Supplementary Material

S1. Stiffness of the sample derived from force curves

Figure S1

Force curve on a cell while at maximum force a z step is performed (see yellow circle). The figure shows the same data as in figure 3, here in the conventional scheme of deflection versus z height as is usually done in force curves. The slopes and the corresponding stiffness of the sample, which can be calculated from the approach and retract part of the data are also indicated in the annotation.

Figure S2

Force curve on a cell while at maximum force a magnetic step is performed. The figure shows the same data as in figure 4, here in the conventional scheme of deflection versus z height as is usually done in force curves. Since in a magnetic step, only the deflection changes due to the magnetic force (z is kept constant) the effect of the step is harder to see than in fig S1. The slopes and the corresponding stiffness of the
sample, which can be calculated from the approach and retract part of the data are also indicated in the annotation.

In a force curve we can derive the stiffness of the sample from the slope of the plot deflection versus z-height. The slope $s$ is defined as:

$$ s = \frac{\Delta d}{\Delta z} $$

(A1.1)

The stiffness of the sample will be:

$$ k_s = k_c \cdot \frac{s}{1 - s} $$

(A1.2)

Since the slope may be different on approach or retract, we distinguish both. In cells, due to a high viscous contribution $k_{\text{approach}}$ and $k_{\text{retract}}$ will always be substantially different.
S2. Analysis of Creep response Data from z steps

We apply a step force when in contact with the sample at a z-position $z_1$, the deflection will be $d_1$, and the indentation is $\delta_1$. Before the step the force equilibrium will be:

\[ k_c \cdot d_1 = k_s \cdot \delta_1 = k_s \cdot (z_1 - d_1) \]

(A2.1)

We have used here the general relation between z-height, deflection and indentation, which will be always obeyed:

\[ z = d + \delta \]

(A2.2)

Since the forces are in equilibrium at this point, we can simplify our calculations by redefining the origin such, that

\[ d_1 = z_1 = \delta_1 = 0 \]

(A2.3)

Conventional step by increasing z-height: z-step

When applying a z-step we will change the z-height to:

\[ z_2 = z_1 + \Delta z = \Delta z \]

(A2.3)

After relaxation, the deflection will have a new value $d_2$:

\[ d_2 = d_1 + \Delta d = \Delta d \]

(A2.3)

We model our sample by a combination of two springs and a dashpot, termed the general linear solid model, which is the minimum model to reproduce the measured creep behaviour above. After relaxation, the spring $k_2$ will be relaxed due to the creep of the viscous damping element $f$, so the force balance looks like:
(A2.4) \[ k_c \cdot d_2 = k_1 \cdot \delta_2 = k_1 \cdot (z_2 - d_2) \]
\[ k_c \cdot \Delta d = k_1 (\Delta z - \Delta d) \]

Figure S3
Creep response after applying a loading step in z height at \( t = 1.5s \) and an unloading step at \( t = 2.0 \) s. The indentation is calculated as the difference between z height and deflection. The deflection data are fitted with an exponential function, which will give \( k_1, k_2 \) and \( f \) as results (same data as in figure 2).

So, we can derive the spring constant \( k_1 \) from the measurable quantities \( \Delta d \) and \( \Delta z \):

(A2.5) \[ k_1 = k_c \frac{\Delta d}{\Delta z - \Delta d} \]

Right after (the infinitely step) z-step, the viscous element can be considered as a stiff rod. Thus the force balance looks like:

(A2.6) \[ k_c \cdot d_3 = (k_1 + k_2) \cdot \delta_3 \]
\[ k_c (\Delta d + a) = (k_1 + k_2) \cdot (z_2 - d_3) \]
\[ k_c (\Delta d + a) = (k_1 + k_2) \cdot (\Delta z - \Delta d - a) \]

So, we can derive the spring constant \( k_2 \) from the measurable quantities \( \Delta d, \Delta z \) and of the initial value \( a \) of the deflection:

(A2.7) \[ k_2 = k_c \frac{\Delta d + a}{\Delta z - \Delta d - a} - k_1 \]
For describing the creep response, we use the following ansatz for the relaxation process:

\[(A2.8)\]
\[
d = d_2 - d_1 + a \cdot e^{-t/\tau} = \Delta d + a \cdot e^{-t/\tau}
\]

The amplitude \(a\), and the relaxation time \(\tau\) can be obtained by an exponential fit of the data.

