SUPPORTING INFORMATION

NEWTONIAN TO NON-NEWTONIAN FLUID TRANSITION OF A MODEL TRANSIENT NETWORK

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Abstract

The viscosity of gel-forming fluids is notoriously complex and its study can benefit from new model systems that enable a detailed control of the network features. Here we use a novel and simple microfluidic-based active microrheology approach to study the transition from Newtonian to non-Newtonian behavior in a DNA hydrogel whose structure, connectivity, density of bonds, bond energy and kinetics are strongly temperature dependent and well known. In a temperature range of 15 °C, the system reversibly and continuously transforms from a Newtonian dispersion of low-valence nanocolloids into a strongly shear-thinning fluid, passing through a set of intermediate states where it behaves as a power-law fluid. We demonstrate that the knowledge of network topology and bond free energy enables to quantitatively predict the observed behavior using established rheology models.

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S1. DNA sequences and sample preparation

DNA nanostars (NS) are obtained via the hybridization of the following three oligomers:

S1: 5'-p CTACTAAGCGTGGGCGTAAAAACGGTAACCGTTGCGTCATCC CGATCG-3'S2: 5'-p GGATGACGCAACGGTTACCGAACTCAAGAAGGTATTTATAGC CGATCG-3'S3: 5'-p GCTATAAATACCTTCTTGAGAATTTACGCCCCACGCTTAGTAG CGATCG-3'

Each of these oligomers is designed to bind to the two others in 20-base pairs sections. The complementary sections are highlighted in the sequences above by colored characters. The hybridization of the sequences yields 3-arms structure in which every arm terminates in a 6-base-long auto-complementary sequence (CGATCG, bold in the sequence above).

DNA oligomers were synthesized by NOXXON Pharma. DNA was hydrated using NaCl electrolyte solutions to yield a total ionic strength of about 20 mM. The molarity of each sequence was carefully measured and equalized before mixing. NS were annealed by slowly cooling the samples from 90 °C to room temperature (rate 0.3 °C/min). The fraction of resulting NS was determined via gel electrophoresis as detailed in previous works ¹. We obtain solutions where more than 86% of DNA are aggregated in NS.

S2. Dynamic Light Scattering measurements

An aqueous dispersion of DNA NS at the ionic strength and concentration used in this work was characterized by Dynamic Light Scattering (DLS). The sample was sealed in a glass tube of 2.4 mm internal diameter (outer diameter 3 mm) and placed in a metal chamber in which the temperature was controlled by water circulation and heating resistor. The scattered light intensity I(t) ($\lambda = 532 \text{ nm}$) was collected at 90° by an optical fiber. The intensity correlation function $g_2(t)$ was calculated by a digital correlator (flex-03d Correlator.com). The intermediate scattering function $g_1(t)$ is than determined through the equality:

$$g_2(t) = \langle I(t')I(t'+t) \rangle_{t'} / (\langle I \rangle_{t'}^2) = 1 + b[g_1(t)]^2$$
 eq. S2.1

where *b* is the Siegert coefficient, which depends on the experimental setup. g_1^2 measured at different T, matching the same T window used for viscosity measurement, is shown in Fig. S1



Figure S1 **a**. Intermediate scattering functions g_1^2 measured at different T. Two relaxation processes are clearly visible. **b,c**. Characteristic time τ_{DLS} of the slower process and height of the intermediate plateau h as a function of T.

The correlation functions were fitted using a double step relaxation model obtained as a sum of an exponential and of a stretched exponential decay as detailed in Refs. 1,2:

$$g_{2}(t) = 1 + b \left[(1-h)e^{-t/\tau_{fast}} + h e^{-\binom{t}{\tau_{DLS}}^{0.8}} \right]^{2}$$
eq. S2.2

where h is the plateau height of the first relaxation and τ_{fast} , τ_{DLS} are the two characteristic times of the fast and slow relaxation modes respectively. In transient networks, the thermal concentration fluctuations are not fully dissipated by diffusion (first decay in figure S1a), but need to wait for the relaxation of the network which depends on the opening of the bonds and thus on their lifeime¹. Indeed, the characteristic time of the slower decay τ_{DLS} is of the same order of magnitude for the expected lifetime of the hybridized overhangs³ and scales with T as an activated process with the enthalpy expected for such bonds¹ (Fig. S1b). Moreover, the height of the intermediate plateau *h* grows markedly with 1/T (Fig. S1c) in a way similar to the expected melting curve for the overhangs (see section S7). These combined evidences indicate that the slow mode of the concentration fluctuations is intimately related to the formation of the network and reflects the dynamics of the network rearrangement^{1,2}.

