Supplementary Information (SI) for Soft Matter.
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Supporting Information for

Hydrogel Formation by Liquid–Solid Phase Transition of Hagfish Intermediate Filament Protein Condensates

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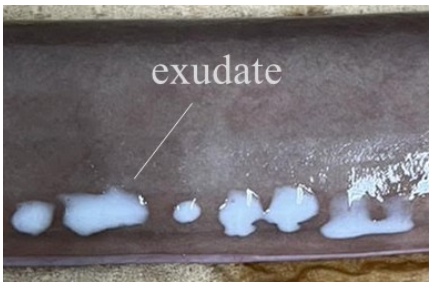

Figure S1. Image of hagfish exudate. Exudate released along the abdomen of hagfish following its sedation and subsequent mild electrical stimulation.

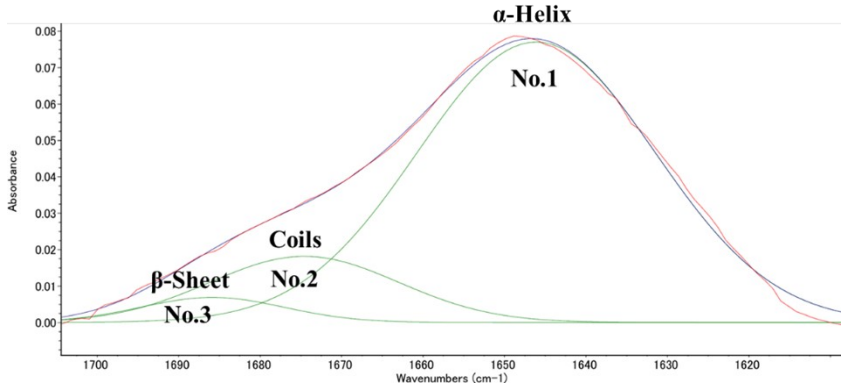

Figure S2. Peak resolution for the amide I band of purified hagfish IF protein samples. The secondary structure was identified from the amide I band of the IF protein spectrum recovered after the purification process in Figure 1A. The secondary structure was identified from the amide I band of the IF protein spectrum recovered after the purification process in Figure 1A, where the presence of a high-wavenumber shoulder around 1690 cm^{-1} indicates the formation of antiparallel β-sheet structures.

Table S1. Parameter values for the amide I component peaks of the wave spectra of purified and treated IF proteins (Figure 1A purple line).

No. of peaks	Center (cm^{-1})	Height	Width	Area	% Area
1	1646	0.0770	34.4	2.818	80.6
2	1674	0.0181	27.3	0.527	15.1
3	1685	0.0069	20.7	0.151	4.3

TKα：

MSISQTVSKSYTKSVSRGGQGVSYSQSSSHKVGGGSVRYGTTYSSGGISRVLGFQGGAGGAASA
GFGGSVGGSGLSRVLGGSMVSGYRSGMGVGGLSLSGTAGLPVSLRGVGAGKALHAITSAFRTR
VGGPGTSVGGYGVNYSFLPSTAGPSFGGPFGGPFGGPFGGPLGPGYIDPATLPSPDTVQHTRIREK
QDLQTLNTKFANLVDQVRTLEQHNAILKAQISMITSPSDTPEGPVNTAVVASTVTATYNAQIEDL
RTTNTALHSEIDHLTTIINDITTKYEEQVEVTRTLETDWNTNKDNIDNTYLTIVDLQTKVQGLDEQ
INTTKQIYNARVREVQAAVTGGPTAAYSIRVDNTHQAIDLTTSLQEMKTHYEVLATKSREEAFT
QVQPRIQEMAVTVQAGPQAIIQAKEQIHVFKLQIDSVHREIDRLHRKNTDVEREITVIETNIHTQS
DEWTNNINSLKVDLEVIKKQITQYARDYQDLLATKMSLDVEIAAYKKLLDSEETRISHGGGITIT
TNAGTFPGGLSAAPGGGASYAMVPAGVGGVGLAGVGGYGFRSMGGGGGVGYGAGGGGVGY
GVGGGFGGGMGMSMSRMSMGAAVGGGSYGSGSGYSGGFGLSSSRAGYSASRKSYSSARSSSRI
Y

