Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Information

Sample solution constraints on motor-driven diagnostic nanodevices

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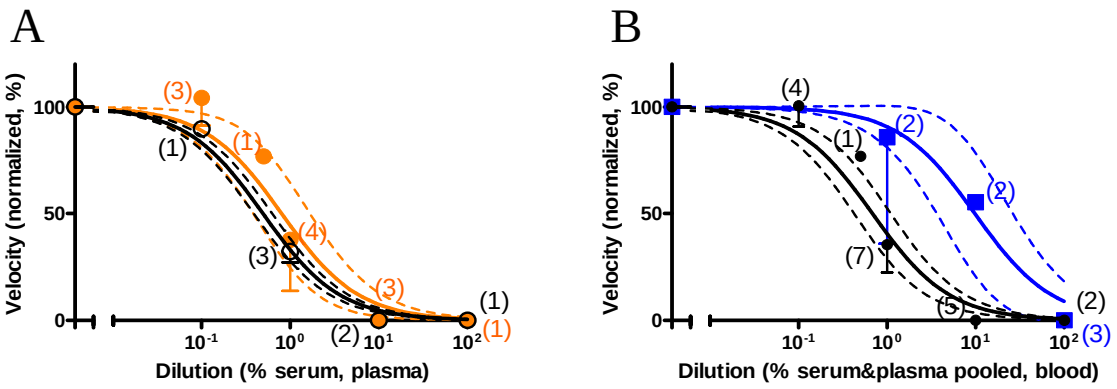
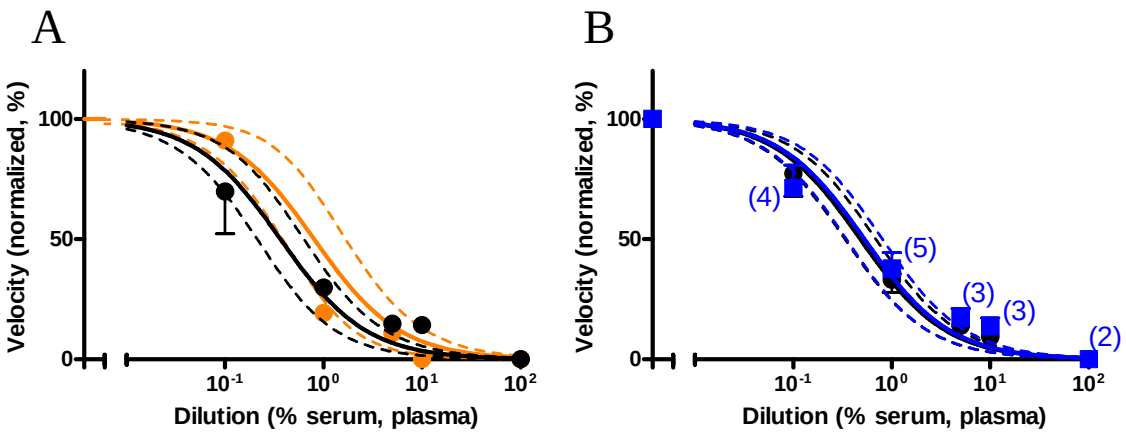


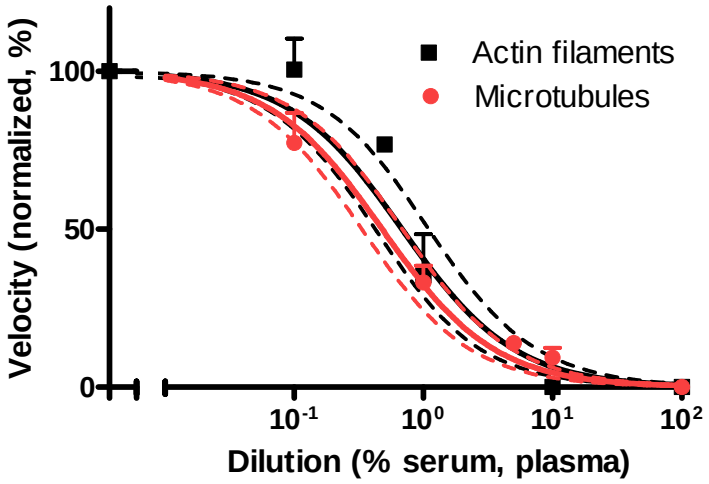
Supplementary Figure 1. Non-linear regression analysis of the effects of different concentrations of plasma, serum and blood on actomyosin motility. The mean speed of the filaments in each given flow cell was first normalized to control data with the same HMM preparation and temperature (see Experimental Methods in main paper). The mean speed values between flow cells under control conditions varied between 4.1 and 9.1 µm/s mainly due to temperature differences between experiments (temperature constant to ± 0.5 °C during each experiment). Control speeds were: 1. Serum: 6.68 ± 1.86 µm/s (mean ± standard deviation; n = 7 flow cells), 2. Plasma: 7.06 ± 1.52 µm/s (n=7) and 3. Blood: 4.30 ± 1.11 µm/s (n=6). (A) The effects of serum (orange) and plasma (black) did not differ when data was normalized to the control conditions as clear from overlapping 95 % confidence intervals (CI; dashed lines) of the non-linear regression fit (full lines) of the following equation:

