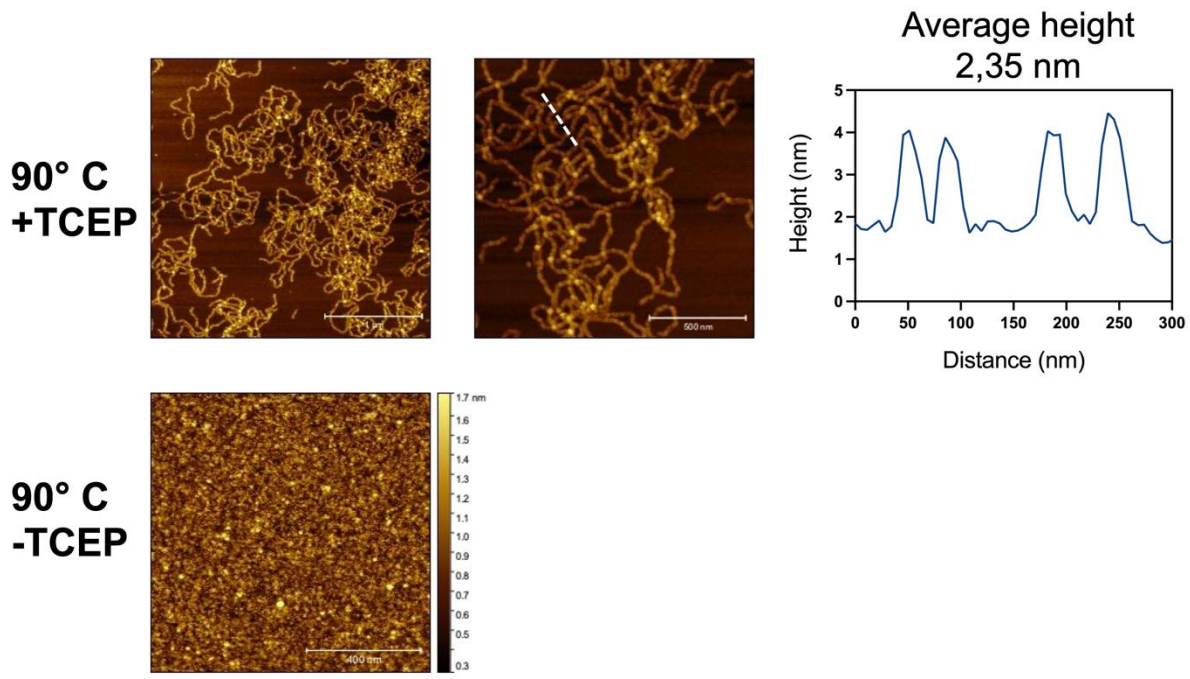

Figure S1. Atomic force microscopy images of amyloid-like BSA fibrils prepared by treating BSA with 40 mM TCEP at pH 3.6 and heating at 90°C for 24 hours (top), or for 24 hours without TCEP (bottom). The fibrils were deposited on a mica surface and gently air dried prior to imaging in tapping mode. A height profile corresponding to the white dashed line is presented at the top left and the average height, determined from 50 measurements, was calculated to be 2.35 nm.

