Supplementary Information: Effects of Monovalent and Divalent Cations on the Rheology of Entangled DNA

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I. PARTICLE TRACKING MICRORHEOLOGY

A. Removing Localization Errors

Savin and Doyle [1] describe sources of static and dynamic error in particle tracking microrheology, and suggest methods of how to minimise or remove them. Static errors arise due to noise to signal ratio of the videos. There are a variety of sources of noise in the experimental setup such as out of focus particles, photon shot noise, and CCD noise. We corrected for static errors by taking videos of tracer probes fixed to the glass substrate and removing their MSD from the one of the probes in the bulk; however, the contribution of static errors is negligible as the MSD of the stuck probes are at least 2 orders of magnitude smaller than the MSD of the particles in the bulk (see violet trace in Figure 1). It was ensured that the videos of the stuck beads were identical in their noise to signal contributions as the original videos.

We also assessed the dynamic error (also suggested in [1]) by taking different videos at varied exposure times. We show in Figure 2 that the exposure starts to affect the accuracy of the MSD at 500 ms, at which point "blurring effects" come into play. All videos were taken at 4ms exposure time, to avoid introducing any dynamic errors in this way.
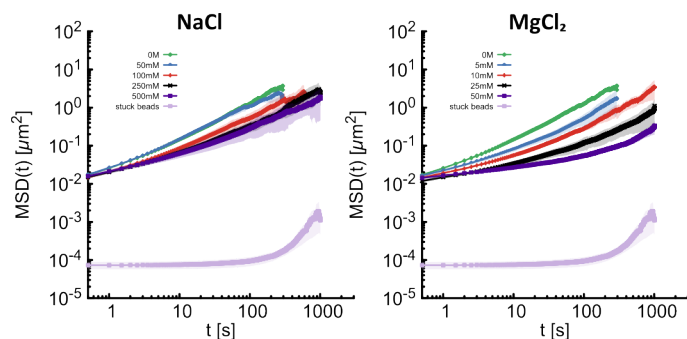
B. Probability Distribution Functions

An assumption of passive microrheology is the particles are moving via random thermal fluctuations in a homogeneous material. One way to assess the homogeneity of the sample is to plot the probability distribution function (PDF) of the probe displacement at a given lag time, if the material is homogeneous this will follow a Gaussian distribution [2]. We plotted the PDFs of both x and y displacements at a lag time of 10s for all tracers in the field of view, for at least 4 different video repeats. The results can be seen in Figures 3 and 4.

II. HOMOGENEITY OF λDNA WITH CATIONS

The homogeneity of the λDNA samples was ensured by leaving the samples on a roller bank for at least 48hrs prior to utilization as well as heating to 65°C and thorough mixing. However, through analysis of the microrheology probe PDFs, we find the emergence of inhomogeneities only after the addition of cations. This is shown in Figure 3 a-c which depicts the PDFs when the cations are mixed into the homogeneous λDNA solution for a maximum of 30 minutes before microrheology

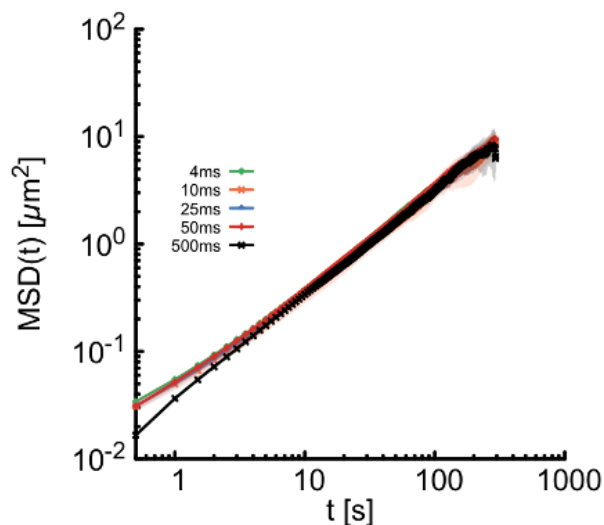FIG. 1. Mean squared displcement (MSD) as a function of lag time, t[s], of tracer probes in solutions of DNA with varying NaCl (left) and MgCl2 (right) concentrations, compared with MSDs of particles stuck at the glass interface (violet trace at the bottom). One can appreciate that they are at least 100x smaller than the MSD of the probes in the bulk. The long lagtime upturn of the violet traces is due to drift correction.

