# Supplementary information for: Flexibility induced effects in the Brownian motion of colloidal trimers

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## A Modelling and analysis of the hydrodynamic properties of flexible trimers

We used three different models to describe the hydrodynamic properties of the flexible trimers: a bead model (Figure S1 a), a bead-shell model for a rigid trimer using HydroSub [3] (Figure S1 b) and a bead-shell model for flexible trimers (Figure S1 c). For the bead-shell models, the results were evaluated for multiple small bead sizes, so that the result could be linearly extrapolated [11, 12] to the limit where the small bead radius approaches zero (see the Methods in the main text for details).

In Figure S1 d, the calculated diffusivities are shown for all three models. The bead model predicts higher diffusivities compared to both bead-shell models for all different elements of the diffusion tensor. The bead-shell models agree qualitatively, but predict different magnitudes of the diffusivities due to differences in hydrodynamic interactions between the outer beads, which are higher for the flexibly linked clusters [5, 13–15]. We have used the bead-shell model of Figure S1 c (solid line) to model our experimental data, because it best describes our experimental data and because it can be used to model conformational changes, which are not yet implemented in the HydroSub model.

For our results described in the main text, we used the center of mass as reference point. For purely rotational and conformational terms, the diffusivity is expected to be independent of the chosen reference point, however, for terms that include translation, the location of the reference point has a large effect on the measured diffusivity [10, 16]. This can be seen in Figure S2: in panels a-c), we show the diffusivities calculated using the central particle as reference point. The results are remarkably different from the center of mass based results shown in Figure S2 d-f), where we have used the diffusivities relative to the central particle to calculate the diffusivities relative to the center of mass using the coordinate transformations determined by Harvey and coworkers:[10]

$$D[tt]_{CM} = D[tt]_0 + D[t\alpha]_0^{\mathsf{T}} \cdot U - U \cdot D[t\alpha]_0 + U \cdot D[\alpha^2] \cdot U + D[t\theta]_0^{\mathsf{T}} \cdot W + W^{\mathsf{T}} \cdot D[t\theta]_0$$

$$-U \cdot D[\alpha\theta]^{\mathsf{T}} \cdot W + W^{\mathsf{T}} \cdot D[\alpha\theta] \cdot U + W^{\mathsf{T}} \cdot D[\theta^2] \cdot W \tag{1}$$

$$D[t\alpha]_{CM} = D[t\alpha]_0 + D[\alpha^2] \cdot U + D[\alpha\theta]^{\mathsf{T}} \cdot W$$
<sup>(2)</sup>

$$D[t\theta]_{CM} = D[t\theta]_0 + D[\alpha\theta] \cdot U + D[\theta^2] \cdot W$$
(3)

We have made this comparison because the coupling terms are expected to be larger in the central particle frame. The results indeed show this larger coupling and exclude the possibility that the coupling modes we observed are artifacts of the coordinate system we used. Because the rotational and conformational diffusivities are independent of the reference point, localization uncertainties in the determination of the position of the reference point may have a larger effect on  $D[xx, yy, xx, x\alpha, y\alpha, x\theta, y\theta]$  than on  $D[\alpha^2, \theta^2, \alpha\theta]$ . Because of the uncertainties that are propagated when we first determine the diffusivity with respect to the central particle and then transform this to the diffusivity with respect to the center of mass (in Figure S2 d-f), the error is larger for this method compared to the direct calculation of the diffusivities with respect to the center of mass.



Supplementary Figure S1: Comparison of the diffusion tensor calculated by different hydrodynamic models. Renderings made using FreeWRL [4] of a) the simple bead model, b) the bead-shell model (minimum radius of the small spheres r = 55 nm) used by HydroSub [3] for rigid trimers, c) the bead-shell model (radius of the small spheres r = 31 nm to 54 nm, r = 45 nm is shown) we used for calculating hydrodynamic properties of flexible trimers. For all models, the radius of the large particles is  $R = 1 \,\mu\text{m}$ . d) Top row, left to right: the translational diffusivity, rotational diffusivity and coupling between translational and rotational diffusivity for the bead model (a, dotted lines), the rigid bead-shell model generated with HydroSub (b, dashed lines) and the segmentally flexible bead-shell model (c, solid lines). Bottom row, left to right: the joint flexibility, coupling between shape changes and rotation and couplings between shape changes and translational diffusion.