TKγ：

MASHSSVSYRSVRTGGTSAMIGSSGYGGSSSSRAMGLGMGAAGLSMGGGSFRVGSAGIGGMG
ISSGIGGMGISSRAGGMSAYGGAASGGAGGFVSGGVPMLGYGGGAGGFIGGVSPGIMASPAFT
AGRAITSAGMSGVVGTLGPAGGMVPSLVSRDEVKNILGTLNQRLASYVDKVRQLTIENETMEE
ELKNLTGGVPMSPDSTVNLENVETQVTEMLTEVSNLTLERVRLEIDVDHLRATADEIKSKYEFE
LGVRMQLETDIANMKRDLEAANDMRVDLDSKFNFLTEELTFQRKTQMEELNTLKQQFGRLGP
VQTSVIELDNVKSVNLTDALNVMREEYQQVVTKNVQEAETYCKMQIDQIQGISTQTTEQISILD
KEINTLEKELQPLNVEYQRLLTTYQTLGDRLTDLQNRESIDLVQFQNTYTRYEQEIEGNQVDLQ
RQLVTYQQLLDVKTALDAEIATYKKLLEGQELMVRTAMADDFAHATVVRSGTLGGASSSSVG
YGASSTTLGAISGGYSTGGGASYSAGAGGASYSAGAGGASYGVGGGYSGGSSAMMEGSSSGG
HSMYSSSSMKRSSSKSASASAGGYGTSGHDSTIILQQ

Figure S3. Amino acid sequences of hagfish thread keratin α and γ used to predict phase separation propensity.

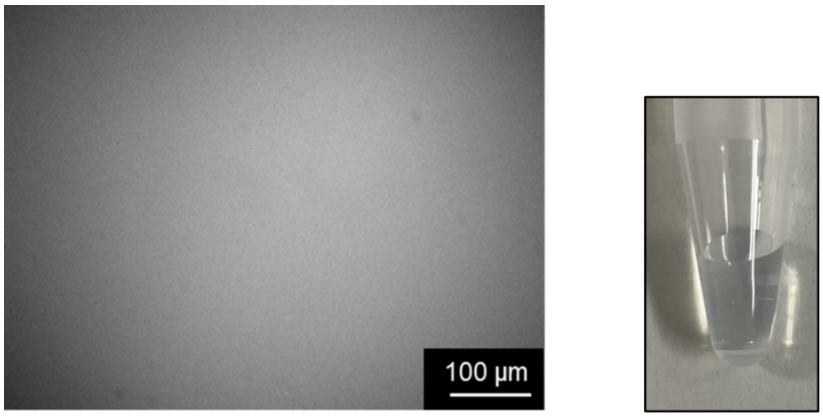

Figure S4. Differential interference contrast microscopic image and photographs of the solution in a system lacking intermediate filament proteins. DIC image (left) and photograph (right) of a solution containing 10% (w/v) DEX and 100 mM NaCl. In the absence of monomerized IF proteins, no phase separation dynamics occurred, and no precipitates formed in the system.

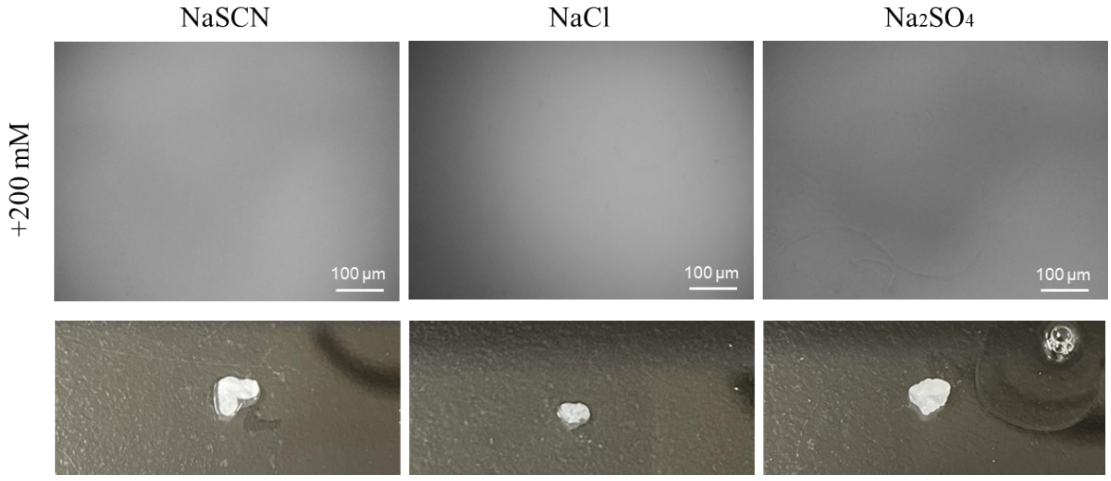

Figure S5. Hofmeister effect on intermediate filament protein precipitate formation. DIC images (top) and photographs (bottom) of precipitates formed by the addition of NaSCN (a chaotrope), NaCl, and Na_2SO_4 (a kosmotrope) in a Hofmeister series aimed at verifying the salting out effect on IF proteins. The samples consisted of each electrolyte dissolved in PBS containing 6% (w/v) IF proteins and no DEX.