Speed = Bottom + (Top-Bottom)/(1+10^(Log(Concentration)-Log(IC50))) (Eq. 1)

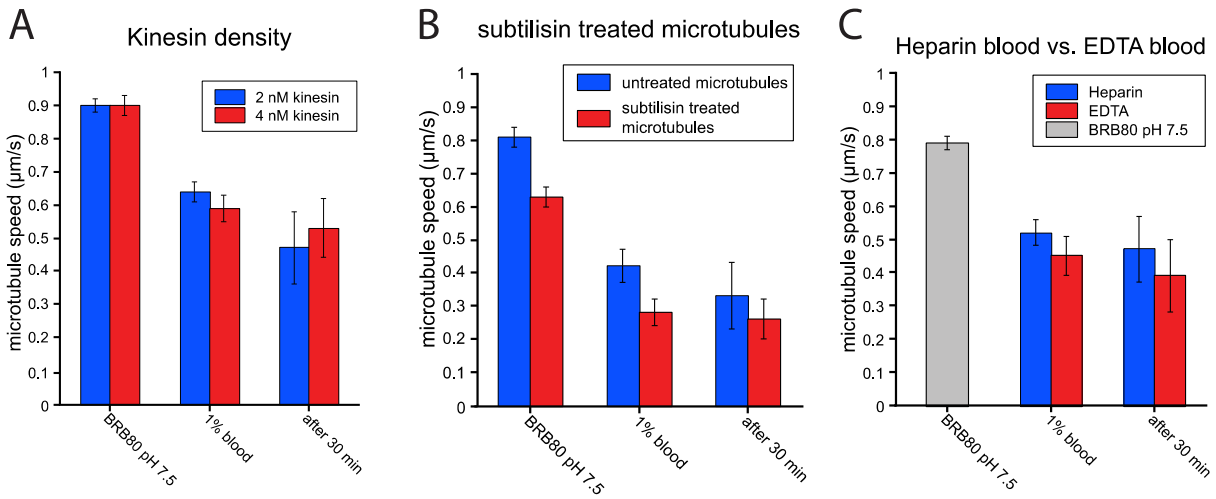
Here, only the parameter ”IC50” was adjusted in the fit with ”Bottom” and ”Top” held constant at 0 % and 100 %, respectively. Whereas we do not claim that this model is capturing all aspects of the data it is useful for an approximate insight into the possible effect of a mixture of inhibiting substances. Moreover, a replicates test for lack of fit failed to detect a statistically significant deviation from the model ($p>0.05$). The error bars for individual data points in the figure represent SEM to indicate the uncertainty in estimated mean value for a population of flow cells subjected to a given concentration of blood samples. (B) Data for serum and plasma pooled (black) and compared to data for blood (blue). The best estimate for the IC_{50} was 0.68% (95 % CI: 0.42 % to 1.12 %) for plasma/serum and 9.77 % (95 % CI: 3.08 % to 31.0 %) for blood. Whereas this indicates a significantly different inhibitory effect for the different blood samples ($p< 0.05$) we refer the effect to the complexity of whole blood such as the risk of haemolysis. This idea is consistent with the large variability for one of the data points and the appreciable difference between the actin filament and microtubule data.



