Supporting information to Two-Stage Collapse of PNIPAM Brushes: Viscoelastic Changes Revealed by an Interferometric Laser Technique

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SI.1 Brush thickness determination: cross-section and imaging

We use focussed ion beam (FIB) and scanning electron microscopy (SEM) to determine the thickness of the PNIPAM layer. First, we disperse the beads in water. Then, we deposit a small droplet of the dispersion onto a silicon substrate and let it dry. To prevent charging of the sample during imaging, we sputter a 10 nm thick Pt-layer onto the beads. Then a thicker Pt-layer with a µm-thickness is locally deposited by ion-assisted Pt-deposition at 30 kV and 1 nA. Then, a section is step-wise milled away at 30 kV and 3 nA. After this coarse milling procedure, we polish the surface in two steps, with 0.5 nA, and 0.3 nA, both at 30 kV. Finally, we image with SEM at 2 kV at an angle of 35° (SI.Figure 1). Before analysis, the imaging angle is corrected by scaling the image to a circular cross-section of the bead. The dark region around the bead corresponds to the PNIPAM layer (SI.Figure 1A, B). Pristine beads did not show this dark region (SI.Figure 1C). We measured the layer’s thickness over the bead’s perimeter 20 times. By this procedure, we measured a mean polymer layer thickness of 51 ± 3 nm. We also tested the effect of prolonged dispersion in water on the brush layer. Similar to our experiments, we left the beads in water for one day. The PNIPAM layer thickness did not change significantly (SI.Figure 1D).

SI.Figure 1 SEM images of cross-sections of PNIPAM brush-coated beads. A A cross-section of PNIPAM-coated beads. The platinum layer is indicated. A zoom-in of the red rectangle is shown in B. B We attribute the dark layer around the beads to PNIPAM. C A cross-section of beads that were not coated with a PNIPAM brush layer. No dark layer can be seen here. D A cross-section of a PNIPAM-coated bead that had been in water for one day.

SI.2 The beads are sticking to the wall

We observe that most beads stick to the wall by themselves. As the beads stick, they do not move parallel to the wall. Thus, the formation, the relative placement, of a number of beads is constant (SI.Figure 2A). During a measurement, the beads do not drift out of focus. For non-sticking beads we observed that the beads aggregate somewhere in the capillary (SI.Figure 2B). Thus, the formation of
the beads changes. All measurements that are presented in the paper were on beads that were sticky at all measured temperatures.

SI. Figure 2 Optical microscope images of beads. A) The beads are sticking to the bottom of the capillary. B) Beads that do not stick to the wall will aggregate. In this case, the mean bead diameter is about 8 µm. Here, the beads could move parallel to the wall, and consequently collected at one of the capillary edges. The white scale bar corresponds to 10 µm.

The most likely reason for the sticking of the beads is physical interactions between the brush and the wall, sometimes referred to as bridging. Two possible types of such interactions can be considered: attractive Van der Waals forces between the brush and the wall, and the formation of hydrogen bonds between the amide moieties of PNIPAM and the hydroxyl groups of the silica wall. Such attraction has been observed before between a planar PNIPAM brush and a silicon nitride AFM tip, a silica colloidal-probe AFM, or an SFA with mica surface.

SI.3 Blocking of the PSD data
Before we fit Eq. (2) to the measured PSD, we “block” the data. Blocking makes each data point statistically independent and Gaussian distributed. As such, we can use fitting methods such as a least-square method. The original PSD is linearly distributed over the frequency range. The blocked PSD is logarithmically distributed. Each order of magnitude in frequency has a fixed number of data points. In our case we block to 50 data points per frequency decade; each blocked data point is the mean value of the original PSD points that are closest to it. We fit from 1 Hz up to 25 kHz. At 1 Hz, the data is averaged. 25 kHz is half of the sampling rate of the set-up.

