Supporting Information

Structure and nanomechanics of dry and hydrated intermediate filament films and fibers produced from hagfish slime fibers

L J Böni[1‡], A Sanchez-Ferrer[2], M Widmer[1], M D Biviano[3], R Mezzenga[2], E J Windhab[1], R R Dagastine[3], and P Fischer[1]

[1]Food Process Engineering, Department of Health Science and Technology, ETH Zürich, 8092 Zürich, Switzerland
[2]Food and Soft Materials, Department of Health Science and Technology, ETH Zürich, 8092 Zürich, Switzerland
[3]Department of Chemical and Biomolecular Engineering, University of Melbourne, Melbourne, Australia

[‡]Corresponding author email: lukas.boeni@hest.ethz.ch

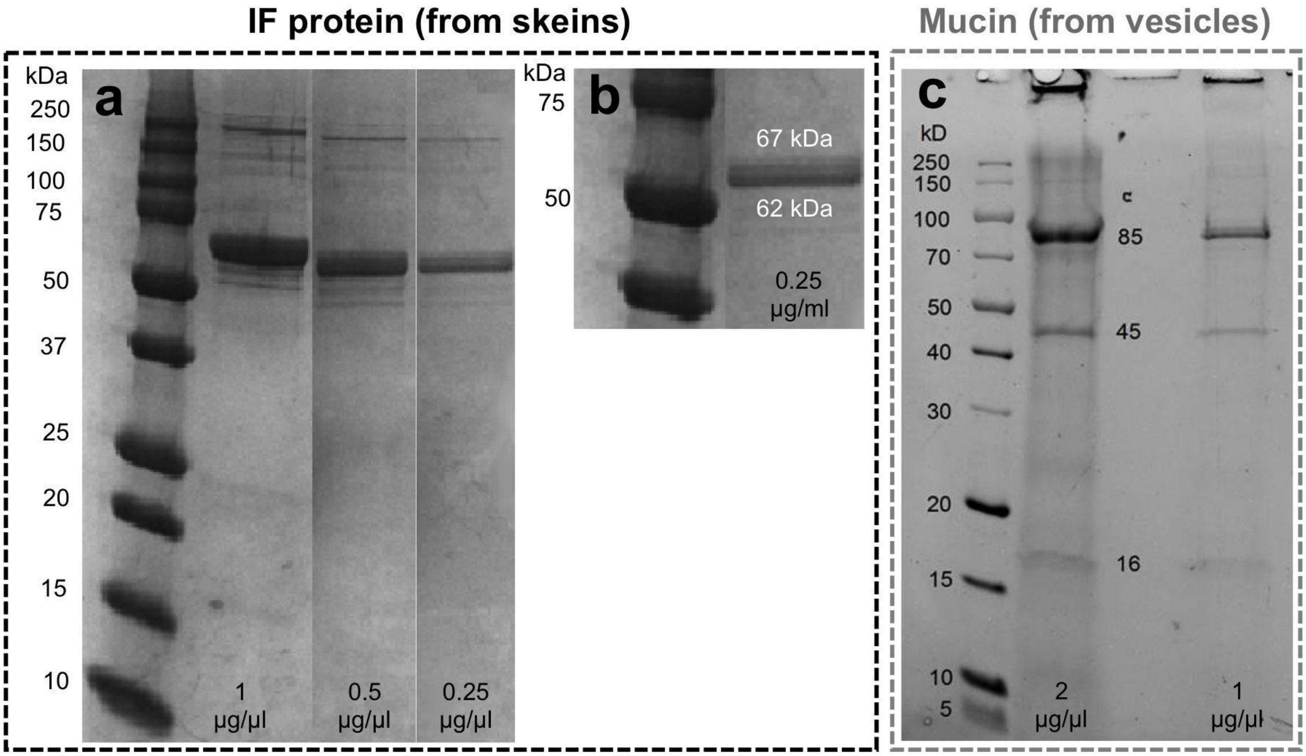

Figure S1: SDS-PAGE of freeze-dried hagfish skeins and mucin. (a) Skeins (hagfish fiber intermediate filament protein) at various concentrations consistently show a double band around 62 to 67 kDa. **(b)** Magnification of double band from (a) at a sample concentration of 0.25 µg/µl. **(c)** Dialyzed hagfish mucin