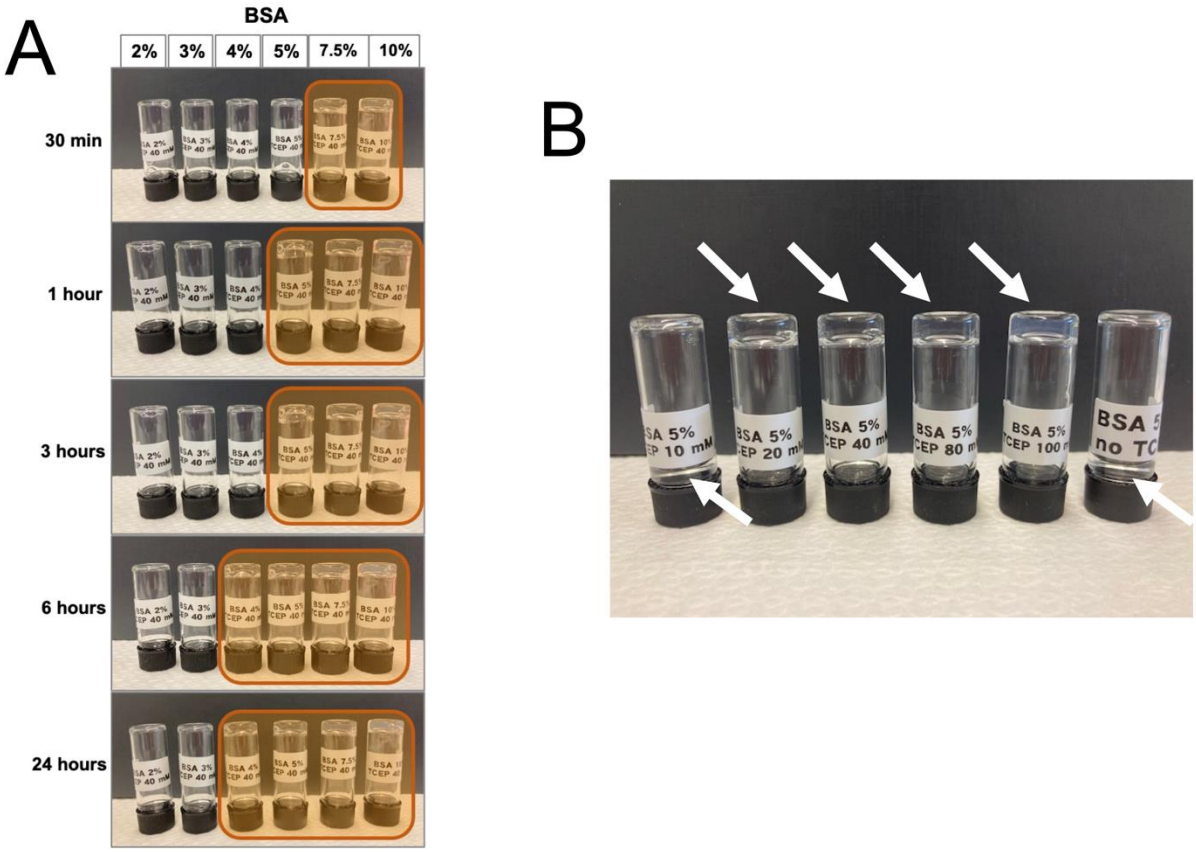

Figure S2. A) Inverted tube assay for the determination of gelation of aqueous BSA solutions with different BSA concentrations and 40 mM TCEP. Vials were kept at room temperature and inverted at different time points to determine whether a gel was formed (highlighted yellow). Solutions with BSA concentration equal or above 4% w/v formed gels within 6 hours. **B)** Inverted tube assay showing the effect of TCEP concentration on gelation of aqueous 5% w/v BSA solutions 24 hours after TCEP addition. A threshold concentration of 20 mM was required to induce gelation.