The combination of the data in Fig. S1 and of the matching between slow relaxation time and bond lifetime indicates that at all the temperatures considered in the rheological measurements the network retains its transient character, thus leading to ergodicity. This notion was explored in depth in Ref. 2, where we explicitly tested the thermodynamical equilibrium – and thermal history independence – of an analogous DNA nanostar hydrogel.

Fig. 3e of the main text shows that τ_{DLS} and the parameter τ extracted from the microrheology measurement - representing the network restructuration time relative to the length-scale of the bead ($\sim 10 \mu m$) - not only display the same scaling with T, but interestingly have also comparable values. It might appear surprising that two phenomena occurring on different length scales have comparable relaxation times. However, we have recently demonstrated that length-scale independence of the network relaxation time is a remarkable property of transient networks ⁴. This peculiar dynamic behavior enables the matching of viscous and structural relaxation times in a large range of length-scales.

S3. Viscosity calibration and data fitting procedure for Newtonian fluids

The raw data emerging from the particle tracking are given by the space-time curves x(t) of the beads recorded during the shooting procedure. Being the system overdamped, we can neglect inertial effects. In the case of Newtonian fluids, the optical force $F(x,P_L)$ is thus balanced by the viscous friction:

$$F(x,P_L) = 6\pi\eta R \frac{dx}{dt}$$
 eq. S3.1

When non-Newtonian fluids are considered Eq. S3.1 can still be used, provided that the viscosity is understood as an apparent viscosity η_A .

Since the optical force depends on the bead position x but not on t, it is possible to separate the two variables, obtaining an equation that can then be integrated between the initial coordinates (x_i , t_i) and those of a generic instant of the shooting experiment (x^* , t^*):

$$\int_{t_{i}}^{t^{*} < t_{f}} dt = \int_{x_{i}}^{x^{*} < x_{f}} \frac{6\pi R\eta}{F(x, P_{L})} dx ,$$
 eq. S3.2

where x_f and t_f represent the final bead position and time of the acquired data. The optical force can be written as $F(x,P_L) = AP_Lg(x)$, where A is the calibration constant, P_L is the laser power and g(x) is the force profile obtained both from simulations of paraxial optics and experiments ⁵.

The determination of the force profile g(x) via paraxial optics in the case of large beads (i.e. with a diameter much larger than the wavelength) can be generally decomposed in three steps ⁶. First, the optical beam is decomposed into a set of optical rays. Each ray is associated to a fraction of the total optical power, defined thanks to the analysis of the far-field intensity distribution, and has a specific direction, derived by analyzing the gradient of the optical phase. Second, the interaction of each ray with a microbead, in a given position, is considered and the optical forces (scattering force and gradient force) exerted on the microbead are calculated by summing up the contributions from each ray. Third, the force-calculation procedure is repeated for every microbead position considered interesting for the experiment ⁶. It is worth underlining that in order to properly use this approach a careful characterization of the in-chip optical parameters must be carried out, so as to include the effect of possible losses or system fabrication imperfections in the analysis. A careful description of the procedure is reported in ^{5–8}.

To determine the calibration constant A we define the function $R(t^*, x^*, x_i, t_i, \eta, A)$ based on eq. S3.2:

$$R(t^*, x^*, x_i, t_i, \eta, A) = \int_{t_i}^{t^* < t_f} dt - \frac{6\pi R\eta}{AP_L} \int_{x_i}^{x^* < x_f} \frac{dx}{g(x)},$$
eq. S3.3

We performed experiments on fluid with known viscosity (e.g. pure water at various controlled $\int_{0}^{t} R^{2} dt^{*}$

temperatures) and determined A by minimizing the value of t_i

Conversely, once A is known, the viscosity η of an unknown fluid can be obtained through the same minimization procedure.

The calibration procedure was repeated after every measurement campaign to check the stability of the system in terms of force and calculated viscosity. We find the setup to be very stable (difference between calibration constant A before and after the experiments is less than 3%) also thanks to the monolithic alignment between the optical components and to the laser stability.

S4. Data fitting procedure for shear thinning fluids

The data analysis described in the previous section cannot be simply extended to non-Newtonian fluids, where η varies with the speed of the bead as explained in the main text.

