A

Ligand-receptor	F_u (pN)	ΔH (kcal/mol)	ΔG (kcal/mol)	r_{eff} (Å)
Avidin-biotin	160 ± 20	−21.5	−20.4	9.3
Avidin-iminobiotin	85 ± 10	−11.6	−14.3	9.5
Streptavidin-biotin	257 ± 25	−32.0	−18.3	9.3
Avidin-desthiobiotin	94 ± 10	−13.5	−16.5	10
Streptavidin-iminobiotin	135 ± 15	NA	−12.2	

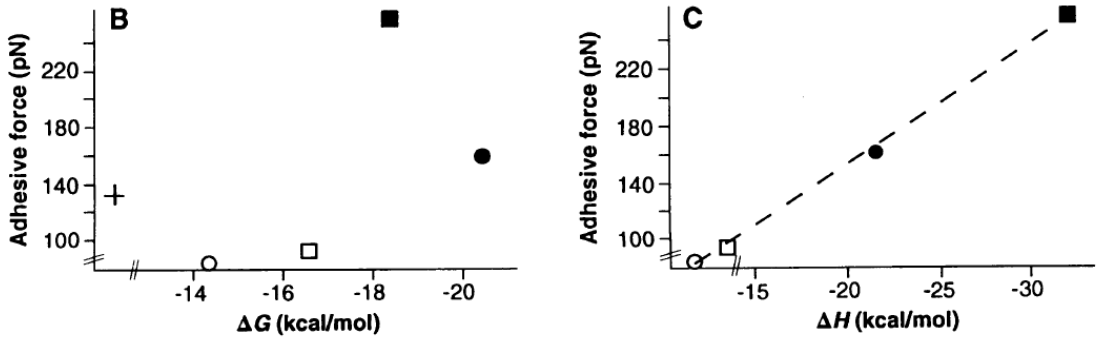

Fig. 1. (**A**) Tabulation of ligand-receptor unbinding forces and the corresponding thermodynamic values. Force measurements were carried out with a scanned-stylus–type AFM (*14*). Thermodynamic values were taken from Green (*5*), except those for streptavidin-biotin, taken from Weber *et al*. (*15*). Calorimetric measurements for avidin-desthiobiotin were performed at 25°C in a MicroCal Omega titration calorimeter. Forty 2-μl injections of ligand solution were titrated at 4-min intervals into 60 μM solutions of receptor. (**B**) Plot of unbinding force versus free energy for avidin-biotin (●), avidin-iminobiotin (○), streptavidin-biotin (■), avidin-desthiobiotin (□), and streptavidin-iminobiotin (+). (**C**) Plot of unbinding force versus enthalpy.

5. N. M. Green, *Adv. Prot. Chem.* **29**, 85 (1975).

14. There is as much as 20% variability among the different methods used in the calibration of cantilevers [V. T. Moy, E.-L. Florin, H. E. Gaub, unpublished results; J. P. Cleveland, S. Manne, D. Bocek, P. K. Hansma, *Rev. Sci. Instrum.* **64**, 403 (1993); J. L. Hutter and J. Bechhoefer, *ibid.*, p. 1868; T. J. Senden and W. A. Ducker, *Langmuir* **10**, 1003 (1994)]. The values reported here are based on cantilevers calibrated with a macroscopic reference lever in a method that does not depend on the high-frequency response of the cantilever.

15. P. C. Weber, J. J. Wendololoski, M. W. Pantoliano, F. R. Salemme, *J. Am. Chem. Soc.* **114**, 3197 (1992).