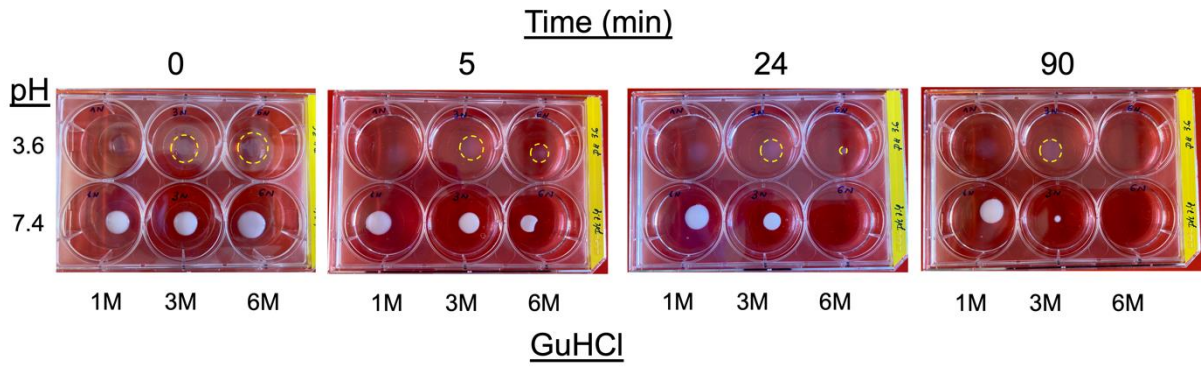

Figure S3. Images of 5% BSA hydrogels prepared on circular glass coverslips with 40 mM TCEP at pH 3.6 or 7.4. The hydrogels were placed in a 6-well plate and GuHCl solutions in PBS with indicated molarity were added. Images were acquired at different time points to determine the time that was required for complete dissolution of hydrogels. Here, images at 0, 5, 25 and 90 minutes after GuHCl are presented.

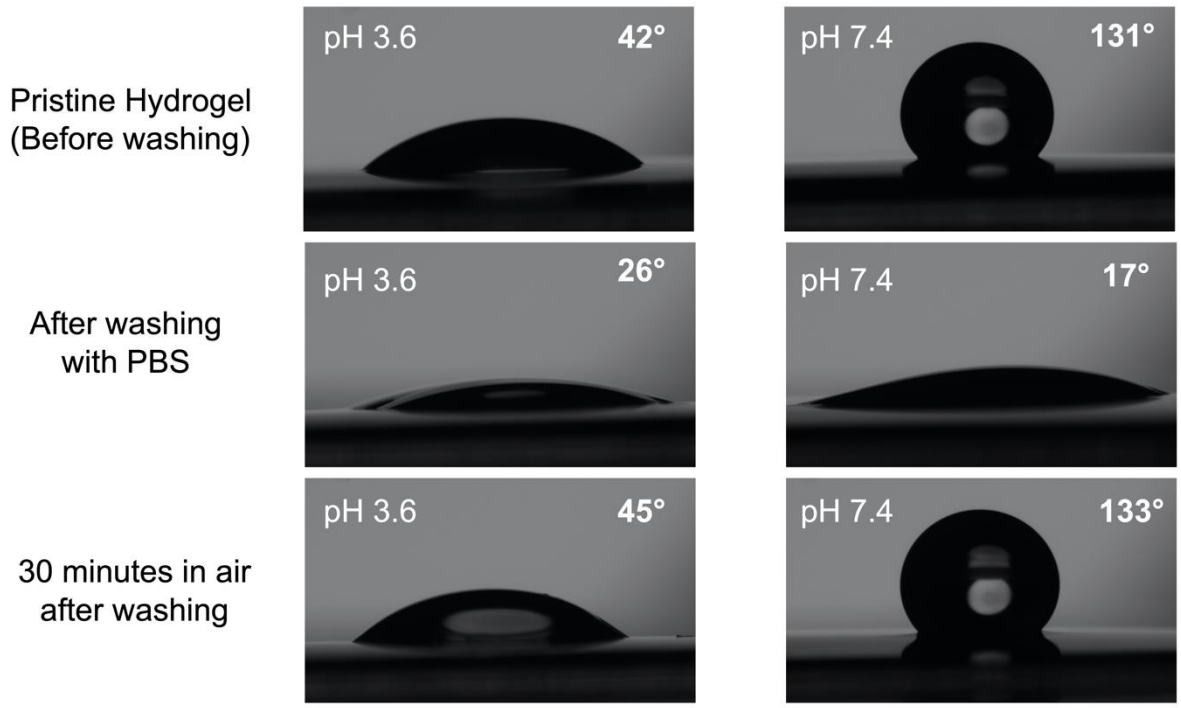

Figure S4. Contact Angle measurements on 5% BSA / 40 mM TCEP hydrogels prepared at pH 3.6 or 7.4. A drop of PBS was pipetted on top of the hydrogel 24 hours after formation, immediately after washing with PBS or 30 minutes after washing in air. Washing with PBS led to a decrease in contact angle indicating a more hydrophilic surface. The surface hydrophobicity recovered with time in air.