Nevertheless, we can replace the viscosity η in S.Eq. 3.1 with an apparent viscosity for which we have adopted the Cross model functional dependence:

$$\eta_A = \frac{\eta_0 - \eta_\infty}{1 + \frac{\nu\tau}{R}} + \eta_\infty$$

eq. S4.1

We obtain a quadratic equation $av^2 + bv + c = 0$ whose coefficients are given by:

$$a = \frac{\eta_{\infty}\tau}{R} \quad b = \eta_0 - \frac{g(x)AP_L\tau}{6\pi R^2} \quad c = -\frac{g(x)AP_L}{6\pi R}$$
eq. S4.2

Again, we can separate the variables and define the functional R:

$$R(t^*, x^*, \eta_0, \tau) = \int_{t_i}^{t^*} \int_{t_i}^{eq. S4.3$$

to be minimized with respect of the parameters η_0 and τ over the trajectory x(t). The results and the accuracy of the fit are visible in Fig. 3a of the main text, while the extracted parameters are shown in Fig. 3e.

S5. Simplified derivation of the shear thinning viscosity model

The quantitative interpretation of the data in this paper makes use of the "Soft Glass Material" (SGM) model, introduced and developed in Ref.⁹. The use of such model provides an accurate evaluation of the temperature where the system becomes non-Newtonian and of the T range involved in such crossover, as shown in Fig. 3f and described in the main text.

In the spirit of facilitating the appreciation of the physics included in these models, we propose in this section a simplified description of the viscosity of a transient network such as the DNA NS hydrogel. This rough model lacks the accurate evaluation of the statistical distribution of the quantities of the SGM model of Ref. ⁹ and of the extended study of the predicted viscoelastic behavior, but it is more transparent in terms of physical meaning.

As in the SGM model, we picture the connected NS as a set of trapped elements that can escape – i.e. open the NS-NS bonds – either because of a spontaneous activated process or as a result of an external shear. The shear exerted by the bead motion produces a deformation on the NS network which responds elastically. The transient and reversible nature of the network plays here a crucial role since it leads to the dissipation of the stored elastic energy, which in fact is revealed by our experiments as an apparent viscous force. Such dissipation can take place either because of the finite lifetime of the bonds, or via their yielding due to the shear.

Thus, we model the DNA NS gel as a network of connected springs of elastic constant k and density $3/2^{n_{NS}}$ (n_{NS} is the number density of NS and 3/2 takes in account for their valence f=3). We assume that each spring has a mean lifetime τ before disconnecting and a maximum elongation l before breaking.

Let's then consider a bead that moves forward of Δx with a speed v while immersed in such a network, thus straining the network locally. We can assume that the average frictional force that the bead experience in the time of the experiment (set by $\Delta x/v$) is the sum of the elastic responses of the springs strained by the motion. More precisely the springs belong to two categories:

- springs that, during the bead displacement, are elongated more than l, and thus yield ($^{N_{broken}}$).
- springs with elongation l < l (^Nunbroken).

We assume here that the lifetimes of the springs are exponentially distributed, with a characteristic time given by the average lifetime τ . In particular, we assume a distribution of lifetimes m(t) :

$$m(t) \div e^{-t/\tau}$$
 eq. S5.1

We are here focussing only on the bonds that are strained by the motion of the bead. Although the shear rate induced by the bead motion depends on the distance from the bead, it has been often approximated by assuming that, in the volume involved in the deformation, $\dot{\gamma} = v/2R$. This assumption leads, in the case of power-law fluids, to errors within 50% ¹⁰. Each spring is thus deformed with a rate proportional to vL/2R, where L is the mean mesh size of the network. We can therefore replace t with the ratio between the elongation l of the spring and cvL/R, where C is a (unknown) dimensionless coefficient, to obtain a distribution of spring strain:

$$m(l) \div e^{-\frac{lR}{cvL\tau}}$$
eq. S5.2

Within this distribution, some of the springs are not strained enough during their lifetime to be actively broken by the bead motion (l < l), and thus spontaneously open. Their fraction over the total is:

$$m_{unbroken} = \frac{\int_{0}^{l} e^{-\frac{lR}{cvL\tau}} dl}{\int_{0}^{\infty} e^{-\frac{lR}{cvL\tau}} dl} = 1 - e^{-\frac{lR}{cvL\tau}}$$
eq. S5.3a