SI.4 Discussion of the model, the fit, and the parameters $D$, $f$, $A$, and $C$
For each temperature, we printed all four fit parameters (SI Table 1), along with their errors (the 95% confidence interval, as noted in the main text). All PSD analysis was done with Matlab 9.2.0.538062 (R2017a). Furthermore, two goodness-of-fit values are printed, the root mean squared error (RMSE), and the adjusted R-squared value (adj. R^2). The RMSE is the root mean-square deviation of the fit from the fitted data points in decades. Thus, the unit of the RMSE is log(V^2/s), where a value of 1 would mean that the RMSE value between data and fit is one decade; a value of 0 describes a perfect match between data and fit. Independent of temperature, the RMSE value is roughly 0.01 decade, indicating a good fit. Alternatively, the adj. R^2 value describes the goodness of the fit in a range of 0-1, with a value of 1 describing a perfect match between fit and data.
SI Table 1 The fitted parameters for the PSD vs. frequency of each curve (corresponding to Figure 3 of the main text). The columns labelled with “err” are the 95% confidence interval values of the previous column.

| T [°C] | D [V²/s] | err | f_k [Hz] | err² | A [V²/s] | err3 | C [V²/Hz] | err4 | RMSE [log(V²/s)] | adj. R² |
|--------|----------|-----|----------|-----|----------|-----|----------|-----|----------------|--------|
| 22.9   | 5.94E-04 | 3.99E-05 | 1.21E+02 | 6.82E+00 | 2.80E-08 | 4.35E-09 | 1.88E-10 | 1.74E-12 | 1.16E-02 | 0.899 |
| 25.0   | 2.52E-04 | 3.34E-05 | 1.10E+02 | 1.04E+01 | 8.74E-09 | 2.17E-09 | 1.72E-10 | 2.14E-12 | 1.58E-02 | 0.963 |
| 28.0   | 9.45E-05 | 1.58E-05 | 1.15E+02 | 1.23E+01 | 7.57E-09 | 1.13E-09 | 1.47E-10 | 1.30E-12 | 1.14E-02 | 0.967 |
| 29.9   | 3.55E-05 | 1.35E-05 | 9.38E+01 | 2.11E+01 | 9.13E-09 | 1.57E-09 | 1.39E-10 | 1.57E-12 | 1.48E-02 | 0.936 |
| 31.0   | 3.28E-05 | 9.69E-06 | 1.14E+02 | 1.97E+01 | 5.05E-09 | 5.82E-10 | 1.37E-10 | 9.23E-03 | 9.36E-03 | 0.960 |
| 32.8   | 3.69E-05 | 1.07E-05 | 1.04E+02 | 1.74E+01 | 3.34E-09 | 5.06E-10 | 1.40E-10 | 1.16E-02 | 1.08E-02 | 0.942 |
| 34.0   | 4.97E-05 | 1.08E-05 | 9.24E+01 | 1.21E+01 | 7.20E-09 | 1.05E-09 | 1.31E-10 | 1.16E-02 | 1.16E-02 | 0.964 |
| 35.8   | 3.15E-05 | 7.80E-06 | 5.69E+01 | 8.62E+00 | 8.15E-08 | 2.45E-09 | 1.41E-10 | 1.24E-02 | 1.16E-02 | 0.977 |
| 38.3   | 3.71E-06 | 1.53E-06 | 2.07E+01 | 6.62E+00 | 1.11E-08 | 1.59E-09 | 1.65E-10 | 1.28E-02 | 1.05E-02 | 0.969 |
| 41.3   | 1.54E-06 | 4.09E-07 | 1.04E+01 | 2.99E+00 | 4.60E-08 | 1.17E-09 | 1.84E-10 | 1.48E-12 | 1.10E-02 | 0.950 |
| 44.5   | 2.69E-06 | 1.15E-06 | 1.80E+01 | 6.62E+00 | 1.82E-08 | 2.37E-09 | 1.72E-10 | 1.31E-12 | 1.03E-02 | 0.975 |
| 49.6   | 2.46E-05 | 9.19E-06 | 7.48E+01 | 1.73E+01 | 2.88E-08 | 3.61E-09 | 1.36E-10 | 1.31E-12 | 1.27E-02 | 0.972 |
| 54.7   | 2.27E-05 | 6.42E-06 | 7.53E+01 | 1.53E+01 | 9.44E-08 | 6.71E-09 | 1.43E-10 | 9.10E-13 | 8.32E-03 | 0.993 |
| 22.5   | 1.69E-03 | 7.71E-05 | 1.04E+02 | 5.29E+00 | 7.42E-08 | 1.47E-08 | 1.56E-10 | 1.77E-12 | 1.37E-02 | 0.994 |

In addition to these goodness-of-fit values, we also plotted the residuals of the data presented in Fig. 2 of the main text (SI Figure 3). Here, the fit deviates from the data around the noisy region around 5 Hz. Furthermore, at frequencies below 3 Hz, the fit has a slight, but nonetheless systematic deviation. The low frequency regime is dominated by the drift term, $A/f_k^2$. The deviation can mean the drift term does not perfectly describe the data at these frequencies. However, the residuals do not deviate much at other frequencies and, more importantly, around $f_k$. Thus, we can use the damped harmonic oscillator model to describe the Brownian motion.

