Supporting Information for "Fundamental High-Speed Limits in Single-Molecule, Single-Cell and Nanoscale Force Spectroscopies"

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Time-varying forces when the displacement acts on the cantilever support

Figure S1. Dependence of the reconstructed time-varying force as a function of the frequency at which the distance is modulated for different environments. (a) The cantilever support is displaced at ϖ = 5, 10, 20 (*Q*= 100). (b) The sample support is displaced at ϖ = 100 (*Q*=100). The dashed line shows the model force. (c) The sample support is displaced at $\varpi = 5, 10, 20$ ($Q=1$). (d) The sample support is displaced at $\varpi = 100$ ($Q=1$). The dashed line shows the interaction force (model). The insets show the force in the vicinity of the snap-off region. Simulation parameters $a_0=0.2$ nm $E_{\text{eff}}=1$ GPa, $H=0.5$ eV, $f_0=150$ kHz, $k=5$ N/m, $Q=$ 100, $R = 5$ nm, $A_m = 15$ nm, $z_c = 10$ nm.

Unbinding forces for a triangular tip-sample displacement

Triangular waves involve several Fourier components but they provide constant approach and retraction speeds. They are very commonly used in single molecule force spectroscopy.

Figure S2. (a) Time-varying forces for different distance modulation frequencies in single-molecule force spectroscopy using a triangular modulation. We consider the first 26 harmonics. (b) Reconstructed force curve for f_m =0.5 kHz (ϖ =100) and force curve of the model (c) Dependence of the unbinding and peak forces on the frequency ratio. Parameters of the interaction force and cantilever dynamics, $F_{umb} = 50 \text{ pN}, \beta = 0.8 \text{ nm}^{-1}$, $D_0 = 10$ nm, $a_0 = 0.4$ nm, $E_{\text{eff}} = 1$ MPa, for $f_0 = 150$ kHz, $k = 0.7$ N/m, $Q=1$, $R = 5$ nm, $A_m = 15$ nm, $z_c = 10$ nm.

Figure S3. (a) Model and reconstructed Young modulus map spanning an eight order of magnitude range. Simulations for both sample support and cantilever base displacement. (b) Relative error $(E_{\text{eff}} - E_{\text{meas}}) / E_{\text{eff}}$ The vertical dashed line separates experiments that should be performed in liquid from those performed in air. The dashed line separates the simulations in liquid $(Q = 1)$ and air $(Q = 100)$. In general the theoretical error is in the 1-10 % range. The error in the Young modulus comes from the the transient component of the deflection and from the uncertainty in the determination

of the indentation. For stiff materials the indentation should be corrected using the intermolecular distance *a0*. In an experiment there are additional sources of incertitude that have not been accounted here. For example, the error in the calibration of the force constant or the instrumental noise (laser, photodetector).