

### Electronic Supplementary Information

#### Bell-Evans Calculations

The Bell-Evans equation<sup>1</sup> is expanded to obtain a similar form to the straight line equation  $y=mx+c$ ,

$$F(r) = \left(\frac{k_B T}{x_\beta}\right) \ln r + \left(\frac{k_B T}{x_\beta}\right) \ln \frac{x_\beta}{k_{off} k_B T}$$

Therefore when  $F(r)$  is plotted against  $\ln r$ , the slope of the best fit line equals  $\left(\frac{k_B T}{x_\beta}\right)$  and the y-intercept equals  $\left(\frac{k_B T}{x_\beta}\right) \ln \frac{x_\beta}{k_{off} k_B T}$ .

All values are converted to standard units, N and N/s. The width of the energy barrier,  $x_\beta$  is determined by the equation,

$$x_\beta = \frac{k_B T}{m}$$

Where  $m$  is the slope of the line.  $k_{off}$  was determined by rearranging the equation.

$$k_{off} = \frac{x_\beta}{k_B T e^{c x_\beta / k_B T}}$$

Where  $c$  is the y-intercept.

Once  $k_{off}$  is determined, we applied the following equation to determine the height of the energy barrier,  $\Delta G^1$ .

$$k_{off}(F) = \left(\frac{k_B T}{h}\right) e^{-\frac{\Delta G - x_\beta F}{k_B T}}$$

Rearranged,  $\Delta G$  can be determined as

$$-\Delta G = k_B T \ln \frac{k_0 h}{k_B T}$$

Where  $h$  is Planck's constant. For our purposes, we used  $k_B T$  as units for  $\Delta G$ .

#### Error Analysis

For the Bell-Evans model, errors were determined by the square root of the sum of the squares of the standard error<sup>2</sup>. For example, the standard error of  $x_\beta$  was calculated as follows:

Since  $x_\beta$  is a function of the slope,  $m$ ,

$$x_\beta = \frac{k_B T}{m}$$

With  $m$  being the slope of the line using a least squares fit of the data points, the standard error of  $x_\beta$  is given by the equation,

$$\frac{\delta x_\beta}{x_\beta} = \sqrt{\left(\frac{\delta m}{m}\right)^2 + \left(\frac{\delta T}{T}\right)^2}$$

The standard error of the slope,  $m$ , was calculated using the graphing software. The standard error of the temperature was negligible, but still used and estimated as 1 K.

The standard error for all parameters using the Dudko-Hummer-Szabo model<sup>3</sup> were calculated using Origin v 9.0.

Hutter's thermal noise method<sup>4</sup> of cantilever spring constant calculation typically results in a standard error of 10-20%<sup>5</sup>. To

calculate the standard errors for the Friddle-De Yoreo model<sup>6</sup>, a 15% error was estimated for the cantilever spring constant. Since  $\Delta G$  is a function of the cantilever spring constant and  $f_{eq}$ , the standard error in  $\Delta G$  was estimated as

$$\delta \Delta G = \Delta G \sqrt{\left(\frac{\delta f_{eq}}{f_{eq}}\right)^2 + 0.15^2}$$

The remainder of standard errors were calculated in a manner similar to the Bell-Evans model.

#### Supplementary Information References

- 1 E. Evans, and K. Ritchie, *Biophys J.* 1997, **72**, 1541.
- 2 J. Taylor J. An Introduction to Error Analysis, 1997, Sausalito: University Science Books.
- 3 O. Dudko, G. Hummer, and A. Szabo A, *Proc. Nat. Acad. Sci.*, 2008, **105**, 15755.
- 4 J. Hutter, and J. Bechhoefer, *Rev. Sci. Instrum.*, 1993, **64**, 1868.
- 5 JPK Instruments AG. Technical Summary: Calibration of atomic-force microscope tips.
- 6 R. Friddle, A. Noy, and J. De Yoreo, *Proc. Nat. Acad. Sci. USA.*, 2012, **109**, 13573.