**SI Figure 3** PSD of a PNIPAM brush-coated glass bead with a 5-µm diameter sticking to a glass surface (blue circles). The solid black line is the fit of Eq. (2) in the main text. The data and the fit are the same as in Figure 2 in the main text. The residuals of the fit are plotted below the PSD. Each individual component of the Eq. (2) has been plotted as well, each component indicated in the legend. The effect of changing each of the fitted parameters is indicated by the double arrows. Noise dominated data are plotted with the solid grey line.

To discuss the sensitivity of the data, we discuss the individual terms that we presented in Eq. (2) in the main text (plotted in SI Figure 3). From the individual plots, it can be appreciated that each term has a unique influence on the shape of the fit. For example, in a measurement with high drift, only the fit parameter $A$ will increase, shifting this part of the line parallel to the y-axis (dash-dotted line in SI Figure 3). The Brownian motion of the bead is described by $D$, and $f_k$, which move this part of the line up-down or left-right, respectively (dotted line in SI Figure 3). Finally, the detection limit, or white laser
noise is described by $C$, which will raise that part of the line parallel to the y-axis (dashed line in SI Figure 3). Thus, each fitted parameter has a unique influence on the fit. Naturally, if the signal of the Brownian motion is shrouded by the drift, or close to the detection limit, finding values for $D$, and $f_k$ will result in higher errors. In the presented measurements, we find that $f_k$ has an error between 5-30%, depending on the clarity of the Brownian motion signal.

In the main text, the fit parameter $f_k$ is thoroughly discussed. In this section the other parameters, $D$, $A$, and $C$ will be discussed. Potentially, $D$ can be used to describe a Brownian particle. Right now, we cannot measure $D$ accurately. We measure $D$ in “arbitrary units” of $V^2/s$, instead of the typical $m^2/s$. Berg-Sørensen and Flyvbjerg\textsuperscript{38} describe the formalism that we use in the main text. They state about optical tweezers calibration “...by equating the fitted values for $D$, which is determined in arbitrary units, to the value in physical units known from Einstein’s relation, Eq. (6).”, where Eq. (6) is $D = k_B T/\beta$ (section V in the reference). Indeed, this equation is the well-known and established Stokes-Einstein equation. However, using this equation requires precise knowledge about the environment of the bead. In our case, we would require $d$, and $\eta_{rel}$. Thus, we do not measure $D$ in insightful units, nor can we accurately calculate it. There is another reason why we do not investigate $D$. The signal that we measure is the intensity of the laser that reflected off the wall and the bead. The reflection depends on the refractive index of the PNIPAM, which changes during collapse.\textsuperscript{14, 32}

The parameters, $A$ and $C$, describe secondary effects not involved in the Brownian motion of the bead. The parameter $A$ describes the magnitude of drift of the set-up during a measurement. Our measurements involve heating the sample, which results in thermal expansion of e.g. the glass microscope slide. We start each measurement with the laser centred on one bead. However, due to thermal drifts, after a certain time the laser may hit the bead off-centre, which results in a change of the reflected intensity. Such drifts are included into our fitting through the term $A/f^2$ (in Eq. (2) of the main text). This value may change from measurement-to-measurement. We did not observe a significant trend with temperature, however (SI Table 1). The parameter $C$ describes the limit of detection of the set-up, caused by e.g. noise in the laser or limits and noise of the APD detector. The value of $C$ may change, depending on how much light is incident on the detector. Values of $C$ during the temperature measurement are $1.5 \cdot 10^{-10} \pm 0.2 V^2/Hz$ (SI Table 1). The value of $C$ in Fig. 2 of the main text is higher. We ascribe this difference to the fact that the measurements were taken on different samples at different times. Thus, the alignment of the set-up could be different. However, in both cases, the value of $C$ does not exceed the signal of the Brownian motion, and values for $D$ and $f_k$ could still be found.
SI.5 Data of measured PSD spectra when varying the temperature

![PSD spectra graph](image)

**SI. Figure 4** Data that is used Figure 3 of the main text. The PSD of a PNIPAM brush-coated bead vs. temperature. The offset for each subsequent measurement here is 3 decades on the y-axis. The colours are the same as the used in Figure 3. The thin black lines are the fits with Eq. (2). The fitted values for $f_k$ and their errors are plotted as well.

The references in this supporting information correspond to references in the main text.