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# AFM protein-protein interactions within the EcoR124I molecular motor

**Aneta E. Sikora,[a] James R. Smith,*[b] Sheelagh A. Campbell[b] and Keith Firman[a]**

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## Bell-Evans Model Parameters

The dissociation rate under the applied external force $k_{diss}(f)$ can be described by Eq. S-1:

$$k_{diss}(f) = k_{diss}(0)\exp\left[\frac{f\,x_{diss}}{k_B T}\right] \qquad \text{(S-1)}$$

where $k_{diss}(0)$ is the natural dissociation and association rate, $f$ is the applied force, $x_{diss}$ is the distance of the energy barrier to rupture, $k_BT$ is thermal energy (4.1 pN nm).

$k_{diss}(0)$ of the bond is characteristic for any particular pair of molecules and varies between $10^{-6}$ and $10^{1}$ $s^{-1}$.[1,2] $\tau(0)$, in the absence of an external force, for ligand-receptor complexes varies between $10^{-4}$ s and a few min and can be measured using Eq. S-2:

$$\tau(0) = \frac{1}{k_{diss}(0)} \qquad \text{(S-2)}$$

The measured forces necessary to rupture the bond between molecules in the complex increase over time during a pulling experiment with increasing loading rate $R_f$,[3] defined as the product of the effective spring constant $k_s$ (stiffness) of the system (the elasticity of both cantilever $k_c$ and bound molecules $k_b$)[4] and the velocity $\upsilon$ in which the force is applied (in practice, the retraction speed of the AFM tip) (Eq. S-3):

$$R_f = k_s\upsilon \qquad \text{(S-3)}$$

The rupture force $f^*$ is related to $R_f$ (Eq. S-4):

$$f^* = \frac{k_BT}{x_{diss}}\ln\left(\frac{x_{diss}}{k_{diss}(0).k_BT}\right) + \frac{k_BT}{x_{diss}}\ln R_f \qquad \text{(S-4)}$$

[a] *School of Biological Sciences, University of Portsmouth, King Henry Building, King Henry I Street, Portsmouth PO1 2DY, UK.*
[b] *School of Pharmacy and Biomedical Sciences, University of Portsmouth, St Michael's Building, White Swan Road, Portsmouth PO1 2DT, UK. E-mail: james.smith@port.ac.uk; Fax: +44 (0)23 9284 3565; Tel: +44 (0)23 9284 2556*

A plot of $f^*$ vs. $\ln(R_f)$ enables $x_{diss}$ and $k_{diss}(0)$ to be calculated from the gradient and intercept, respectively (Eqs. S-5–S-8):[5]

$$a = \frac{k_BT}{x_{diss}}\ln\left(\frac{x_{diss}}{k_{diss}(0).k_BT}\right) \qquad \text{(S-5)}$$

$$b = \frac{k_BT}{x_{diss}} \qquad \text{(S-6)}$$

$$k_{diss}(0) = \frac{1}{b}e^{-\frac{a}{b}} \qquad \text{(S-7)}$$

$$x_{diss} = \frac{k_BT}{b} \qquad \text{(S-8)}$$

According to the theoretical predictions,[5] $f$ rises linearly with the $R_f$ on a half-logarithmic scale, which is characteristic for a single-energy barrier in the thermally activated regime.[6] A change in slope is observed for each new energy barrier. For example, avidin-biotin and streptavidin-biotin complexes have shown multiple energy barriers in the dissociation pathway.[6]

The Bell-Evans model parameters $k_{diss}(0)$ and $x_{diss}$ were determined from linear regression of a $f^*$ vs. $\ln(R_f)$ plot, the intercept $a$ (2.29) and slope $b$ (3.04) (Fig. 8, main text) being obtained from Eqs. S-9 and S-10:

$$a = \frac{\sum y_i}{n} - b\frac{\sum x_i}{n} \qquad \text{(S-9)}$$

$$b = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{n\sum x_i^2 - (\sum x_i)^2} \qquad \text{(S-10)}$$

where $x_i$ and $y_i$=$R_f$ and $f^*$, respectively, and $n$=number of measurements. The $\Delta a$ and $\Delta b$ values correspond to the standard errors in $a$ and $b$ obtained from the linear regression fit of $f^*$ vs. $\ln(R_f)$. $\Delta a$ (2.47) and $\Delta b$ (0.37) were calculated using Eqs. S-11 and S-12:

$$\Delta a = \sqrt{\frac{1}{n}\Delta b^2 \sum x_i^2} \quad \text{(S-11)}$$

$$\Delta b = \sqrt{\frac{n\left[\sum y_i^2 - b\sum x_i y_i - a\sum y_i\ \right]}{(n-2)\left[n\sum x_i^2 - (\sum x_i)^2\right]}} \quad \text{(S-12)}$$

The standard error in $\Delta x_{diss}$ (0.17 nm) was calculated using Eq. S-13:

$$\left(\frac{\Delta x_{diss}}{x_{diss}}\right)^2 = \left(\frac{\Delta b}{b}\right)^2 \quad \text{(S-13)}$$

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