Supplementary Figure S2: Influence of the reference point on the diffusion tensor. a-c) The translational (a), translational-rotational (b) and translational-conformational (c) diffusivities with the reference point chosen in the center of the central particle. d-f) The translational (d), translational-rotational (e) and translational-conformational (f) diffusivities with the reference point at the center of mass of the cluster. For these graphs, we transformed the data from panels a-c using the coordinate transformation described in the text from the "center particle"-based to the "center of mass"-based diffusivity. Note that the combination of experimental errors of the  $D[t\alpha]$ ,  $D[\alpha^2]$ ,  $D[t\theta]$ ,  $D[\theta^2]$  and  $D[\alpha\theta]$  terms lead to large uncertainties and deviations, especially for the translational diffusivities. In all panels, the points show the experimental data and the lines are the predictions of the bead-shell model.

#### **B** Near-wall diffusion: friction factors



Supplementary Figure S3: **Distribution of friction factors** as given by equation (3) of the main text. The mean value for  $\phi_{tt} = 0.29 \pm 0.04$  is close to the lower bound of 0.39 (indicated by the dotted line) as predicted by Equation 4. We find an average rotational friction factor  $\phi_{\alpha\alpha} = 0.55 \pm 0.07$ . The average flexibility friction factor  $\phi_{\theta\theta} = 0.40 \pm 0.12$  shows a broader distribution, which we attribute to a spread in DNA linker concentration. The average friction factor of the rigid clusters is also indicated ( $\phi_{tt,r} = 0.254 \pm 0.004$ ,  $\phi_{\alpha\alpha,r} = 0.49 \pm 0.02$ ) and coincides with the friction factors we find for flexible clusters.

As a first approximation to compare the experimental diffusion of freely-jointed trimers above a substrate to models of trimers diffusing in the bulk, we use Faxen's theorem: [6]

$$\frac{D_w(h)}{D_0} = 1 - \frac{9}{16} \frac{R}{h+R} + \frac{1}{8} \left(\frac{R}{h+R}\right)^3 - \frac{45}{256} \left(\frac{R}{h+R}\right)^4 + O\left(\left(\frac{R}{h+R}\right)^5\right),\tag{4}$$

with  $D_0$  the translational diffusion coefficient in the bulk,  $D_w(h)$  the in-plane translational diffusion coefficient near a wall at height h and R the particle radius. We calculate an effective particle radius  $R_{eff} = \frac{k_B T}{6\pi\eta D} = 1.8 \,\mu\text{m}$ from the short-time translational diffusion coefficient [9], with  $k_B$  Boltzmann's constant, T the temperature,  $\eta$  the viscosity of the medium and  $D = 0.136 \,\mu\text{m}^2 \,\text{s}^{-1}$  the lowest short-time translational diffusion coefficient of the trimer as predicted by the bead-shell model.

The expected Debye length of our medium (at I = 200 mM) is  $\kappa^{-1} = \frac{0.304}{\sqrt{I}} \approx 0.7 \text{ nm}$  [8], and so we neglect electrostatic interactions between the trimer and substrate. Therefore, the height of the particle above the substrate is set by balancing the effect of sedimentation and thermal fluctuations as expressed by the gravitational length  $l_g = \frac{k_B T}{g \Delta \rho V}$ , with g the gravitational acceleration constant,  $\Delta \rho$  the density difference between the particle and the medium and V the volume of the particle.

Using the appropriate values for the trimer, we find  $l_g = 20 \text{ nm}$ . By setting this as input for h in Equation 4, we obtain a upper bound for  $\frac{D_w(h)}{D_0}$ , equal to 0.40. A lower bound is found at h = 0, which gives a value of 0.39. The translational friction coefficient  $\frac{D_w(h)}{D_0}$  that we find has an average value of  $0.29 \pm 0.04$ , as shown in Figure S3, which is close to the lower bound we have calculated above. The experimental value is slightly lower than the predicted lower bound, because Equation 4 accounts for hydrodynamic interactions only and real experiments typically show lower diffusivities because of additional sources of friction [7], which in the present case could be explained by additional friction between the polymer coating and the particles.