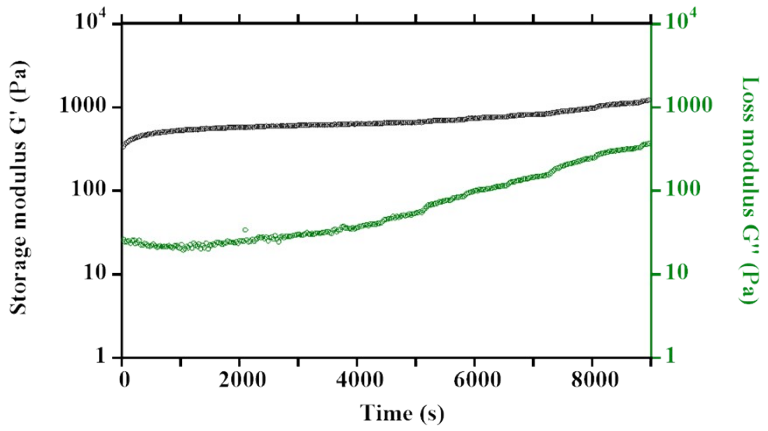

Figure S6. Time sweep test of storage G' and loss G" moduli associated with LSPT. The measurement was performed under oscillatory shear at γ, 1%; ω, 1 rad/s; and 25 °C with a measurement time of 30–9,000 s. The components were 6% (w/v) hydrogel sample, 10% (w/v) DEX, and 1000 mM NaCl.

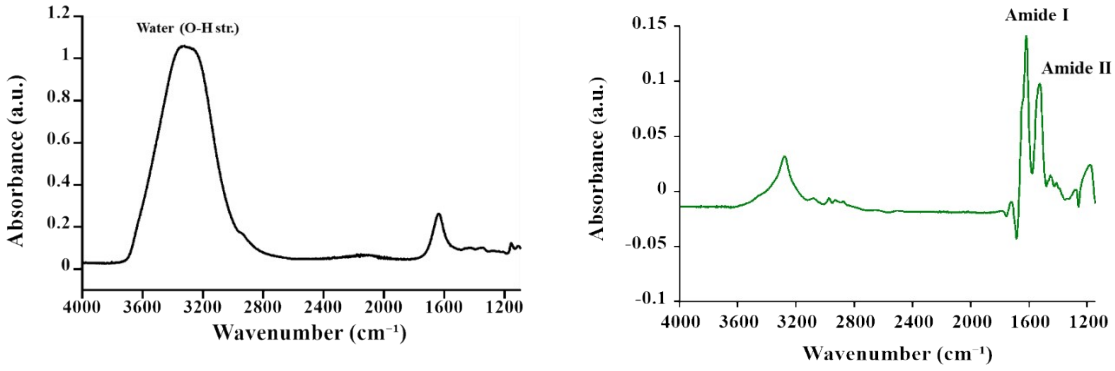

Figure S7. ATR-FTIR spectra of the background and the full spectrum of Hagfish IF proteins within the hydrogel sample.
left: Background spectrum of the DEX–PBS solution. right: Full ATR-FTIR spectrum of the Hagfish IF protein within the hydrogel sample.

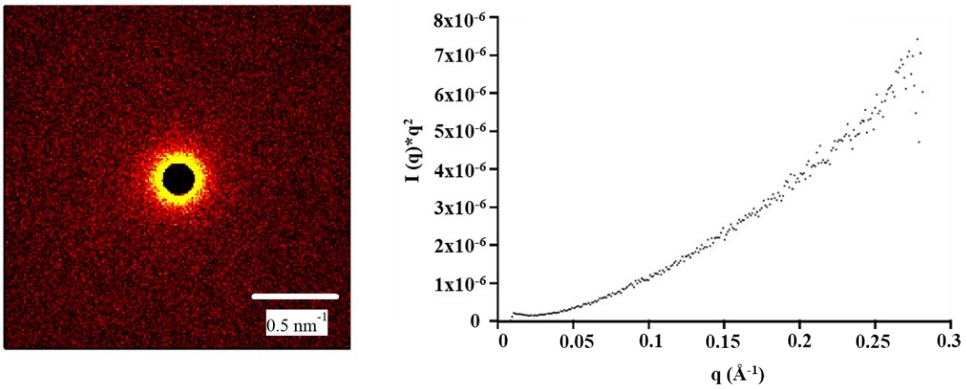

Figure S8. Small-angle X-ray scattering intensity plot of the internal IF protein-based hydrogel structure.

Left: Two-dimensional pattern reflecting the structure of IF proteins inside a hydrogel, as obtained via SAXS measurements. The concentrations of system components were 6% (w/v) IF protein, 10% (w/v) DEX, and 1000 mM NaCl. Right: Kratky plot obtained by analyzing the SAXS intensity data. IF, intermediate filament; SAXS, Small angle X-ray scattering; DEX, dextran.