Supporting Information
Effect of ionic strength on structure and elongational kinetics of vimentin filaments

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I. S.1 HYDRODYNAMIC PROPERTIES OF FILAMENTS

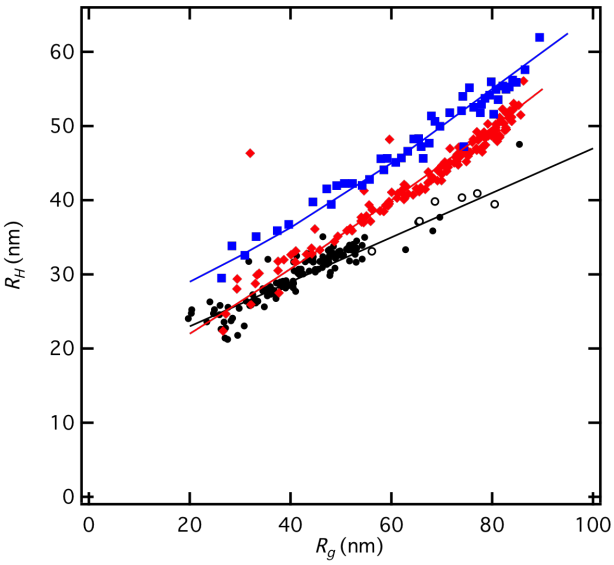

FIG. S1. R_H versus R_g for kinetic runs at different salt concentrations. Blue symbols (c_S = 200 mM), red symbols (c_S = 100 mM) and black symbols (c_S = 50 mM). Lines are guides to the eye.

Figure S1 shows a plot of R_H versus R_g for the three kinetic runs at c_S = 50, 100 and 200 mM discussed in the main manuscript. R_H and R_g values have been binned for clarity (5 points averaged into one for c_S = 50 and 100 mM and 3 points averaged into one for c_S = 200 mM) excluding some spurious points which display abnormally high values of R_g and/or R_H due to the presence of dust. The values of R_H for a given R_g (which serves as a measure of the length of filaments) are seen to increase with increasing salt concentration. This is consistent with the positive correlation between of M_L and c_S.

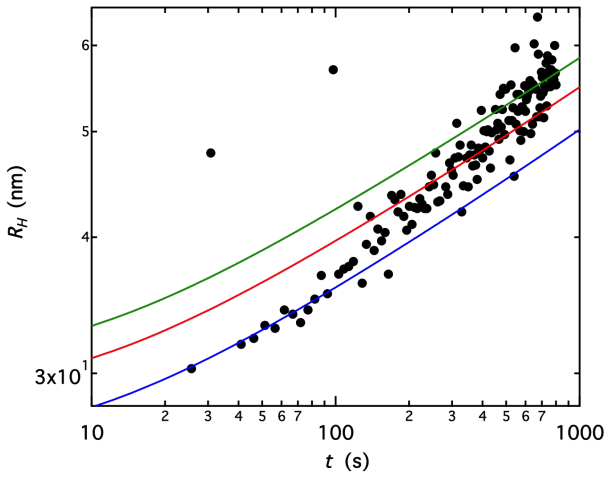

FIG. S2. R_H as a function of time for c_S = 200 mM. Green line corresponds to kinetic model fit with d_H = 60nm, red line for d_H = 52nm and blue line for d_H = 42 nm.

Figure S2 shows fits to R_H as a function of time using the kinetic model with different values for the hydrodynamic filament cross sectional diameter. The value of d_H = 52 nm taken to be the best fit value over–predicts experimental data at short times and under–predicts it at long times. The best fit values of d_H at short and long times correspond to d_H = 42 nm and d_H = 60 nm.

II. S.2 BACKGROUND THEORY AND KINETIC MODELLING

For rods following a Schultz–Zimm distribution of lengths[1, 2], the weight fraction of rods with length L is:

$$w(L) = \Big(\frac{z+1}{L_w}\Big)^{z+1} \frac{L^z}{\Gamma(z+1)} exp\Big(-\frac{(z+1)L}{L_w}\Big) \tag{S1}$$

where Γ is the gamma function, L_w is the weight averaged rod length, L_n is the number averaged rod length and $z = (L_w/L_n - 1)^{-1}$. $\langle R_g^2 \rangle_z$ and D_z are given by:[1, 2]

$$\langle R_g^2 \rangle_z = \frac{\int w(L,z) R_g^2(L) L dL}{\int w(L,z) L dL} = \frac{(z+3)(z+2)}{(z+1)^2} \frac{L_w^2}{12}. \tag{S2}$$

$$D_z = \frac{\int w(L,z) D(L) L dL}{\int w(L,z) L dL} \tag{S3}$$

$$\begin{aligned} D(L) = \frac{k_B T}{3\pi\eta L} ln\Big(2x - 1 + [(2x-1)^2 + 1]^{1/2}\Big) + x^{-1}\Big(x + \sqrt{2} - [(2x-1)^2+1]^{1/2}\Big) + \\ \frac{1}{2x} ln\Big[\frac{(\sqrt{2}-1)^2)\big(1 + [(2x-1)^2+1]^{1/2}\big)}{2x - 1 + [(2x-1)^2+1]^{1/2}}\Big] \end{aligned} \tag{S4}$$

where η is the viscosity of the solvent and $x = L/d_H$ with d_H the hydrodynamically effective cross sectional diameter of the rod.