The force equilibrium for any point in time is given by:

\[(A2.9)\]
\[
F_c = F_1 + F_2
\]
\[
F_2 = k_c \cdot d - k_1 \cdot \delta
\]

The force in the Maxwell element has to follow the following dynamic equation:

\[(A2.10)\]
\[
\dot{\delta} = \frac{F_2 \cdot F_2}{k_2 \cdot f}
\]

Using eq. (A1.2) we find:

\[(A2.11)\]
\[
\delta = z - d
\]
\[
\delta = \Delta z - \Delta d - a e^{-t/\tau}
\]
\[(A2.12)\]
\[
\dot{\delta} = a \cdot \frac{1}{\tau} \cdot e^{-t/\tau}
\]

The force \(F_2\) in the Maxwell element can be rewritten using our ansatz eq. (A2.8):

\[(A2.13)\]
\[
F_2 = k_c \left( \Delta d + a e^{-t/\tau} \right) - k_1 \left( \Delta z - \Delta d - a e^{-t/\tau} \right)
\]
\[
F_2 = k_c \cdot \Delta d - k_1 \left( \Delta z - \Delta d \right) + k_c a e^{-t/\tau} + k_1 a e^{-t/\tau}
\]

Using the above equilibrium of forces (A2.5) this will reduce to:

\[(A2.14)\]
\[
F_2 = k_c a e^{-t/\tau} + k_1 a e^{-t/\tau}
\]
\( F_2 = -k_c \frac{a}{\tau} e^{-t/\tau} - k_1 \frac{a}{\tau} e^{-t/\tau} \)

Entering the expressions from eq. A2.15, eq. A2.14, and eq. A2.12 in eq. A2.10 we get:

\[
\frac{a}{\tau} e^{-t/\tau} = -\frac{k_c \frac{a}{\tau} e^{-t/\tau} + k_1 \frac{a}{\tau} e^{-t/\tau}}{k_2 \frac{a}{\tau} e^{-t/\tau} - k_1 a e^{-t/\tau}} - \frac{-k_c \frac{a}{\tau} e^{-t/\tau} - k_1 a e^{-t/\tau}}{f}
\]

\[
1 = -\frac{k_c + k_1}{k_2 + k_c + k_1} \cdot \frac{k_c + k_1}{f} \cdot \tau
\]

\( f = k_2 \tau \cdot \frac{k_c + k_1}{k_2 + k_c + k_1} \)

The relaxation time constant \( \tau \) is the apparent in the experimental setup, which not only depends on the materials properties \( k_1, k_2, \) and \( f \), but also on the cantilever force constant \( k_c \), i.e. experimental parameters. The intrinsic relaxation time constant is usually defined by the ratio of friction coefficient and \( k_2 \):

\( \tau^* = \frac{f}{k_2} = \tau \cdot \frac{k_c + k_1}{k_2 + k_c + k_1} \)
S3. Analysis of Creep response Data from magnetic force steps

For the magnetic force step eq. (A2.1) for the situation before the step still holds:

After the step, since $z$ is not changed we get:

\[(A3.1)\]
\[
\begin{align*}
  z_1 &= z_2 = 0 \\
  \Delta z &= 0
\end{align*}
\]

Figure S4

Creep response after applying a force step at $t = 1.5$s and an unloading step at $t = 2.0$ s. The indentation is identical with deflection (opposite sign) since the $z$ height is not changed in this experiment. The deflection data are fitted with an exponential function, which will give $k_1$, $k_2$ and $f$ as results (same data as in figure 2).

When applying the external magnetic force $F_m$, we will first see a sudden jump to a deflection $d_1$ and then a relaxation to a deflection $d_2$. Since $z$ is zero during the entire process, indentations and the deflection are directly linked:

\[(A3.2)\]
\[
\begin{align*}
  \delta_1 &= -d_1 = 0 \\
  \delta_2 &= -d_2 \quad d_2 = \Delta d + a \\
  \delta_3 &= -d_3 \quad d_3 = \Delta d
\end{align*}
\]

For the loading step the change of deflection $\Delta d$ is negative, since the indentation change is positive. The creep amplitude $a$ is positive, since $d_2$ is larger than $d_3$. 
After relaxation to deflection \(d_2\) the force balance looks like:

\[
(A3.3) \quad k_c \cdot d_3 = k_1 \cdot \delta_3 + F_m \\
A3.4) \quad k_c \cdot \Delta d = -k_1 \cdot \Delta d + F_m \\
\]

The initial response after the force step obeys the following force balance:

\[
(A3.5) \quad k_c \cdot d_3 = (k_1 + k_2) \cdot d_3 + F_m \\
A3.6) \quad k_c \cdot (\Delta d + a) = (k_1 + k_2)(\Delta d + a) + F_m \\
A3.7) \quad (k_1 + k_2) = \frac{F_m}{\Delta d + a} - k_c \\
\]