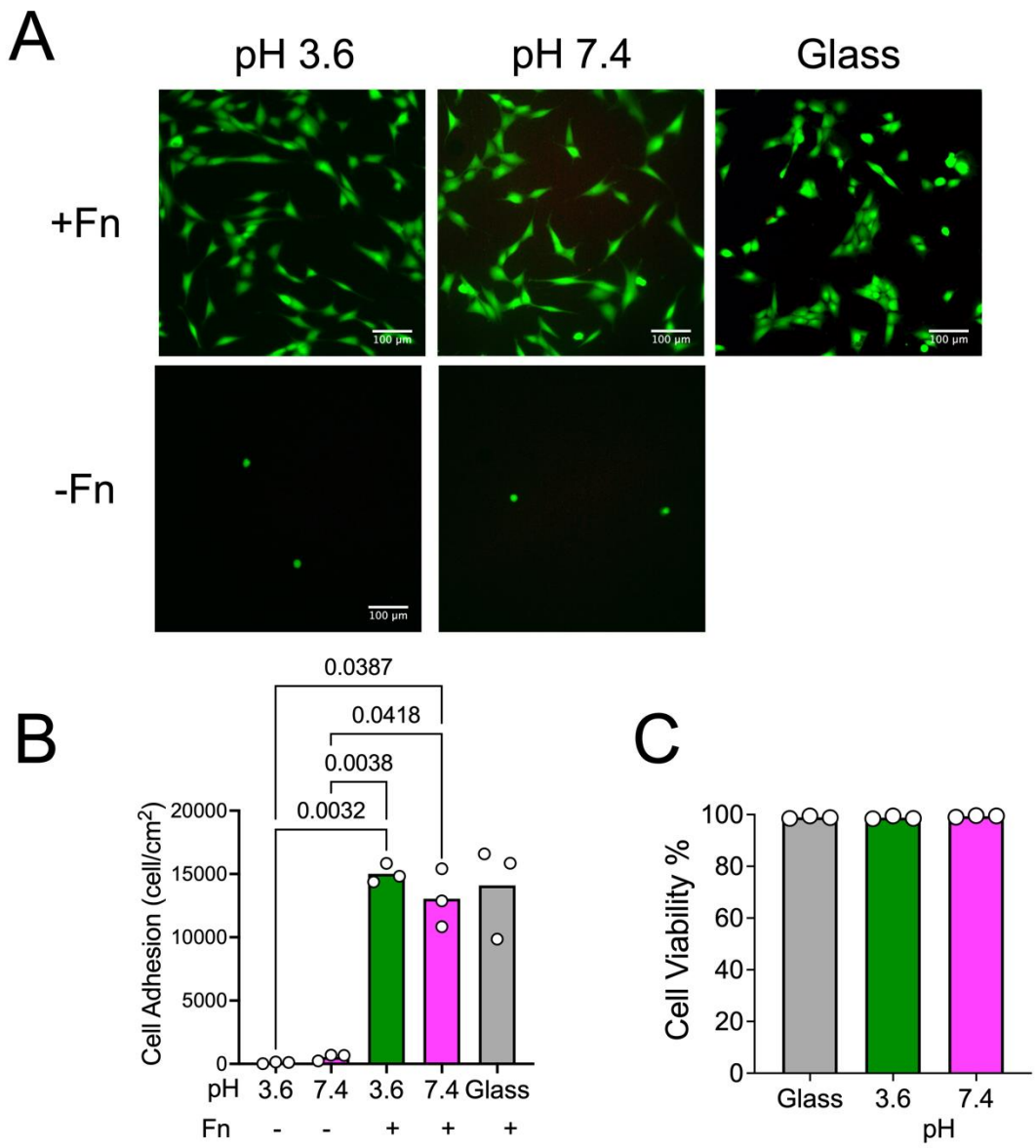

Figure S5. A) Epifluorescence microscopy images of live NIH3T3 cells stained with calcein (green) and dead U2OS cells stained with ethidium homodimer (red), 20 hours after cell seeding. Cells were seeded on Fn-coated or uncoated hydrogels prepared at indicated pH or Fn-coated glass as a control. **B)** Surface concentration of live NIH3T3 cells on different substrates 20 hours post-seeding. **G)** Viability of U2OS cells on different substrates 20 hours post-seeding was calculated as the ratio of live to