Electronic Supplementary Material

AFM protein-protein interactions within the EcoR124I molecular motor

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Bell-Evans Model Parameters

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

\[
10 \ k_{\text{dis}}(f) = k_{\text{dis}}(0) \exp \left( \frac{x_{\text{dis}}}{k_B T} \right)
\]

(S-1)

where \( k_{\text{dis}}(0) \) is the natural dissociation and association rate, \( f \) is the applied force, \( x_{\text{dis}} \) is the distance of the energy barrier to rupture, \( k_B T \) is thermal energy (4.1 pN nm).

\( k_{\text{dis}}(0) \) of the bond is characteristic for any particular pair of molecules and varies between \( 10^{-6} \) and \( 10^1 \) s\(^{-1}\). \( \tau(0) \), in the absence of an external force, for ligand-receptor complexes varies between \( 10^{-5} \) s and a few min and can be measured using Eq. S-2:

\[
20 \ \tau(0) = \frac{1}{k_{\text{dis}}(0)}
\]

(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 \( R_f \), defined as the product of the effective spring constant \( k_s \) (stiffness) of the system (the elasticity of both cantilever \( k_s \) and bound molecules \( k_b \)) and the velocity \( v \) in which the force is applied (in practice, the retraction speed of the AFM tip) (Eq. S-3):

\[
30 \ \frac{R_f}{k_s v}
\]

(S-3)

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

\[
40 \ f^* = \frac{k_BT}{x_{\text{dis}}} \ln \left( \frac{x_{\text{dis}}}{k_{\text{dis}}(0) k_B T} \right) + \frac{k_BT}{x_{\text{dis}}} \ln R_f
\]

(S-4)

According to the theoretical predictions, \( f^* \) rises linearly with the \( R_f \) on a half-logarithmic scale, which is characteristic for a single-energy barrier in the thermally activated regime. 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.

The Bell-Evans model parameters \( k_{\text{dis}}(0) \) and \( x_{\text{dis}} \) were determined from linear regression of \( f^* \) 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:

\[
50 \ a = \frac{\Sigma y_i}{n} - b \frac{\Sigma x_i}{n}
\]

(S-9)

\[
60 \ b = \frac{n \Sigma x_i y_i - \Sigma x_i \Sigma y_i}{n \Sigma x_i^2 - (\Sigma x_i)^2}
\]

(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:

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

$$\left(\frac{\Delta x_{\text{dist}}}{x_{\text{dist}}}\right)^2 = \left(\frac{\Delta b}{b}\right)^2$$  \hspace{2cm} (S-13)