FIG. 2. Mean squared displacement (MSD) as a function of lag time, t[s], with varying exposure times of λDNA at $450ng/\mu L$ with no cations present .
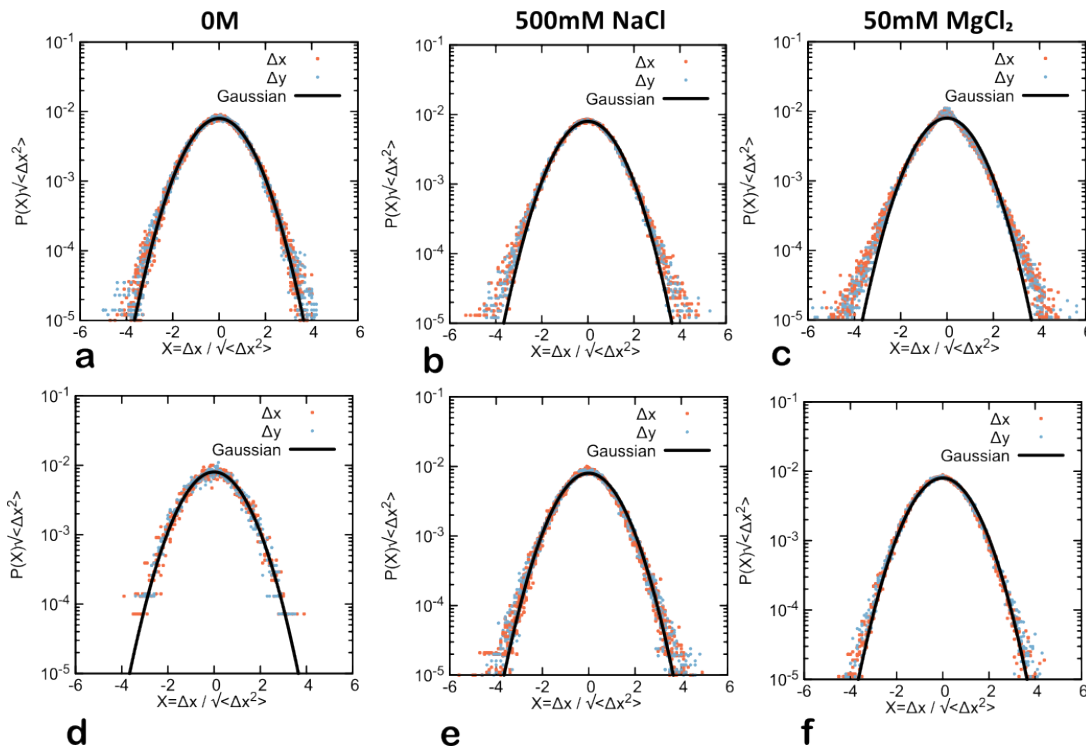
FIG. 3. Comparison of probability distribution functions, P(X), of microrheology tracers x (orange) and y (blue) displacements after mixing λDNA solutions with cations for different time durations: less than 30 minutes (a-c) and more than 24 hours (d-f). The figure displays controls with 0M (no cations) (a, d), monovalent cations (b, e), and divalent cations (c, f). Distributions are expected to follow a Gaussian function with zero mean and unit invariance (plotted in black) for freely diffusing probes in a homogenous material.

is performed. We observed departures from Gaussian behaviour as the cation concentration increased, this being particularly pronounced for the highest concentration of divalent cations (Figure 3c). This points to the emergence of inhomogeneities in the λDNA sample only following the introduction of high concentrations of divalent cations. To address this, the sample was subjected to an additional 24-hours mixing period on the roller after the cations had been added into the solution. The PDFs are now notably more Gaussian after the extra mixing step, thereby indicating a homogeneous sample as seen in Figure 3d-f. Figure 4 shows the PDFs for all concentrations of both monovalent and divalent cations, demonstrating how all samples now exhibit homogeneous behaviour once mixed for an extra 24hr period after cations are added.

All data in the paper is therefore from λDNA sample mixed for 24hrs with cations present in solutions ensuring Gaussian (homogeneous) behaviour. However, we found this adjustment had no major impact (within errors) on the overall scaling of viscosity with cation concentration. This is shown in Figure 5, where f(x) is fitted to samples mixed with cations for 30 minutes, and f1(x) for samples mixed for 24hrs. For monovalent cations we find $f(x) \sim x^{0.70\pm0.26}$ and $f1(x) \sim x^{0.82\pm0.19}$. For divalent cations $f(x) \sim x^{1.62\pm0.40}$ and $f1(x) \sim x^{1.35\pm0.09}$. Indeed, the average behaviour of the probes is in line with the previous (heterogeneous) systems, albeit in the heteroge-