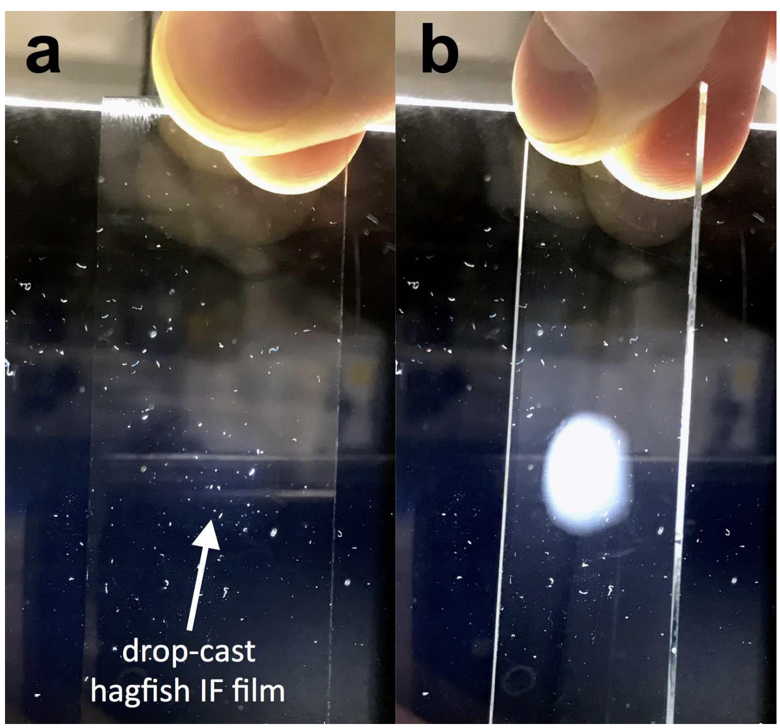

Figure S2: Birefringence of hagfish IF film drop-cast on glass. (a) Holding the glass slide parallel to the cross-polarizers results in no birefringence pattern. **(b)** When the glass slide is tilted birefringence is observed. This suggests that the β-sheets in the IF β-crystallites are parallel to the glass substrate. Therefore, when the film is held parallel to the cross-polarizers, the incident light is perpendicular to the β-sheets and thus no birefringence is observed.