Supplementary Figure 2. Non-linear regression analysis of the effects of different concentrations of plasma, serum and blood on kinesin-microtubule motility. The mean speed of the filaments in each given flow cell was first normalized to control data with the same kinesin preparation and temperature (also same flow cell; see Experimental Methods in main paper). The mean speed values between flow cells under control conditions varied negligibly. Control speeds were: 1. Serum: 0.65 ± 0.072 µm/s (mean ± standard deviation; n = 11 flow cells), 2. Plasma: 0.67 ± 0.065 µm/s (n=12) and 3. Blood: 0.62 ± 0.061 µm/ (n=14). (A) The effects of serum (orange) and plasma (black) did not differ as clear from overlapping 95 % confidence intervals (CI; dashed lines) of the non-linear regression fit (full lines). The fit was performed as in Supplementary Figure 1. The error bars for individual data points represent SEM to indicate the uncertainty in estimated mean value for a population of flow cells subjected to a given concentration of blood samples. (B) Pooled data for serum and plasma (from A) compared to data for blood (blue). The best estimate for the IC_{50} was 0.48% (95 % CI: 0.33 % to 0.70%) for plasma/serum and 0.53% (95 % CI: 0.34 % to 0.81 %) for blood, indicating no significant difference of the inhibitory effect for the different blood samples ($p > 0.05$).

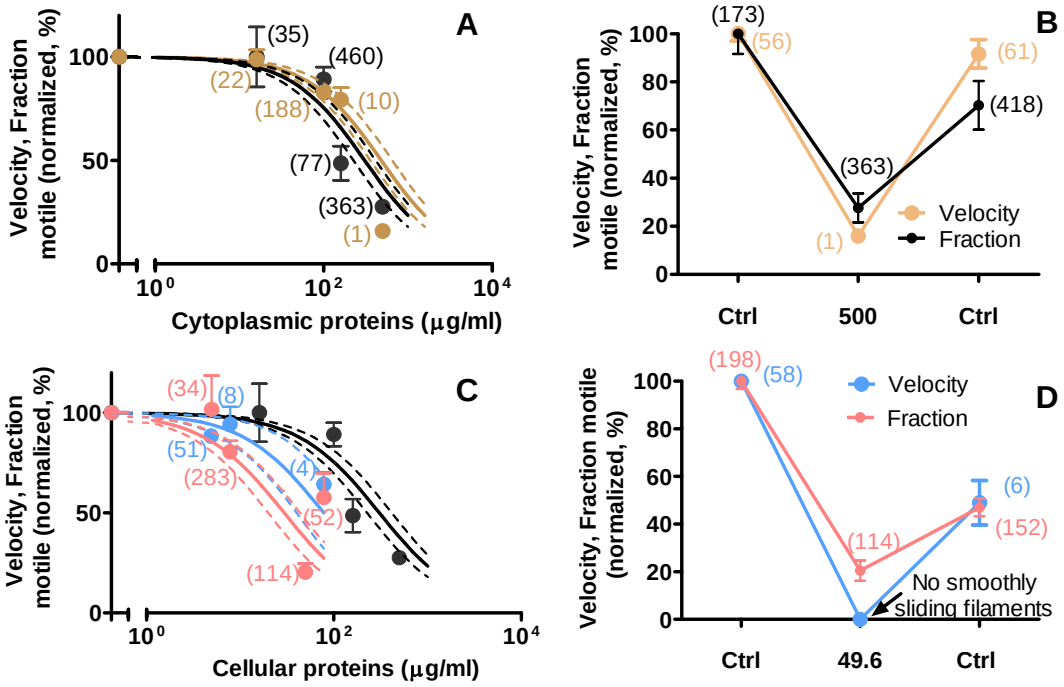


Supplementary Figure 3. Comparison between effects of serum/plasma at different dilutions on actomyosin and kinesin-microtubule motility. The pooled serum+plasma data in Supplementary Figs. 1 and 2 have been replotted. The data show appreciable similarity between data for the two motor systems. The tendency for a somewhat steeper relationship between speed and serum concentration (at 0.1 – 1 % serum/plasma) for actin filaments is not statistically significant. Moreover, as a small difference like this would be without practical relevance, further investigations are outside the scope of the present study.