In an earlier study[3] we employed SLS/DLS to monitor the assembly of vimentin intermediate filaments in aqueous 50 mM KCl solutions. The resulting M_w, R_g and R_H could be fitted to a two step model: in step I, p tetramers assemble laterally to form ULFs, followed by end–to–end elongation in step II. Rate equations for these two steps are:

$$\frac{df_1}{dt} = -pk_n[f_1]^p \tag{S5}$$

$$\frac{dF_i}{dt} = +k_n[f_1]^p \delta_{i,1} - \sum_{j=1}^{\infty} k_{i,j}[F_i][F_j] + \frac{1}{2}\sum_{j=1}^{i-1} k_{j,i-j}[F_j][F_{i-j}] \tag{S6}$$

where k_n is the reaction constant for step I, $k_{i,j}$ is the reaction constant between species i and j in step II, $[f_1]$ is the concentration of non–assembled species (i.e. tetramers) and $[F_i]$ is the concentration of assembled species, with the same cross section as a ULF and with longitudinal degree of polymerisation i. $[F_1]$ corresponds to the concentration of ULFs. Eq S6 without the first term is the Smoluchowski equation, which has been applied to rods in the context of vimentin assembly [3–6] and gold nano–rods [7] to give but two examples. The rate constant $k_{i,j}$ is length dependent and given by [8]

$$k_{i,j} = 4\pi(D_j + D_j)(L_i + L_j)P_{i,j}/2 \tag{S7}$$

where D_j and D_j are the diffusion coefficients of the two particles, L_i is the length of particle i and $P_{i,j}$ is the probability that a collision between particles i and j is successful. Hill derived a scaling law of $P_{i,j} = 1/2(\delta\omega/(L_i + L_j))^2$, assuming a maximum angle (ω) and a maximum distance (δ) for a collision to be successful that are independent of L_i and L_j. Inserting the expression for the diffusion coefficient, D_i, by de la Torre et al[9–11]:

$$D_i = \frac{k_B T}{3\pi\eta} \frac{1}{L_i}\Big(ln(L_i/d_H) + 0.312 + 0.565\frac{d_H}{L_i} - 0.1\frac{d_H^2}{L_i^2}\Big) \tag{S8}$$

together with the scaling expression derived by Hill[8] for $P_{i,j}$, into eq S7 gives for $k_{i,j}$[3, 12, 13]:

$$k_{i,j} = \frac{k_B T N_A (\delta\omega)^2}{3\eta} \frac{1}{L_i + L_j} \Big(\frac{ln(L_i/d_H) + 0.312 + 0.565\frac{d_H}{L_i} - 0.1\frac{d_H^2}{L_i^2}}{L_i} + \frac{ln(L_i/d_H) + 0.312 + 0.565\frac{d_H}{L_j} - 0.1\frac{d_H^2}{L_j^2}}{L_j} \Big) \tag{S9}$$

where d_H is the hydrodynamic filament diameter, N_A is Avogadro's number and k_B is the Boltzmann constant, N_A is introduced in eq S9 as we express concentrations of particles in our rate equations in units of mol/L. In order to reduce the number of equations to be solved, we approximate the average rate constant of the elongation reaction as [3, 12]:

$$\overline{k_{i,j}} = \frac{k_B T N_A (\delta\omega)^2}{3\eta} \frac{1}{\overline{L}^2} \Big(ln(\overline{L}/d_H) + 0.312 + 0.565\frac{d_H}{\overline{L}} - 0.1\frac{d_H^2}{\overline{L}^2} \Big) \simeq \frac{G}{\overline{L}^{2+\mu}} \tag{S10}$$

where $\overline{L}$ is the number average length of the F species and $G = \frac{k_B T N_A (\delta\omega)^2}{3\eta} E$. E and μ are chosen such that $E/\overline{L}^{\mu}$ closely approximates the expression in big brackets. For rods, $D \sim L^{-1} ln(L/d_H)$. Accordingly, a variation of the fit parameter d_H in eq S10 or S11 induces an adjustment of the exponent μ. In the thin rod limit $D \sim L^{-1}$ and for rods of finite thickness an apparent power law exponent ($D \sim \overline{L}^{-\alpha}$) of $\alpha \leq 1$ is expected.

