



# Multiple barriers in forced rupture of protein complexes

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Curvatures in the most probable rupture force ( $f^*$ ) versus log-loading rate ( $\log\{r_f\}$ ) observed in dynamic force spectroscopy (DFS) on biomolecular complexes are interpreted using a one-dimensional free energy profile with multiple barriers or a single barrier with force-dependent transition state.

Here, we provide a criterion to select one scenario over another. If the rupture dynamics occurs by crossing a single barrier in a physical free energy profile describing unbinding, the exponent  $\nu$ , from  $(1 - f^*/f_c)^{1/\nu} \sim (\log r_f)$  with  $f_c$  being a critical force in the absence of force, is restricted to  $0.5 \leq \nu \leq 1$ . For biotin-ligand complexes and leukocyte-associated antigen-1 bound to intercellular adhesion molecules, which display large curvature in the DFS data, fits to experimental data yield  $\nu < 0.5$ , suggesting that ligand unbinding is associated with multiple-barrier crossing.

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