Probing protein-ligand and colloid–colloid interactions by optical tweezers

To quantitatively probe the interaction between colloidal particles, force-sensing optical tweezers have proven to be a versatile tool. Here we discuss two different experiments that demonstrate the principle of force spectroscopy optical tweezers experiments in colloid research.

Optical tweezers platform

We have performed our measurements of colloid-colloid interaction forces using JPK's NanoTracker™, an off-the-shelf optical-tweezers platform designed for high-resolution quantitative nanomanipulation (Figure 1). The NanoTracker™ is equipped with two optical traps, which can be independently steered and used for force measurements. Force detection is performed using back-focal-plane interferometry on a quadrant photodiodes, calibrated online using power spectrum analysis [1]. Apart from online calibration, the NanoTracker™ software allows full control of all instrument hardware, such as trap/sample positioning or trap stiffness adjustments. Moreover, a range of data acquisition and analysis options are built in.

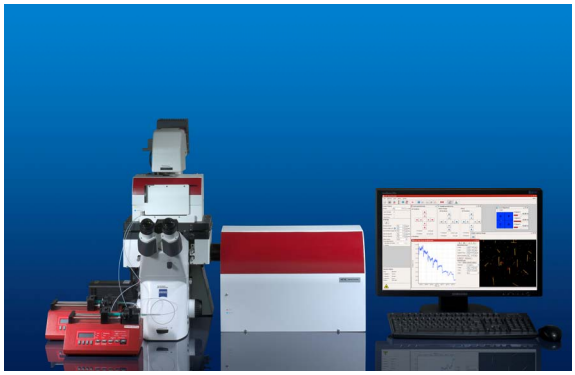
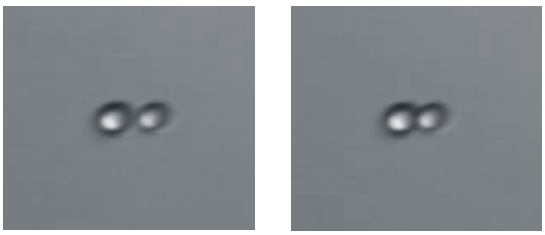


Fig. 1 JPK NanoTracker™ optical tweezers platform.



colloids separated colloids approached

Fig. 2 Microscope images of two differently sized microspheres micro-manipulated in the NanoTracker instrument.

Experiment 1: protein-ligand binding

To probe the strength of protein-ligand binding under physiological buffer conditions, optical tweezers can be used to directly and controllably bring the two species together through 'handle' colloids. After a short interaction time, the colloids are moved apart until rupture of the molecular bond occurs (Figure 2).

The measurements were performed on the following samples: polystyrene colloids coated with Protein A (ProtA) and Immunoglobulin G (IgG) ligands, respectively, similar to previously published results [2]. To allow optical discrimination, the colloids were 2.0 µm and 2.3 µm.
Using the dual-beam optical tweezers supplied by the NanoTracker™, two differently coated colloids were then brought into contact and subsequently pulled apart, to test for protein-ligand interaction. Rupture forces between individual ProtA and IgG antibodies from three different organisms were thus measured and compared.

All measurements have been performed at a trap displacement rate of 500 nm/s, which corresponded with the trap stiffnesses used to loading rates on the order 15-25 pN/s. The trap stiffness for both traps was chosen on the order of 80 pN/µm.

The red traces are the raw force data (with the 'forward' force curve subtracted and therefore freed from the effect of the two colloids pushing against each other). The data shown here are recorded on one of the traps; through Newton's second law the other trap obviously generates the same signal in the opposite direction. The orange curves show traces where the distance channel, which is

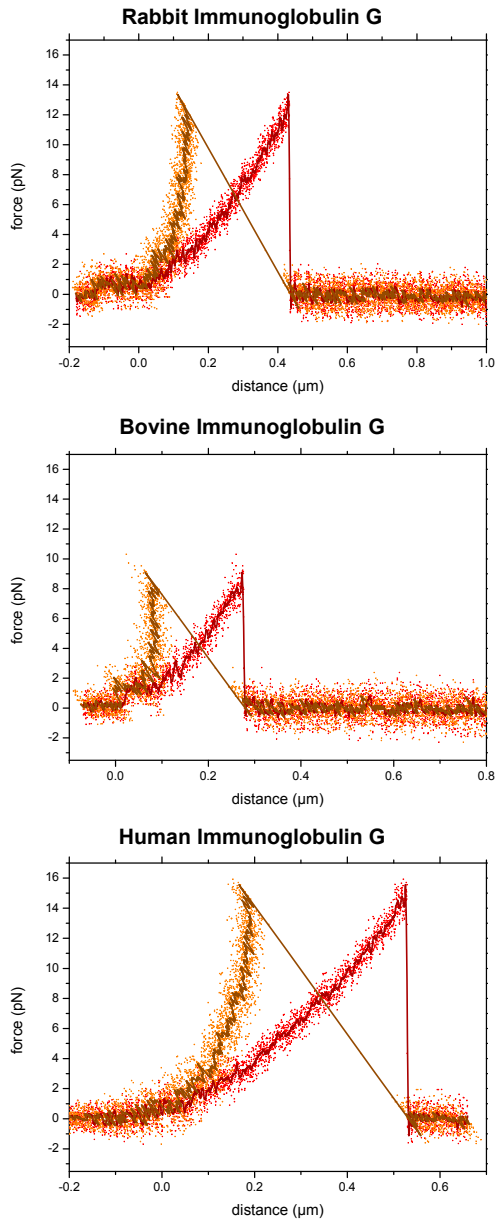


Fig. 3 Typical rupture curves of the three different IgG species.