Viceversa, the fraction of springs that during their lifetime are strained enough to break are:

$$m_{broken} = \frac{\int_{l}^{\infty} e^{-\frac{lR}{cvL\tau}} dl}{\int_{0}^{\infty} e^{-\frac{lR}{cvL\tau}} dl} = e^{-\frac{lR}{cvL\tau}}$$
eq. S5.3b

We can now write the average frictional force experienced by the bead during a displacement Δx as the sum of the contributions of these two class of springs. Using Hooke law:

$$F \sim N_{unbroken} k \delta x + \frac{1}{\Delta x} N_{broken} \frac{1}{2} k l^2$$
eq. S5.4

Where $N_{unbroken}(N_{broken})$ is the number of unbroken (broken) springs and δx is the mean elongation of the strained springs. The populations $N_{unbroken}, N_{broken}$ can be computed as:

$$N_{open} = \frac{3}{2} n_{NS} V_{unbroken} m_{unbroken} \qquad N_{broken} = \frac{3}{2} * n_{NS} V_{broken} m_{broken}$$

$$3$$

where $\overline{2}^{n_{NS}}$ is the number density of springs and V is the volume of hydrogel in which the bonds are strained or broken by the bead motion. V_{unbroken} is the volume encompassing all springs that are affected by the bead motion, which we estimate being of the order of R³. V_{broken} is instead the volume in which bonds are actively broken by the bead motion, which we approximate with 2 R L Δx , as discussed in Section S8. Substituting (S5.3a) and (S5.3b) in (S5.4) we obtain:

$$F = 3/2n_{NS}k \left(R^3 m_{unbroken} \bar{\delta}x + \frac{1}{2} 2RLm_{broken} l^2 \right)$$
eq. S5.5

 δx can be explicitly computed from the distribution m(t):

$$\delta x = \frac{\int_{0}^{l} l e^{-\frac{lR}{cvL\tau}} dl}{\int_{0}^{l} e^{-\frac{lR}{cvL\tau}} dl} = \frac{\frac{cvL\tau}{R} \left(1 - e^{-\frac{lR}{cvL\tau}}\right) - le^{-\frac{lR}{cvL\tau}}}{1 - e^{-\frac{lR}{cvL\tau}}}$$

Inserting the result in (S5.5):

$$F = 3/2n_{NS}kR\left(R^{2}\frac{cvL\tau}{R}\left(1 - e^{-\frac{lR}{cvL\tau}}\right) - l e^{-\frac{lR}{cvL\tau}}(R^{2} - Ll)\right)$$
eq. S5.6

Since $l \ll R$ (l = 14.5 nm, see S6), we can simplify (S5.6) as:

$$F \sim 3/2n_{NS}kcvL\tau R^2 \left(1 - e^{-\frac{lR}{cvL\tau}}\right)$$
eq. S5.7

The viscosity can be then derived via the Stokes equation dividing (S5.7) for $6\pi Rv$ obtaining:

$$\eta = \eta_0 \left(1 - e^{-\frac{lR}{cvLr}} \right)$$
eq. S5.8
where
$$\eta_0 = \frac{3/2 n_{NS} k \tau cLR}{6\pi}.$$

Despite the roughness of the approach, this simple model captures a few features worth mentioning:

lR

eq. S5.8

- it captures the transition from Newtonian to non-Newtonian viscosity, the key quantity being cvLτ, a dimensionless ratio built on the comparison between the maximum strain sustained by the springs and the strain that is produced by the bead during their lifetime;
- it relates the growth of the Newtonian component to the dissipation following the spontaneous opening of the springs. The longer the bond lifetime, the larger the energy that can be incorporated into the spring network, the larger the energy dissipated, as expressed by η₀ ÷ τ;
- it relates the non-Newtonian component of the viscosity to the active breaking of the bonds, which intrinsically yields a force independent on bead velocity, as discussed in the main text and in Section S8 below. Interestingly the formula shows the experimental observed dependence on the lifetime *l*_R

 τ both in the zero-shear viscosity η_0 and in the dimensionless term $cvL\tau$.

If we assume $CL \approx \ell$ we can write Eq. (S5.8) as:

$$\eta = \eta_0 \left(1 - e^{-\frac{R}{v\tau}} \right)$$

and compare it to the Cross model adopted in our interpretation of the data as described in the main text

$$\eta = \frac{\eta_0}{1 + \frac{v\tau}{R}}$$
eq. S5.9

as shown in Fig. S2, where we have used the two dimensionless quantities η/η_0 and $B = \frac{\nu \tau}{R}$. The behavior is quite similar, although the Newtonian to non-Newtonian transition in our simplified model appears to be sharper, a possible consequence of the many rough approximations here introduced, as further discussed in Section S8.