neous samples there was much more variance in terms of individual particles behaviour.
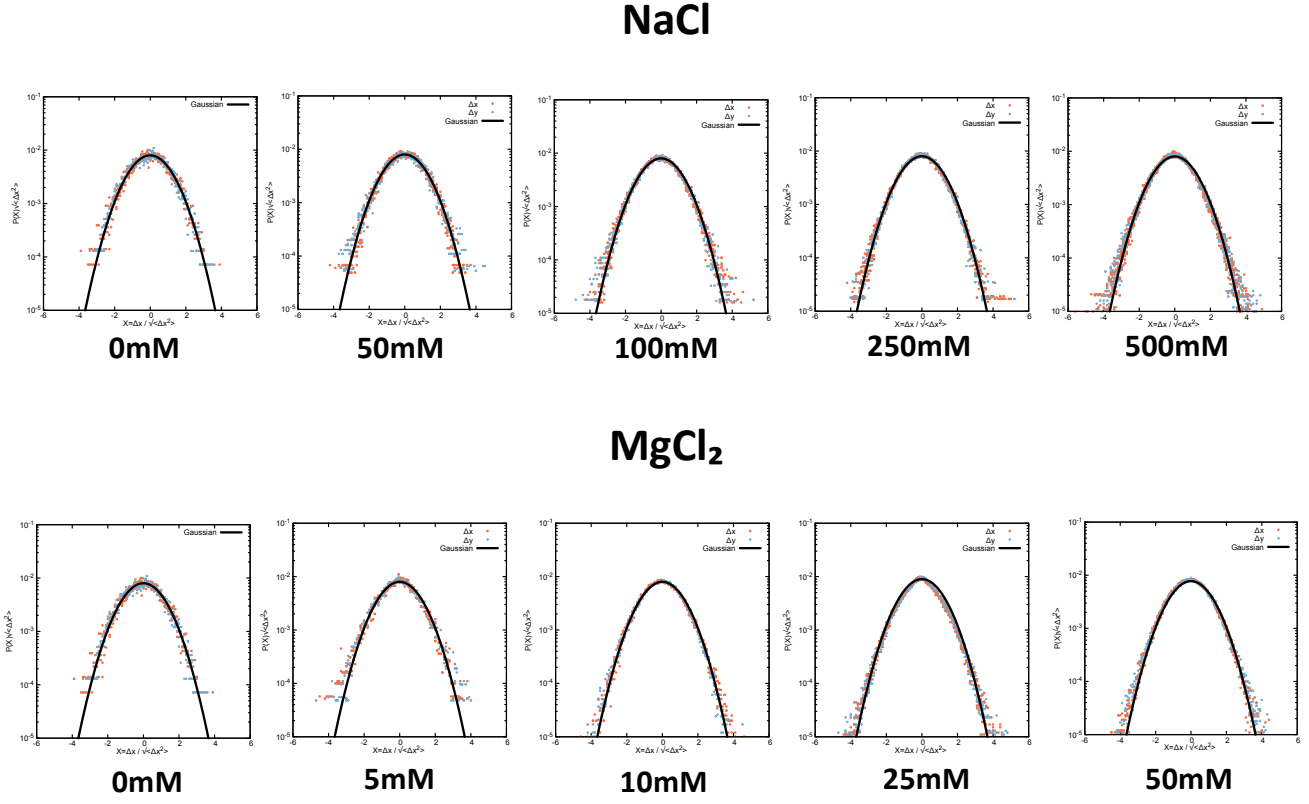
NaCl

| 0mM | 50mM | 100mM | 250mM | 500mM |

MgCl₂

| 0mM | 5mM | 10mM | 25mM | 50mM |

FIG. 4. Probability distribution functions, P(X), of microrheology tracers x (orange) and y (blue) displacements after mixing λDNA solutions with cations for 24hrs. PDFs are displayed for all concentrations of monovalent cations (top) and divalent cations (bottom).
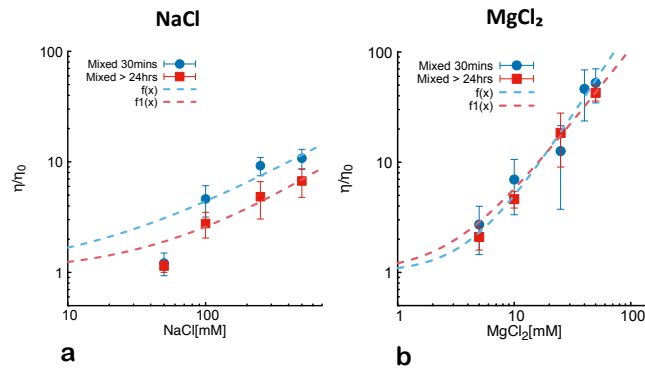

FIG. 5. Viscosity as a function of cation concentration for monovalent (a) and divalent (b) cations. Viscosity values are normalised with respect to the viscosity of λDNA at 450ng/μL with no cations present(η_0). λDNA mixed for only 30 minutes after adding the cations (circles) and λDNA mixed with cations for 24hrs are plotted together with their respective fits, $f(x)$ and $f1(x)$. Points correspond to the experimental data with errors, and the dashed lines display a power law fitted to the data in the form $f(x) = 1 + Ax^b$. We find the best fit parameters for NaCl with 30-minute mixing are $A = 0.134 \pm 0.204$ and $b = 0.703 \pm 0.258$ and 24hr mixing are $A = 0.037 \pm 0.041$ and $b = 0.818 \pm 0.187$. For MgCl₂, we find for 30-minute mixing $A = 0.095 \pm 0.147$ and $b = 1.623 \pm 0.409$, for 24hr mixing $A = 0.211 \pm 0.074$ and $b = 1.352 \pm 0.091$.

III. REFERENCES

[1] T. Savin and P. S. Doyle, Biophysical journal **88**, 623 (2005).

[2] M. T. Valentine, P. D. Kaplan, D. Thota, J. C. Crocker, T. Gisler, R. K. Prud'homme, M. Beck, and D. A. Weitz, Physical Review E **64**, 061506 (2001).