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# Dynamic Force Measurements of Single Molecule Ligand-Receptor Interactions using Frequency Modulation Atomic Force Microscopy

M. Higgins[*], C.K. Riener[**], T. Uchihashi[*], J.E. Sader[***], R.A. McKendry[**], S.P. Jarvis[*]

[*]Centre for Research on Adaptive Nanostructures and Nanodevices, University of Dublin, Trinity College, Dublin 2, Ireland.
[**]London Centre for Nanotechnology, University College London, WC1E 6JJ, U.K.
[***]Department of Mathematics and Statistics, University of Melbourne, Victoria 3010, Australia

The direct measurement of forces between individual receptor-ligand complexes is an emerging new field. With the Atomic Force Microscope (AFM) it has been possible to probe the forces between a range of biological molecules, including complementary oligo-nucleotides, proteins and carbohydrate interactions. To date, the force required to rupture these single ligand-receptor interactions is commonly detected via static mode deflection, and is typically in the picoNewton range. It has been shown that the measured forces vary with the loading rate, and extrapolation to zero loading rate correlates to the thermal dissociation rate (off-rate) [1]. Here, we introduce the first application of dynamic Frequency Modulated AFM (FM-AFM) to investigate the receptor-ligand interactions at high loading rates [2]. The biotin-avidin interaction is exceptionally strong, $K_D = 10^{-15}$ M, and thus serves as a benchmark for molecular recognition studies. AFM tips were covalently functionalised with biotin using a poly(ethylene glycol) (PEG) crosslinker and avidin was electrostatically immobilised on a mica surface (Fig. 1). All measurements were performed in physiological buffer solution [3]. Operating in liquid required the implementation of Magnetically Activated Dynamic mode (MAD mode) [4], whereby a small magnetic particle is attached to the end of a cantilever and an external magnetic field applied via a current carrying coil. Further, by operating the approach in a 'fly-fishing mode' direct tip-substrate contact was usually avoided so minimising non-specific interactions.

Frequency shift curves revealed a positive frequency shift in the form of an adhesion peak that corresponded to an unbinding event. The adhesion peak represented the stretching of the PEG linker and subsequent breaking of the bond (Fig. 2). Using the method of Sader and Jarvis [5] to convert frequency shift into force prior to the bond rupture gave unbinding force values of the order of 300 pN. This is significantly higher than those given in previous studies [3, 6] due to the use of higher loading rates, which in our case correspond to the resonant frequency of the lever (typically of the order of 20 kHz). The significance of these high loading rates will be discussed along with the effect of changing the PEG linker length and oscillation amplitude in these measurements.

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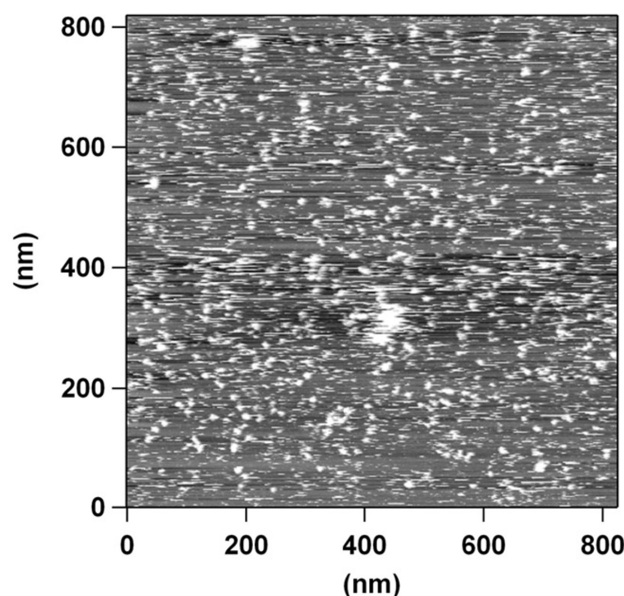


Fig. 1. AFM intermittent contact mode image in phosphate buffer of avidin molecules adsorbed onto cleaved mica surface. A significant number of individual molecules and aggregates are observed per μm$^2$ for a 0.5mg/ml avidin solution.

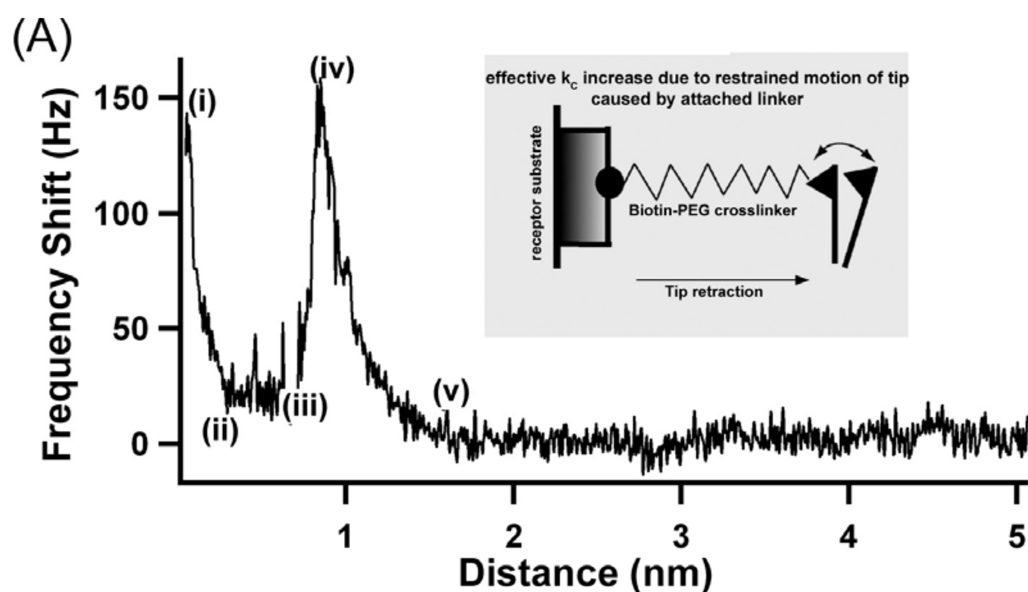


Fig. 2. Frequency shift versus distance curve (retraction curve) showing a single unbinding event for the biotin-avidin interaction. The biotin functionalized cantilever tip interacts with the surface between point (i) and (ii) during which bond formation can occur. Upon retraction of the tip from the surface, the PEG crosslinker is stretched between point (iii) and (iv). Eventually, the elastic restoring force of the PEG linker exceeds the covalent force interaction between the biotin-avidin and the bond breaks at point (iv).

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