Fig. S2. Shear thinning viscosity dependence on the dimensionless parameter B for the Cross model (S5.9) (red line) and the Exponential model (S5.8) (black line)

S6. Structure of a valence-3 network

The aim of this paragraph is to provide an estimate of the density and structure of a dense phase of NS having valence equal to 3. The phase behaviour of low-valence colloids, or "patchy particles", have been extensively described ¹¹. As the valence decreases, the density of the dense phases (liquids, crystals) also decreases, leading to structures that have been described as "empty liquids".

In the case of valence f = 4, density and distances can be deduced from the well-known structure of the diamond lattice. In a diamond lattice, if particles or atoms were to be replaced with spheres whose diameter r matches their nearest-neighbor distance in the lattice, the volume fraction filled by such spheres in a diamond lattice would be $\phi = 0.34$. Although no atomic lattice based on f = 3 is known, studies of the liquid phase of patchy particles indicate that in this case the volume fraction would be approximately $\phi \approx 0.18$ (¹²). On this basis we can calculate, for our system, the distance between bonded NS at low T.

Given the molecular weight of each NS (MW_{NS} = 45000 g/mol) and the concentration (c = 10 mg/ml), we can compute the number density n_{NS} , and from it the distance l between bonded NS:

$$\frac{4}{3}\pi r^{3} = \frac{V}{N} \cdot \phi = \frac{0.18}{n_{NS}} = \frac{0.18}{\frac{c}{MW} \cdot 1000 \cdot N_{A}}$$
eq. S6.1

where N is the total number of NS, V is the total volume, N_A is the Avogadro number. From Eq. S6.1 we obtain r = 6.8 nm, and thus l = 2r = 13.6 nm.

An alternative approach is to develop a lattice structure for f = 3 based on the diamond lattice. This can be done by splitting each f = 4 node into two f = 3 nodes as shown in Fig. S3. The unit cell of the diamond lattice contains 8 atoms/particles (4 are internal, 8 in corners, 6 on faces), has volume a^3 , while the nearest-neighbor inter particle distance is $(\sqrt{3}/4)a$. The new f=3 unit cell resulting from the atom replacement described in Fig.

S3, has instead volume $V_{unit} = a^3 \left(1 + 4 \cdot \frac{\sqrt{3}}{4}\right)$, inter particle distance $l = \sqrt{3}/4a$ and contains 16 atoms/particles (9 are internals, 8 in corners, 10 on faces, 4 on edges).

Here again, from the experimental number density, we can recover the distance between bonded NS by noticing that the ratio between a volume V and the volume of a unit cell V_{unit} must equal the number of NS contained in the volume divided by the number of NS contained in a unit cell:

$$\frac{V}{V_{unit}} = \frac{N}{16}$$
 eq. S6.2

So that, introducing number density $n_{NS} = N/V$ we obtain:

$$n_{NS} = \frac{16}{V_{unit}} = \frac{16}{a^3(1+\sqrt{3})}$$
eq.
S6.3

Remembering that $a = 4/\sqrt{3} l$, solving by l we found l = 15.3 nm a value slightly bigger that the previous evaluation. One possible reason is that in the "modified diamond lattice" approach we force the NS to have a rigid planar structure with angles between arms equal to 120°, while in the actual system this angle can fluctuate.

Since both approaches include some approximation, we decided to adopt the intermediate value l = 14.5 nm. This length should be compared with 16 nm, the inter-NS distance that we would expect when their arms are perfectly straight and aligned.



Fig. S3 The diamond unit cell (left-hand side panel) can be transformed into a valence 3 unit cell (righthand side panel) by splitting the valence 4 nodes into two valence 3 nodes.

S7. Computation of n_3 as a function of temperature

The aim of this paragraph is to evaluate the effective connectivity of the network when only a fraction p(T) of NS terminals are actually connected. p(T) can be computed on the basis of the NS terminal concentration and of the free energy involved in the NS-NS bonds, which can in turn be evaluated using the Nearest-Neighbours model (NN)¹³ as implemented by the computing tool NUPACK ¹³. Fig. S3 shows p(T) computed for the NS overhang sequence CGATCG at the concentration 666 μ M, i.e. three times the NS concentration, since each NS has three overhangs.