#### C The collective diffusion constant depends on size polydispersity

In this manuscript we showed that freely-jointed trimers diffuse slightly faster than rigid trimers; their diffusion constant differs by 3%. When reporting such a small difference it is important to exclude other effects that could lead to similar variations in the diffusion constant. Therefore, in this section we address the effect of size polydispersity on the average diffusion constant of a collection of particles. We consider an ensemble of particles, whose sizes are normally distributed around an average radius,  $\bar{a}$ , and with a standard deviation,  $\sigma$ . We assume that the particles exhibit Stokes diffusion so that each particle *i* has a size dependent diffusion constant  $D_i = \frac{k_B T}{6\pi\eta a_i}$ . The small particles in the ensemble diffuse faster than large particles.

The experimental average diffusion constant of this ensemble of particles,  $\bar{D}$ , can be found by tracking the motion of many individual particles, calculating their individual diffusion constants and averaging those. One might assume that this average diffusion constant equals the diffusion constant of a monodisperse sample of particles with the same average size, but this turns out to be generally not true:  $\bar{D} \neq \frac{k_B T}{6\pi\eta\bar{a}} \equiv D_{\bar{a}}$ . The reason for this inequality is that the diffusion constant scales nonlinearly with size. Therefore, the diffusion constants of small particles are weighted more heavily than those of large particles, which skews the distribution of diffusion constant and shifts the average away from  $D_{\bar{a}}$ .

We asked how much the collective diffusion constant of a polydisperse sample would deviate from that of a monodisperse sample and how this deviation depends on size polydispersity. To this end, we first define the relative polydispersity as  $\sigma' = \sigma/\bar{a}$ , which is a value between 0 and 1. The normalized distribution of particle sizes is then

$$f(a) = \frac{1}{\sigma' \bar{a} \sqrt{2\pi}} \exp\left[-\frac{1}{2} \left(\frac{a-\bar{a}}{\sigma' \bar{a}}\right)^2\right].$$
(5)

Because the size is normally distributed and the diffusion constant scales with size as 1/a, the diffusion constant exhibits a reciprocal normal distribution:

$$g(D) = \frac{D_{\bar{a}}}{D^2 \sigma' \sqrt{2\pi}} \exp\left[-\frac{1}{2} \left(\frac{D_{\bar{a}}}{\sigma'} (1/D - 1/D_{\bar{a}})\right)^2\right].$$
 (6)

Figure S4a shows the hypothetical size distributions of three sets of particles with an average radius of 1  $\mu m$  and relative polydispersities of 5%, 10%, and 20%. Figure S4b shows the diffusion constant distributions that correspond to these particle ensembles. Note that the diffusion constant is — unlike the size — not normally distributed, but has a tail of fast diffusion, corresponding to small particle sizes. Note also that the most probable diffusion constant shifts with polydispersity. This is also due to the 1/a scaling of the diffusion constant and can intuitively be explained by the fact that a range of large particles give a similarly small diffusion constant. This increases the probability of measuring this small diffusion constant and shifts the peak in the distribution. These properties of the distribution cause the average diffusion constant of a polydisperse sample (indicated by dashed lines in Figure S4b) to shift compared to the monodisperse case (indicated by a black dashed line). How much the diffusion constant is underestimated depends on the size polydispersity. We intentionally chose large polydispersities to show the effect clearly. Note that at for a size polydispersity of 5% the distribution of diffusion constants still looks rather symmetric.

The average diffusion constant of the particle ensemble is

$$\bar{D} = \int_{D=-\infty}^{D=\infty} D g(D) dD.$$
(7)

The integral in Equation 7 cannot be solved analytically, but we solved it numerically and compared it to the diffusion constant corresponding to particles with an average size  $D_{\bar{a}}$ . As integration limits we used 0 and  $100 \times D_{\bar{a}}$  in order to probe all non-zero elements of the distribution function. We found that a 5% polydispersity results in an underestimate of the diffusion constant by only 0.25%. To underestimate the diffusion constant by 3%, the relative polydispersity needs to be at least 17%. We found that these results are independent of the particle size. This finding indicates that the measured 3% increase of flexible trimers compared to rigid trimers cannot be due to size polydispersity alone, because the employed particles have a size polydispersity of only 2.6%.