total cells. Data in panels **F** and **G** were analyzed using a Brown-Forsythe and Welch ANOVA statistical test, comparing all data sets with each other. Only P values from statistically significant comparisons ($P<0.05$) are plotted. Scale bars: 20 µm.

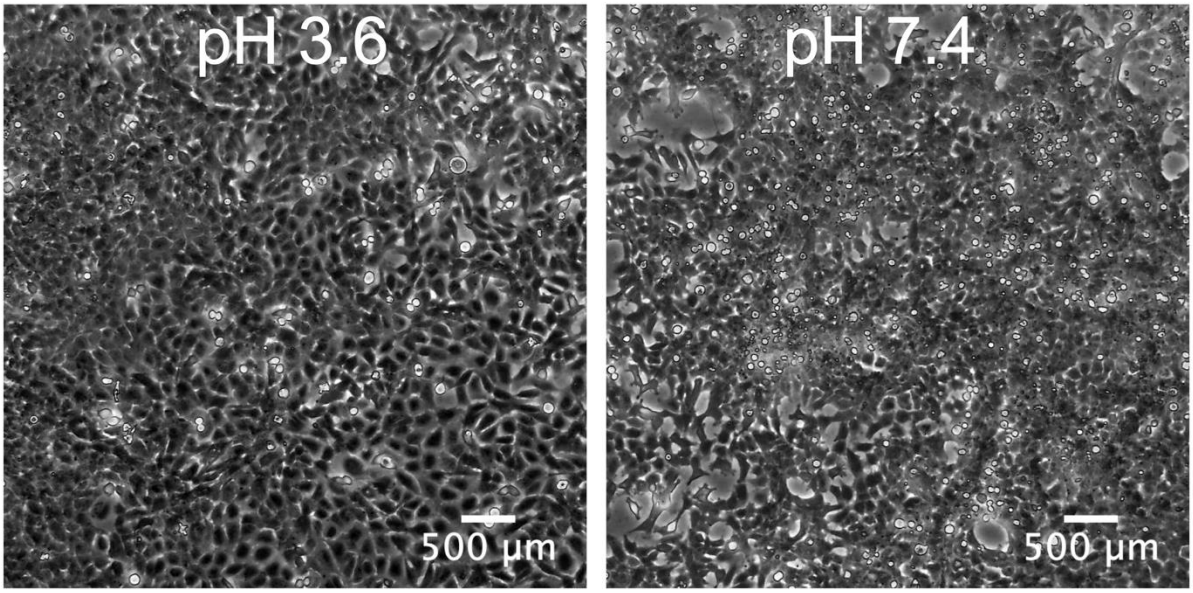

Figure S6. Phase contrast images of U2OS cells growing for 10 days on 5% BSA hydrogels, prepared at indicated pH values and subsequently coated with 10 µg/ml FN. Cells were initially seeded at 2,000 cells/cm^2 corresponding to approximately 10% confluency.