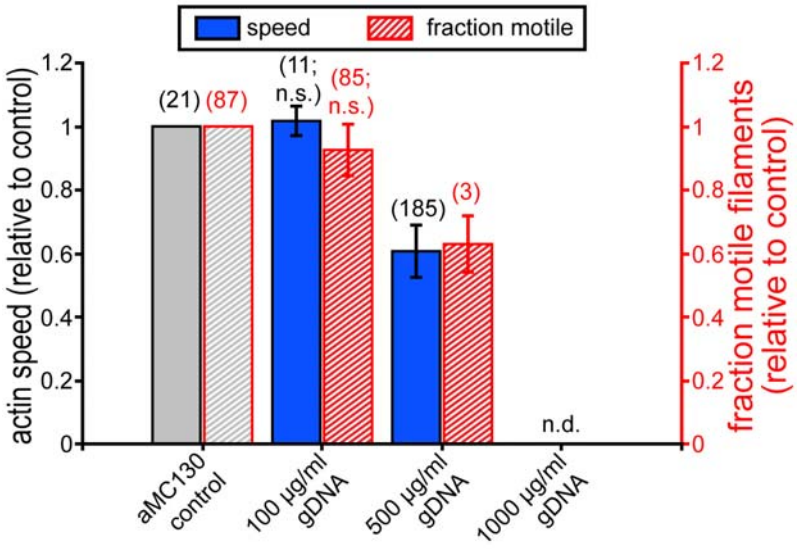


Supplementary Figure 4: Optimizations of motility assay in 1% blood that did not have an effect. (A) Effect of 1% blood on microtubule speed on surfaces coated with 2nM or 4 nM kinesin. (B) Effect of 1% blood on the speed of subtilisin treated microtubules compared to untreated microtubules. (C) Effects of 1% blood either with anticoagulant heparin or with anticoagulant EDTA on microtubule speed were measured before, directly after (1% blood) and 30 min after injection (after 30 min) of 1% blood diluted in BRB80 pH7.5. (A-C) Speeds are given as mean ± standard error of the mean of 16 randomly selected microtubules. All data points are statistically significantly ($p<0.05$) different from the respective controls.

No significant effect of kinesin density or anticoagulant could be shown. Subtilisin treatment did slow down microtubule speed in all cases, but the relative speed difference was the same with or without blood. Therefore, we conclude that these factors did not have an effect on the slow down of microtubule gliding speeds caused by blood.



Supplementary Figure 5. Effect of cytoplasmic and nuclear proteins on actomyosin motility. (A) Actin filament speed and fraction of motile actin filaments at different concentrations of cytosolic proteins. Speed data (brown) or fraction of motile filaments (black) normalized to control data measured in each given flow cell before incubation with proteins (one flow cell per concentration). Non-linear regression fits (full lines) of Supplementary Eq1 performed as in Supplementary Figure 1. This analysis gave average IC_{50} values of 483 µg/ml (95 % CI: 356 to 656 µg/ml) for actin filament speed and 307 µg/ml (95 % CI: 225 to 418 µg/ml) for the fraction of motile filaments. (B) Reversibility for the highest protein concentration (500 µg/ml) upon re-immersion in ordinary aMC130 solution. **C.** Data for fraction of motile filaments from A (black) re-plotted together with speed data (light blue) or fraction of motile filaments (pink) in the presence of different concentrations of nuclear proteins. Nonlinear regression analysis gave average IC_{50} values of 78.3 µg/ml (95 % CI: 38 to 160 µg/ml) for actin filament speed and 29.5 µg/ml (95 % CI: 20 to 44 µg/ml) for the fraction of motile filaments. (D) Reversibility for the highest protein concentration (78 µg/ml) upon re-immersion in ordinary aMC130 solution. Error bars in A and C: SEM. Error bars in B and D: 95 % CI indicating statistically verified incomplete reversibility. Number of individual filament paths given in parentheses.



Supplementary Figure 6: Effect of gDNA on Actin filament speed. Actin filament speed after preincubation of the myosin surface with 100-1000 µg/ml gDNA. Speeds and fractions of motile filaments are given as mean ± standard error of the mean of the number of filaments given in brackets next to the data points. Unless marked with n.s. (not significant) or n.d. (not determined because no filaments could be observed) all data points are statistically significantly ($p<0.05$) different from the respective controls.