For the relaxation we use the same ansatz as above

\[
A3.8) \quad d = d_2 + a e^{-t/\tau} \\
A3.9) \quad = \Delta d + a e^{-t/\tau} \\
\]

The force balance needs to be expanded because of the magnetic force \(F_m\):

\[
A3.9) \quad F_c = F_1 + F_2 + F_m \\
A3.10) \quad F_2 = k_c \cdot d - k_1 \cdot \delta - F_m \\
\]

The force dynamics for the Maxwell element (A2.10) is also necessary here.

Using our ansatz we can calculate \(F_2\), its time derivative and the time derivative of the indentation:
\[ F_2 = (k_c + k_1) \left[ \Delta d + a e^{-t/\tau} \right] - F_m \]

\[ \dot{F}_2 = (k_c + k_1) \left( -\frac{a}{\tau} \right) e^{-t/\tau} \]

The indentation and its time derivative are given by:

\[ \delta = -d \]
\[ \dot{\delta} = -\Delta d - a e^{-t/\tau} \]
\[ \ddot{\delta} = -\dot{d} = \frac{a}{\tau} e^{-t/\tau} \]

This will be entered in the dynamic equation of the Maxwell element A2.10:

\[ \frac{a}{\tau} e^{-t/\tau} = \frac{k_c + k_1}{k_2} \left( -\frac{a}{\tau} \right) e^{-t/\tau} + \frac{(k_c + k_1) \Delta d}{f} + \frac{(k_c + k_1) a e^{-t/\tau}}{f} - \frac{F_m}{f} \]

This relation can be split in its time dependent part and those terms, which do not depend on time:

\[ \frac{a}{\tau} e^{-t/\tau} = \frac{k_c + k_1}{k_2} \left( -\frac{a}{\tau} \right) e^{-t/\tau} + \frac{(k_c + k_1) \Delta d}{f} + \frac{(k_c + k_1) a e^{-t/\tau}}{f} \]

\[ 0 = \frac{(k_c + k_1) \Delta d}{f} - \frac{F_m}{f} \]

Eq. A3.15b is identical with eq. (A3.4), so it gives no new information and is satisfied.

Eq. A3.15a can be simplified and will give us a relation for the friction coefficient:

\[ \frac{1}{f} = -\frac{k_c + k_1}{k_2} + \frac{(k_c + k_1) \tau}{f} \]

\[ f = k_2 \tau \left( k_c + k_1 \right) \left( k_2 + k_c + k_1 \right) \]

This relation is identical to eq. (A2.17) from above for the case of the z step.
S4 Creep response data from gels

![Graph showing creep response data from gels]

**Figure S5**

Typical creep response of a soft poly-acrylamide gel after applying a z step. The sequence of experiments is identical to the data presented in figure 3. Due to the much smaller viscous response of the gel, the creep after approach, and after the loading and unloading step is much smaller than in the case of cells. Gels predominantly react elastic, and the viscous response is very small to negligible.
Figure S6
Creep response of a gel after a magnetic force step. Experimental parameters are as in figure 5. Again, the creep response after the approach ramp and after applying loading/unloading steps is very small, due to the predominantly elastic nature of the gel.

Figure S7
Analysis of step response data on polyacrylamide gels. Panel A shows a comparison of stiffness value calculated from approach and retract curve with the elastic constant $k_1$ values from step response. Panel B shows the creep response time and the elastic constant $k_2$ values. All values are very close for unloading and unloading, except the approach and retract data calculated from the force curves, as expected.
S4 Thermal noise in AFM cantilevers

Since Boltzmann's equipartition theorem can also be applied to AFM cantilevers there will be thermal fluctuations in the deflection signal. This is often used to calibrate the force constant of cantilevers.

The (time averaged) thermal fluctuation in the deflection signal are given by:

\[ \langle d^2 \rangle = \frac{k_b \cdot T}{k_c} \]

where \( k_b \) is Boltzmann's constant, \( k_c \) is the cantilever force constant, \( T \) is the absolute temperature and \( d \) is the cantilever deflection. The pointed brackets denote the time average of the cantilever deflection squared.

The force fluctuations are given by:

\[ \langle F^2 \rangle = k_b \cdot T \cdot k_c \]

Figure S8

Thermal force and deflection fluctuations as a function of AFM cantilever force constants. These numbers will give a lower limit for noise in the quantities force and deflection.