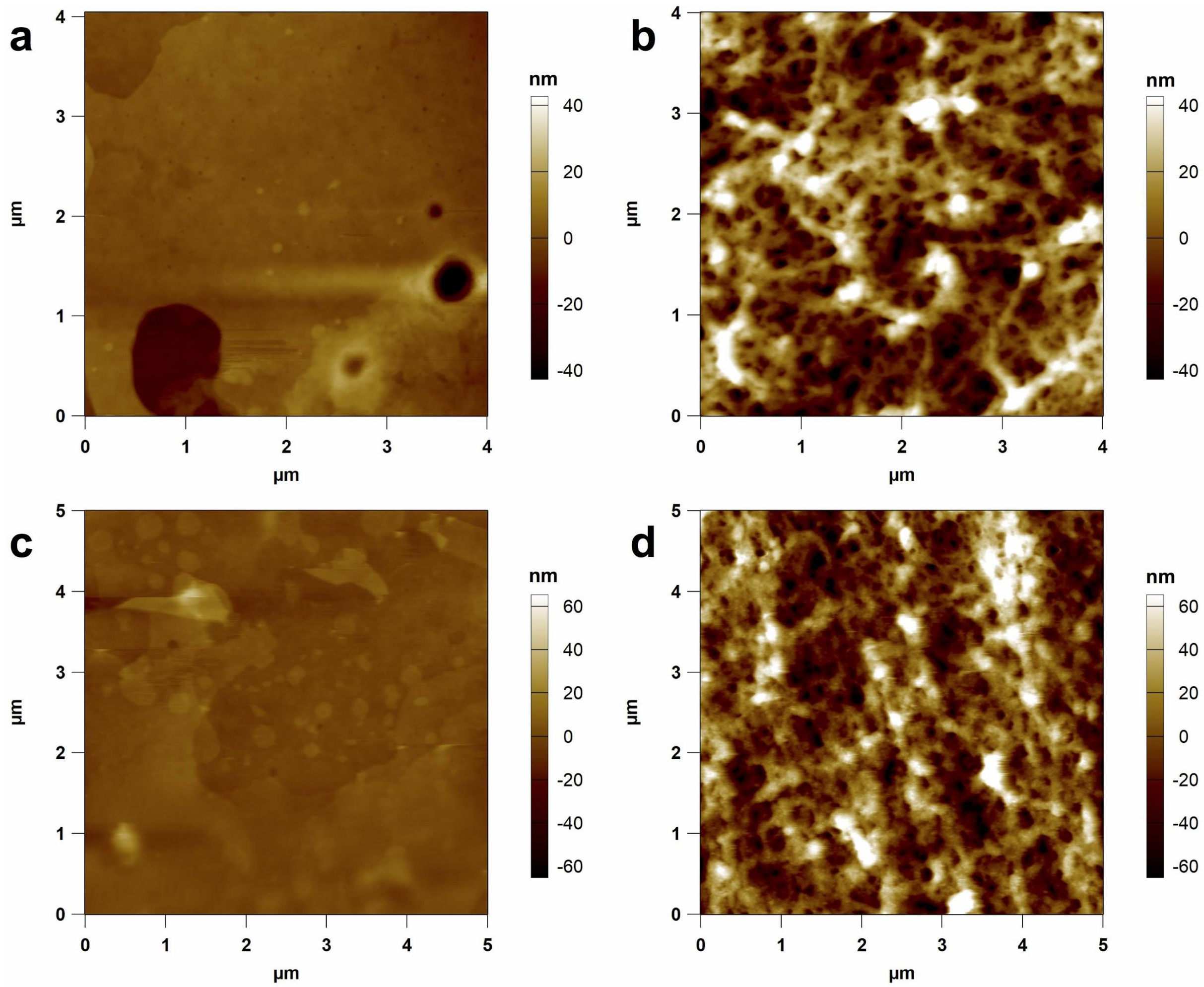

Figure S3: AFM images of a drop-cast film (**a**) and a coagulation film (**b**) in the dry state. AFM images of a drop-cast film (**c**) and a coagulation film (**d**) in liquid (5.45 mM NaCl + 0.1 mM $CaCl_2$).

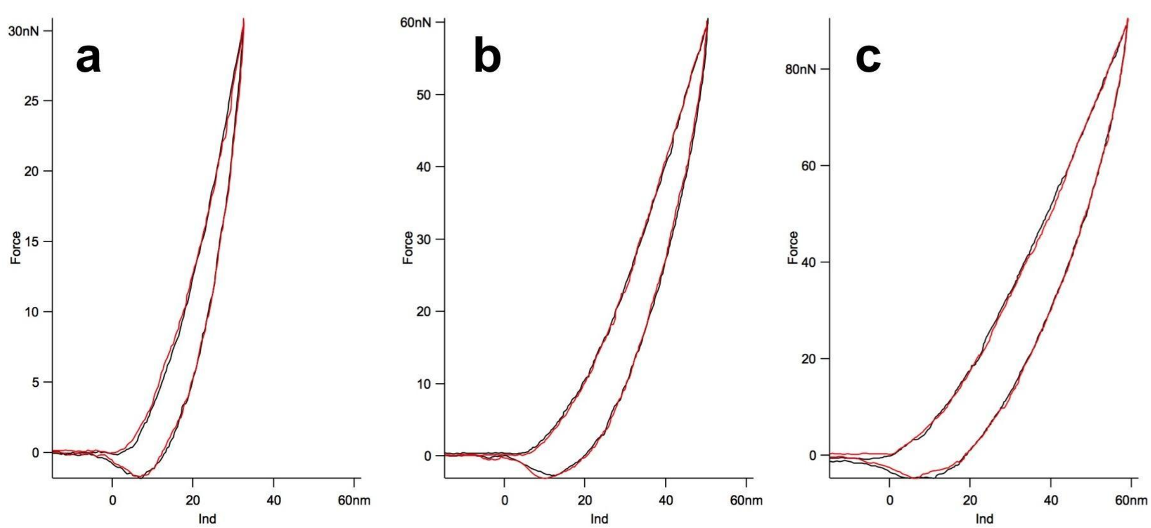

Figure S4: Repeated indentation on the same spot at increasing trigger force on a coagulation film. Red curve - 1st indentation, black curve 6th indentation for increasing trigger forces (a) < (b) < (c). The hysteresis loop does not change with repeated indentations on the same spot, showing that there is no permanent plastic deformation.