The initial conditions are given by the protein concentration and the fraction of tetramers assembled into filaments at $t = 0$. This and similar models have been applied to light scattering [3] and electron microscopy data[4, 5] of vimentin previously. For this study, we leave the power law scaling of $P_{i,j}$ as a free parameter, such that $P_{i,j} = A\overline{L}^{-\beta}/2$, which is equivalent to modifying eq S10 to:

$$\overline{k_{i,j}} = \frac{k_B T N_A A}{3\eta} \frac{1}{\overline{L}^{\beta}} \Big(ln(\overline{L}/d_H) + 0.312 + 0.565\frac{d_H}{\overline{L}} - 0.1\frac{d_H^2}{\overline{L}^2} \Big) \simeq \frac{G'}{\overline{L}^{\beta+\mu}} \tag{S11}$$

where A is a reaction constant with units of nm$^{\beta}$ and $G' = \frac{k_B T N_A A}{3\eta} E$. For two particular values of β, A has a straightforward geometrical interpretation. Hill's model is recovered when $\beta = 2$, leading to $A = (\delta\omega)^2$. For $\beta = 4$, the significance of A can be explained as follows: A sphere of diameter L is drawn around each filament of length L, two areas of $\sqrt{A}$ are placed on two opposing ends of each sphere, corresponding to the ends of the filament. A collision between two filaments is successful if the two areas touch without the spheres overlapping (see Section S3).

The different moments of the tetramers (subscript f) and of the assembled species (subscript F) are given by:

$$\lambda_{fn} = [f_1] \qquad and \qquad \lambda_{Fn} = \sum_{i=0}^{\infty} (ip)^n [F_i]$$

with n indicating the nth moment. The rate equations for the first five moments can be approximated as[12]:

$$\frac{d[f_1]}{dt} = -pk_n[f_1]^p \tag{S12}$$

$$\frac{d\lambda_{F0}}{dt} = k_n[f_1]^p - \frac{1}{2}\overline{k_{i,j}}\lambda_{F0}^2 \tag{S13}$$

$$\frac{d\lambda_{F1}}{dt} = pk_n[f_1]^p \tag{S14}$$

$$\frac{d\lambda_{F2}}{dt} = p^2 k_n[f_1]^p + \overline{k_{i,j}}\lambda_{F1}^2 \tag{S15}$$

$$\frac{d\lambda_{F3}}{dt} = p^3 k_n [f_1]^p + \overline{k_{i,j}}\lambda_{F2}\lambda_{F1} \tag{S16}$$

$$\frac{d\lambda_{F4}}{dt} = p^4 k_n [f_1]^p + \overline{k_{i,j}}(4\lambda_{F3}\lambda_{F1} + 3\lambda_{F1}^2) \tag{S17}$$

Eqs S11–S17 can be used to calculate the observables M_w, $\langle R_g \rangle_z^2$ and R_H via:

$$M_w = \frac{\lambda_{F2} + \lambda_{f2}}{\lambda_{F1} + \lambda_{f1}} M_{tet}$$

$$M_{n,F} = \frac{\lambda_{F1}}{\lambda_{F0}} M_{tet}$$

$$M_{w,F} = \frac{\lambda_{F2}}{\lambda_{F1}} M_{tet} \tag{S18}$$

$$\langle R_g^2 \rangle_z = \frac{\lambda_{F2} R_{g,F}^2 + \lambda_{f2} R_{g,f}^2}{\lambda_{f2} + \lambda_{F2}} \tag{S19}$$

where $M_{n,F}$ and $M_{w,F}$ are the number and weight averaged masses of the assembled population respectively, $R_{g,f}$ is the radius of gyration of a tetramer ($\simeq 17.5$ nm) and $R_{g,F}$ is the square root of the z–averaged squared radius of gyration of the assembled population. This can be calculated from eq S2 (with $1/z = 1 + M_{w,F}/M_{n,F}$) using a (length dependent) mass per unit length of $M_L = M_w/(60 + 43(n_i - 1))$g/(mol nm) [3], where n_i is the longitudinal degree of polymerisation of the assembled species, which requires knowledge only of the 0th to 2nd moments or alternatively via:

$$\langle R_{g,F}^2 \rangle_z = \frac{\lambda_{F4}}{12 n_L^2 \lambda_{F2}}$$

where $n_L = (p/43)$ nm^{-1} is the number of tetramers per unit length. We have chosen to use eq S19 instead to keep our results consistent with previous work [3]. The diffusion coefficient of the entire ensemble is calculated from:

$$\langle D \rangle_z = \frac{\lambda_{F2} D_{z,F} + \lambda_{f2} D_f}{\lambda_{f2} + \lambda_{F2}} \tag{S20}$$

where D_f is the diffusion coefficient of a tetramer (calculated from eq S4 with $d_{tet} = 5$ nm) and $D_{z,F}$ is the z–averaged diffusion coefficient of the assembled population, calculated from eqs S1, S3 and S4.