While polydispersity does not drastically alter the collective diffusion of microparticle suspensions, where  $\sigma'$  is typically around 5%, it could play a large role in the diffusion of nanoparticles, where a  $\sigma'$  on the order of 100% is not uncommon [17]. For example, gold nanoparticles with relative polydispersites on order of 10% are considered very monodisperse and can only be made in a small parameter range[18]. Using Equation 7 we predict that the collective diffusion constant of a sample with 100% polydispersity is 63% larger than a monodisperse sample with the same average size, highlighting the importance of considering this effect in nanoparticle suspensions.



Supplementary Figure S4: The collective diffusion constant depends on size polydispersity. a) Three hypothetical particle size distribution with an average particle radius of 2  $\mu m$  and relative polydispersities of 5%, 10%, and 20%. b) The distributions in diffusion constant corresponding to the three particle size distribution in panel a. The average diffusion constants are indicated by dashed lines. The average diffusion constant of a monodisperse sample is indicated by a black dashed line.

## **D** Supplementary Figures



Supplementary Figure S5: **Probability and free energy as a function of the opening angle of flexible trimers.** Probability and corresponding free energy of the opening angle of the flexible trimers used in this work (with the reference set at 180°). There is no preference for a specific opening angle within the experimental error, meaning the particles are freely-jointed, as was shown before. [1] Note that the slightly lower probability at angles smaller than  $60^{\circ} + \sqrt{2J\tau} \approx 69^{\circ}$  (with J the joint flexibility and  $\tau$  the sampling interval) is caused by boundary effects inherent to our analysis method. [1]



Supplementary Figure S6: **Opening angles of rigid trimers.** The number of rigid clusters of different opening angles used in this study. Six rigid trimers have a 'compact' opening angle (below 120°) while the other six are more extended.



Supplementary Figure S7: Short-time diffusion of rigid trimers. The (a) translational, (b) rotational and (b) translational-rotational diffusivities of rigid trimers with various opening angles (see Figure S6). In all panels, the points correspond to the experimental diffusivities (up to lag times of 0.25 s) and the solid lines correspond to the numerical calculations performed using HydroSub [3]. All points are scaled by the same average friction factor as shown in Figure S3 in order to compare the experiments to the numerical simulations.

## E Supplementary Tables

Identifier	Sequence
DS-B	5'-TCG-TAA-GGC-AGG-GCT-CTC-TAG-ACA-GGG-CTC-TCT-GAA-TGT-GAC-TGT-GCG-AAG-GTG-ACT-
	-GTG-CGA-AGG-GTA-GCG-ATT-TT-3'
DS-S-A	Double Stearyl-HEG-5'-TT-TAT-CGC-TAC-CCT-TCG-CAC-AGT-CAC-CTT-CGC-ACA-GTC-ACA-TTC-
	-AGA-GAG-CCC-TGT-CTA-GAG-AGC-CCT-GCC-TTA-CGA- <i>GTA-GAA-GTA-GG</i> -3'-6FAM
DS-S-B	Double Stearyl-HEG-5'-TT-TAT-CGC-TAC-CCT-TCG-CAC-AGT-CAC-CTT-CGC-ACA-GTC-ACA-TTC-
	-AGA-GAG-CCC-TGT-CTA-GAG-AGC-CCT-GCC-TTA-CGA- <i>CCT-ACT-TCT-AC-</i> 3'-Cy3
PA-A	Cholesterol-5'-TTT-ATC-GCT-CCC-TTC-GCA-CAG-TCA-ATC-TAG-AGA-GCC-CTG-CCT-TAC-GAT-
	-ATT-GTA-CAA-TA-3'-Cy3
PA-B	Cholesterol-5'-CGT-AAG-GCA-GGG-CTC-TCT-AGA-TTG-ACT-GTG-CGA-AGG-GTA-GCG-ATT-TT-3'
DS-H-A	Obtained by hybridization of DS-B and DS-S-A
DS-H-B	Obtained by hybridization of DS-B and DS-S-B
PA-C	Obtained by hybridization of PA-A and PA-B

Table S1: **Overview of all DNA strands used**. Sticky ends are marked in cursive.

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