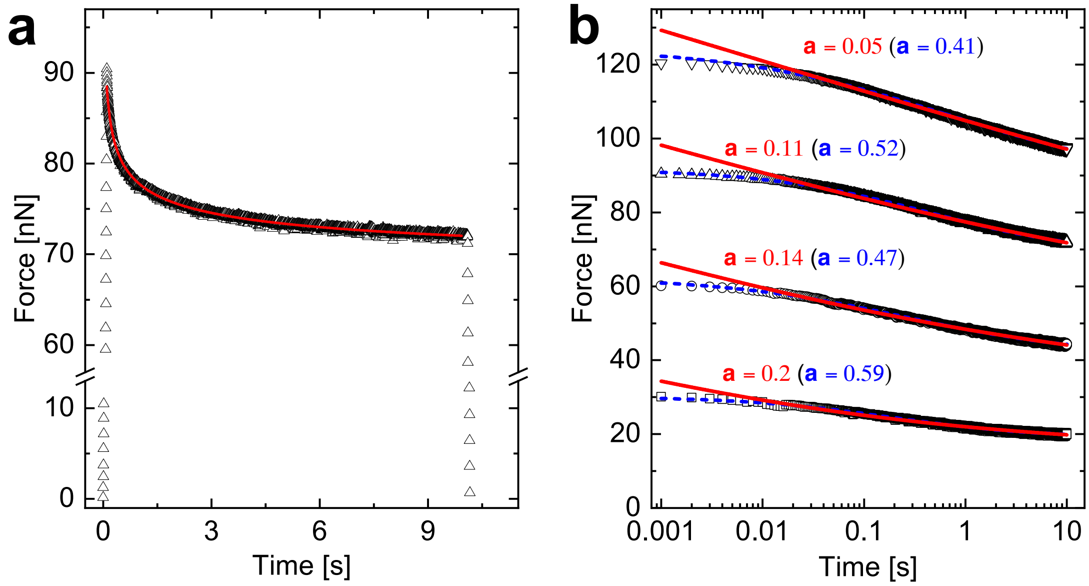

Figure S5: Power-law rheology (PLR) model fitted to AFM stress-relaxation measurements on a hydrated hagfish IF film (coagulation film). (a) Stress relaxation from an initial load of about 90 nN with fitted PLR model. **(b)** Stress relaxation measurements performed at increasing initial peak load (strain) and the corresponding PLR fits. The red lines depict fits where *t'* was held constant at $5*10^{-5}$, similar to the PLR fits used for the F-D curves. The blue lines represent fits where t' was let run free for the fitting, which resulted in better fits but also in substantially higher alpha values (in brackets).