Analytical solutions for the 0th to 2nd moment in the case of instantaneous lateral association ($k_n = \infty$) are given in reference 3.

III. S.3 GEOMETRICAL INTERPRETATION OF $\beta = 4$ EXPONENT

The physical meaning of our empirical observation of a rate dependence depending on the factor $A/\overline{L^{-4}}$ can be interpreted as follows. The rate constant for elongation is dependent on a constant area $\sqrt{A}$ of encounter necessary for a successful link formation. This constant area follows from the approach based on a constant value of a (see bottom schematic in Figure S3), which inevitably means that $\omega \sim \overline{L^{-1}}$. Hill's approach in contrast is based on a constant angle ω (i.e. constant θ_1 and θ_2).

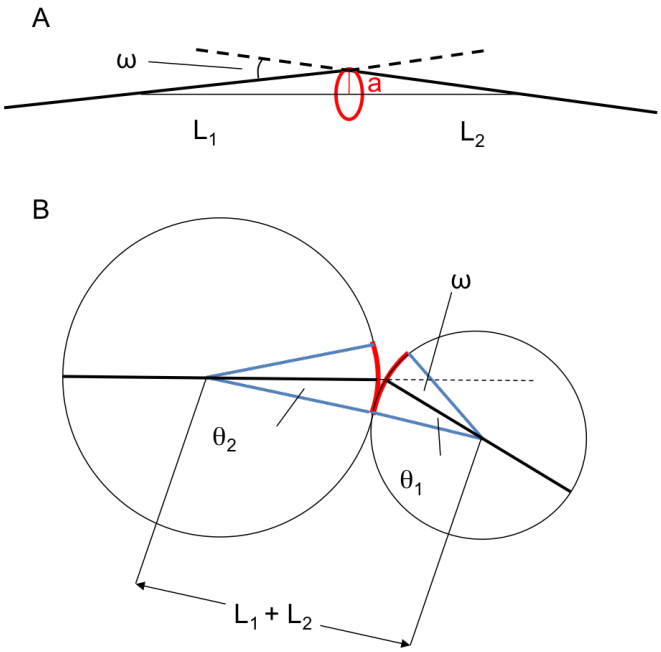

FIG. S3. A: Hill's model, where the maximum angle and maximum distance between the end of the filaments is kept constant, leads to an exponent of $\beta = 2$. B: Schematic of filaments with 'constant reactive area' leading to $\beta \simeq 4$ exponent.

IV. S.4 STEM IMAGES OF VIMENTIN ASSEMBLY

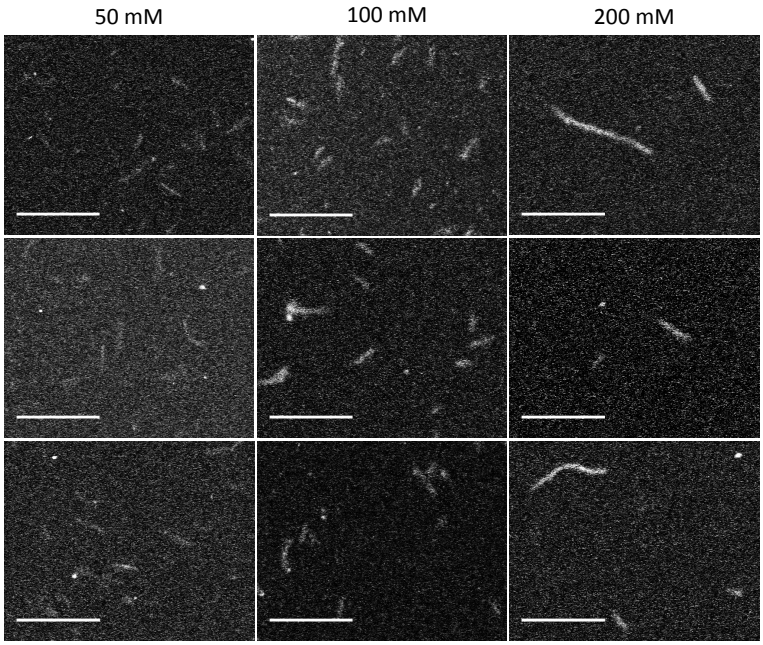

FIG. S4. Representative HAADF STEM images at all values of c_S used for determination of filament M_L. Image contrast has been optimized to improve the visibility of the filaments. Scale bar: 500nm.

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