Fig. S3 Bond fraction p as a function of the temperature T as calculated by the NN model.

The quantity $n_3(T)$ is the number density of NS involved as nodes in a percolating (or large) network. With respect to n_{NS} , in n_3 we need to exclude both NS with less than three bonds and NS that, even fully connected, terminate in a dangling end. $n_3(T)$ is a crucial quantity in the description of the mechanical properties of the

network since the mechanical stress response we observe is given only by the so called "active chains"¹⁴, i.e. by the NS which are put under tension as the bead moves. As also reported in the main text, given the density of "active nodes" $n_3(T)$, the number of active chains $\nu(T)$ is:

$$n_3(T)\frac{3}{2} = v(T)$$
 eq. S7.1

In general, the dependence of the number of nodes n_3 on T in a system with valence 3 (f=3) is expected to be non-trivial as cutting even a few bonds can have a large impact on the network structure, which rapidly becomes loosely connected (large loops), and eventually disconnected.

The fraction of active chains can be estimated using a tree-like approach for f=3 resulting in the expression^{15,16,17}:

$$n_3(T) = n_{NS} \left(\frac{2p-1}{p}\right)^3$$
 eq. S7.2

Where we notice that the formula implies p>0.5, a consequence of the percolation limit of the network, or in other words, the gelling point of the system¹⁵.

We further numerically checked this result by using the modified diamond structure defined in the previous section (S6). We used this crystal structure, we replicated the unit cell in the three dimensions to obtain a lattice of 10^5 nodes, and evaluates the effect on topology and connectivity of p(T) by randomly deleting bonds within the lattice. We cut each bond with equal probability 1-p(T) and explore the resulting connectivity c of each bond. Four possible cases can be found:

- c=0. NS is fully disconnected from the network
- c=1. NS binds one other NS. This is the dangling bond case
- c=2. NS binds 2 other NS. In this case, the NS acts as a "wire" between nodes, but it is not a node itself.
- **c=3.** NS binds 3 NS. This NS potentially is a node of the network.

Clearly, in the first three cases NS do not contribute to the network connectivity as nodes. Even in the fourth case, it can happen that a fully bonded NS is connected to a dangling end, thus not being a real active node. To properly consider this case, we recursively check the neighbours NS of every c=3 element until we found other c=3 NS, thus confirming its role as a node in the network. If instead we find a c=1 NS, we remove the original c=3 NS from the count of the network-connecting nodes.

Fig. S5a compares the T dependence of n_3/n_{NS} calculated from S7.2 (red line) and estimated numerically as described above (blue line). For comparison, we also show the fraction of bonds p(T) (dashed line, same as in Fig. S3) and the number of c=3 nodes without the dangling bond correction (purple line). By adopting the phantom network model introduced in the main text, we can estimate $G' = k_B T3/2n_3(1-2/f)$ from n_3 as computed using the tree-like theory (Fig. S5b, red line), and numerically with dangling bond correction (blue line) and without it (purple line).



Fig. S5. a: fraction n_3/n_{NS} of active nodes as a function of temperature T as calculated according to the tree-like theory (red line), and computed numerically with dangling bond correction (blue line) and without it (purple line). For comparison p(T) as calculated by NUPACK (dashed line) is also shown. b: bulk modulus G' calculated according to the phantom network model for computation of n_3/n_{NS} .

S8. Estimate of the frictional force in the network fracturing regime

By using the number of active nodes $n_3(T)$ calculated in the previous section and the free energy involved in the NS-NS bonds $\Delta G(T)$, it is possible to evaluate the frictional force experienced by a bead of radius R traveling in an NS hydrogel in the limit of long-living bonds. In this condition, the bead can move only by breaking bonds and the frictional force is independent on bead velocity.

The reference model to perform this evaluation of such a force is the classic Lake-Thomas model, as described in the main text. Here we offer a quantitative estimate of the same quantity via a direct intuitive approach.