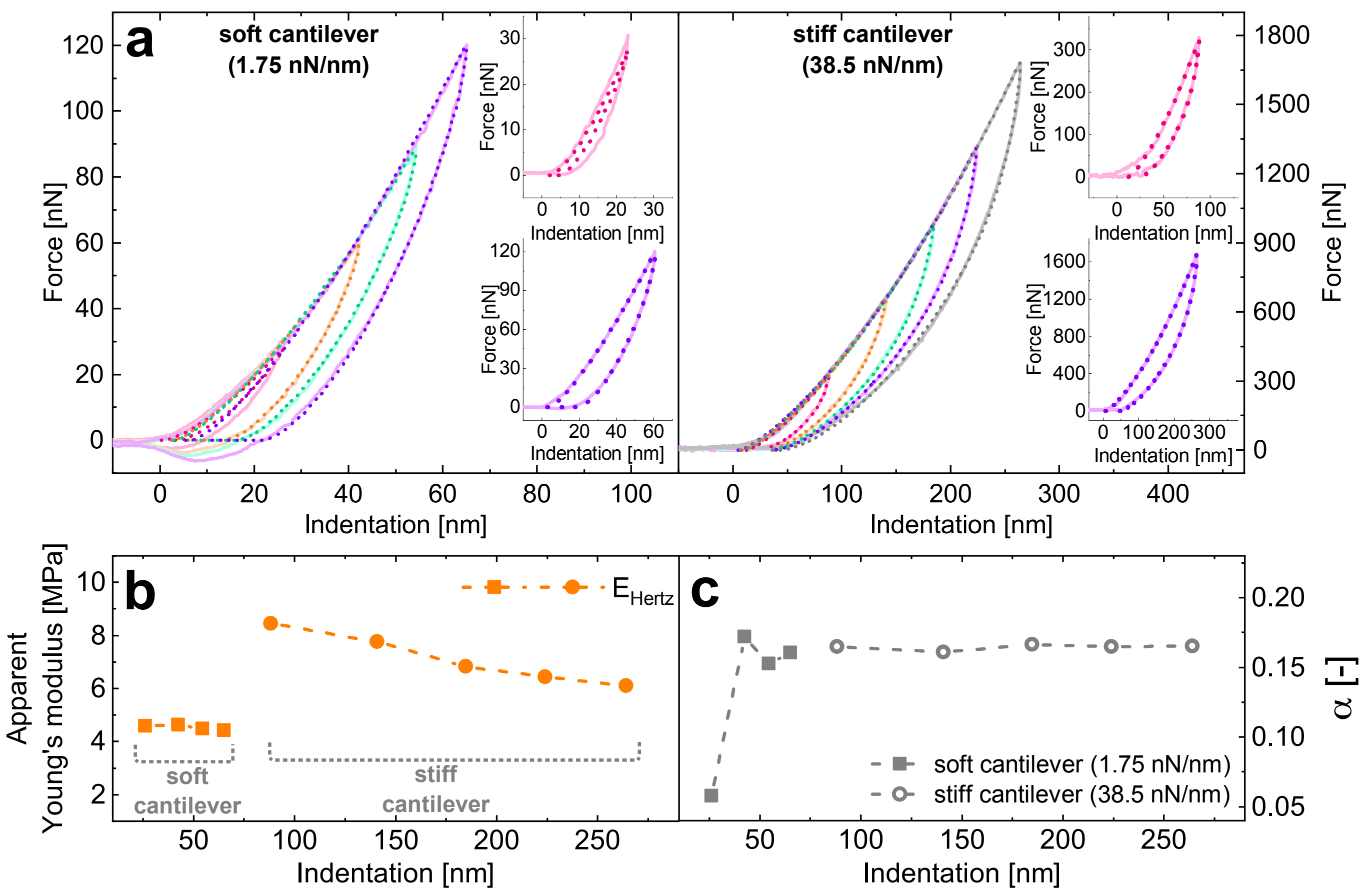

Figure S6: Effect of increasing indentation depth (strain) for two differently stiff cantilevers on the mechanical properties of hydrated coagulation film. (a) Force-distance curves (solid lines) performed at increasing peak forces on a hydrated coagulation film, overlaid with the PLR fit. The graph on the left shows curves obtained with a soft cantilever (1.75 nN/nm) and the graph on the right shows curves obtained using a stiff cantilever (38.5 nN/nm). Both cantilevers had a radius of 500 nm. **(b)** Elastic modulus and **(c)** power-law exponent *α* based on data shown in (a).

Table S1: Root mean square (RMS) and average deviation (ADev) roughness of dry and hydrated hagfish IF films calculated for 1 x 1 μm and 2 x 2 μm window sizes.

	Coagulation film				**Drop-cast film**			
	dry		**hydrated**		**dry**		**hydrated**	
	1 x 1 μm window (n=25)	2 x 2 μm window (n=5)	1 x 1 μm window (n=25)	2 x 2 μm window d (n=25)	1 x 1 μm window (n=25)	2 x 2 μm window (n=5)	1 x 1 μm window (n=5)	2 x 2 μm window n=5)
RMS (nm)	20.8 ± 4.3	19.1 ± 1.3	35.7 ± 7.7	37.0 ± 4.6	6.4 ± 4.3	7.1 ± 3.8	6.2 ±- 3.0	6.7 ± 2.8
ADev (nm)	15.3 ± 3.8	15.1 ± 1.3	22.3 ± 5.7	28.1 ± 6.6	3.8 ± 3.4	4.7 ± 3.0	3.3 ± 1.4	5.2 ± 2.2