the distance between the optical traps, was corrected for the displacement of the colloids out of their traps using the measured forces and stiffnesses through the following equation:

$$x_{corr}(t) = x_{meas}(t) - F_1(t)/\kappa_1 + F_2(t)/\kappa_2,$$

with x(t) being the distance as a function of time and F(t) and κ the force signals and trap stiffnesses of the traps indicated by the suffix.
This distance correction has been successfully crosschecked by comparing the values with those found by a particle-tracking algorithm.

Particularly the bovine IgG species stands out through a significantly lower average rupture force.
Also, in some cases multiples of the rupture forces have been seen, which is probably caused by binding events through multiple protein/ligand complexes. In most of these cases, rupture actually turned out to occur in two or more steps, corroborating this conclusion. These events have been left out of the analysis.

This experimental approach can be extended to include rupture measurements at different pulling rates – so called dynamic force spectroscopy – which allows for a detailed understanding of the binding energy landscape of protein-ligand pairs. In addition, experiments using a force clamp enable one to further probe such interactions in a quantitative manner.

Experiment 2: colloid repulsion

Optical tweezers measurements were performed on plain, uncoated 4.75 µm SiO_2 (silica) colloids and a milliQ water sample (pH 5.8) with 400 µM KCl, to demonstrate the hard-sphere repulsion of these colloids under such buffer conditions, as has been reported elsewhere [3].

Using the dual-beam optical tweezers supplied by the NanoTracker™, the two colloids were brought into contact and pulled apart, to probe for interaction.

When pushing the colloids into each other, a repulsive force is recorded and the colloids move out of their respective trap centers. Up to this point, the approach and retract traces are flat and indistinguishable, as is to be expected for these uncoated particles.

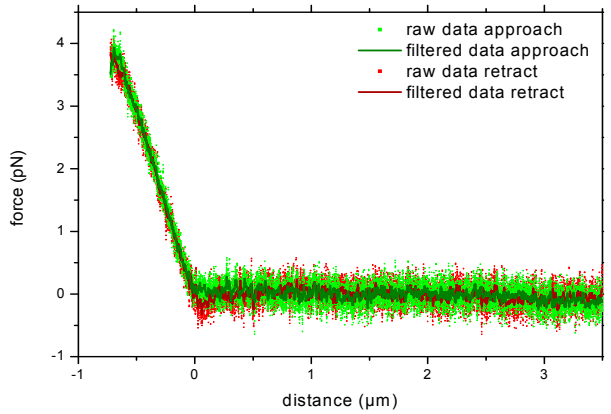


Fig. 4 Typical approach and retract curve of two trapped 4.75 µm SiO_2 colloids.

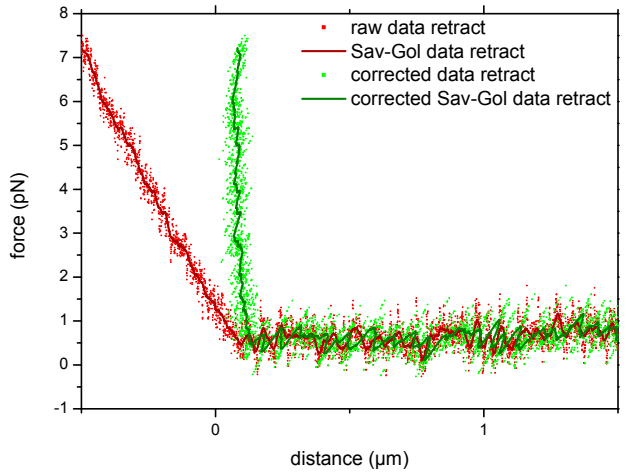


Fig. 5 A retract curve with and without correction for bead displacement from their trap centers under increasing force, showing the expected hard-sphere repulsion.

The repulsive interaction between the hard spheres in the above graphs is artificially softened by not taking into account the finite stiffness of the traps. The distance channel used is the actual distance between the optical traps, which can be corrected for the displacement of the colloids out of their traps to get the inter-colloidal distance as described before. The inset graph shows both original (red) and corrected (green) data for two colloids manipulated with traps of 26.6 and 23.3 pN/µm stiffness, respectively. The corrected trace shows the expected colloid-colloid repulsion.

These measurements prepare the ground for further experimental investigations of the intrinsic repulsion and/or attraction forces for colloids in e.g. various different buffer conditions.

Conclusion

In conclusion, the NanoTracker™ proves to be directly applicable to colloid-colloid interaction measurements for biophysical and materials science research studies. The configurationally freedom provided by the NanoTracker™ to set up the force spectroscopy experiment allows for many different assays, including force clamp or force ramp experiments for interactions between colloids, or interactions between a colloid and (e.g. chemically modified) surfaces.

Acknowledgments

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