The energy dissipated to move the bead forward of a quantity Δx is given by the energy E stored in the bonds that the bead needs to break to advance, which is the same regardless of the bead velocity. We can thus write:

$$F_{FR} = \frac{E}{\Delta x}$$
 eq. S8.1

Thus, to evaluate the force, we need to estimate the number of bonds that are broken as the bead moves forward of a length Δx . Since the network is connected and flexible, the fractured bonds are presumably those needed to form an opening large enough to let the bead move forward. We thus assume the width of such fracture to equal the length 2R, the diameter of the bead. The bonds involved in such fracturing are those within a parallelepiped having volume V

$$V \sim 2R l \Delta x$$
 eq. S8.2

Where l is the characteristic length of the bonds (l =14.5 nm). In other words, the bonds involved are those that would be cut by a blade of width 2R and thinner than the bond length, advancing in the hydrogel of a length Δx . Thus

$$E = N_{bonds} \Delta G(T) = V \frac{3}{2} n_3(T) \Delta G(T)$$
 eq. S8.3

Where V is the volume of the layer, $\Delta G(T)$ is the energy of the bonds at a temperature T, $n_3(T)$ is the number density of active nodes as calculated in the previous section. Substituting S8.2 and S8.3 in S8.1 we obtain:

$$F_{FR} = 2R l \frac{3}{2} n_3(T) \Delta G(T)$$
 eq. S8.4

Since the quantities on the right-hand side of Eq. S8.4 are known, we can calculate the tearing force of the network. For T=22°C, we obtain $F \approx 75 \ pN$, in good agreement with both the experimental findings and the prediction of the Lake-Thomas model.

S9. Estimate of the stored elastic energy

As the bead moves forward, the NS network is deformed, and elastic energy is stored. Evaluating this quantity is useful because when the elastic energy stored in the hydrogel becomes comparable to the energy necessary to break the bonds, the system crosses over from the "stretched elastic network" regime to the "network fracturing" regime, and the viscosity becomes non-Newtonian.

As described in the main text, the stress on the hydrogel can be written as:

$$\sigma = (\eta_w + G\tau_B)\dot{\gamma}$$
eq. S9.1

where τ is the spontaneous lifetime of the NS-NS bonds, which depends on T as an Arrhenius function. If we approximate the shear rate to the one produced on the equatorial region of the bead, which we estimate in v/2R¹⁰, and we consider it to be applied on the surface $4R^2$, we can estimate the total elastic energy E_{tot} to move the bead forward of a length Δx at a speed v as:

$$E_{tot} = F_{el}\Delta x = 4R^2 \left(G' \tau \frac{v}{2R} \right) \Delta x$$
 eq. S9.2

The maximum deformation Δx of the system is also limited by the bond lifetime τ_B , since for longer times the network bonds open and relax. This sets the maximum deformation as $\Delta x_{max} = v\tau_B$. We can thus write the maximum energy as:

$$E_{tot,max} = 2RG'v^2\tau^2$$
eq. S9.3

Taking into consideration the number of bonds (N_{bonds}) in the volume given by Eq. S8.2, it is possible to evaluate the maximum elastic energy stored per bond as:

$$E_{el} = \frac{E_{tot,max}}{N_{bonds}} = \frac{2RG'v^2\tau^2}{2Rl v\tau \rho \frac{3}{2}n_3(T)} = \frac{2G'v\tau}{3\rho n_3(T)l}$$
eq.
59.4

Since the dependence on T of the various parameters ($^{\rho,G,\tau,n_3}$) is known, as discussed in the main text and in the sections S6 and S7, such energy can be explicitly calculated as a function of T. The result of such evaluation is plotted in Fig. 3f of the main text for three different choices of v (blue lines, left-hand axis; dotted line, v=3 µm/s; continuous line, v=10 µm/s; dashed line, v=60 µm/s).

The comparison between maximum stored elastic energy E_{el} per bond and disruption free energy for bond ΔG is shown in Fig. 3f of the main text. When $E_{el} > \Delta G$, the elastic energy becomes larger than the energy required to open the bonds, so the bead motion actively breaks the bonds, the system enters in the "network fracturing" regime and the viscosity becomes non-Newtonian.

Specifically, the crossover is evaluated in Fig. 3f on the basis of the results of SGR model⁹, where it is found that the crossover between Newtonian and non-Newtonian takes place when the elastic energy is within a given range of the bond rupture energy: $\Delta G - 2k_BT < E_{el} < \Delta G - k_BT$. However, the crossover can be satisfactory located even by considering $E_{el} = \Delta G$, i.e. the crossing of the blue line and red line in Fig 3f.

We stress the fact that these evaluation, while possibly suffering of simplifications, are performed with no adjustable parameters, and rely entirely on the geometry of the NS, their concentration, and the general knowledge about DNA hybridization (energy, temperature dependence, bond lifetime) available in literature.

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