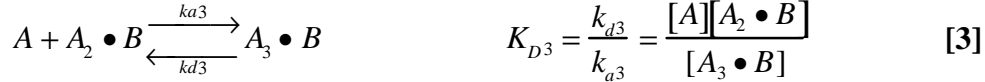
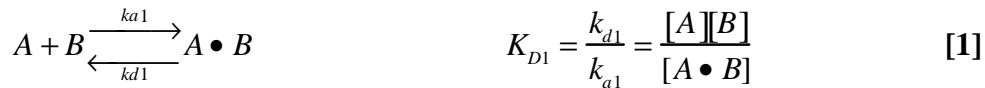


Supporting information for Bennett *et al.* (2002) *Proc. Natl. Acad. Sci. USA*,
10.1073/pnas.182393899.

Equilibrium Analysis of Trivalent Analyte Data

The bivalent analyte model as previously described (1, 2) derives K_{D1} and K_{D2} for the sequential binding reactions that produce single- and double-liganded analyte molecules. We extended this approach to develop a trivalent analyte model that accounts for the possibility of triple-liganded molecules by deriving the additional equilibrium constant K_{D3} . The sequential reactions are as follows, in which A is the ligand and B is the trivalent analyte:



The total ligand concentration, A_{tot} , is given by the expression

$$[A_{tot}] = [A] + [A \bullet B] + 2[A_2 \bullet B] + 3[A_3 \bullet B]. \quad [4]$$

The equilibrium response for a trivalent analyte is proportional to the sum of the concentrations of single-, double-, and triple-liganded analyte,

$$R_{eq} \propto [A \bullet B] + [A_2 \bullet B] + [A_3 \bullet B], \quad [5]$$

which can be rearranged by substituting Eqs. 1-4:

$$R_{eq} \propto \frac{[A_{tot}]}{3} + \left(\frac{2[B]}{3K_{D1}} - \frac{1}{3} \right) [A] + \left(\frac{[B]}{3K_{D1}K_{D2}} \right) [A]^2 \quad [6]$$

The concentration of free ligand, [A], is derived by solving a cubic equation based on Eqs. 1-4. Substituting [A] into Eq. 6 yields the trivalent analyte equation:

$$\begin{aligned} R_{eq} = & \text{Scale} * (A_{tot} + ((-1/3 + (2*B)/(3*KD1)) * (-4*KD3 + (2*2^{1/3}*KD3 * \\ & (-9*B*KD2 - 9*KD1*KD2 + 4*B*KD3)) / \\ & (2*B^3*(27*KD2 - 8*KD3)*KD3^2 + 27*B^2*KD1*KD2*KD3*(2*KD3 + 9*A_{tot}) + \\ & \text{sqrt}(B^3*KD3^2*(4*KD3*(9*B*KD2 + 9*KD1*KD2 - 4*B*KD3)^3 + \\ & B*(2*B*(27*KD2 - 8*KD3)*KD3 + 27*KD1*KD2*(2*KD3 + 9*A_{tot}))^2)))^{1/3} + \\ & (2^{2/3}) * (2*B^3*(27*KD2 - 8*KD3)*KD3^2 + 27*B^2*KD1*KD2*KD3*(2*KD3 + 9*A_{tot}) + \\ & \text{sqrt}(B^3*KD3^2*(4*KD3*(9*B*KD2 + 9*KD1*KD2 - 4*B*KD3)^3 + \\ & B*(2*B*(27*KD2 - 8*KD3)*KD3 + 27*KD1*KD2*(2*KD3 + 9*A_{tot}))^2)))^{1/3}) / \\ & B)) / 6 + \\ & (B * (-4*KD3 + (2*2^{1/3}*KD3 * (-9*B*KD2 - 9*KD1*KD2 + 4*B*KD3)) / \\ & (2*B^3*(27*KD2 - 8*KD3)*KD3^2 + 27*B^2*KD1*KD2*KD3*(2*KD3 + 9*A_{tot}) + \\ & \text{sqrt}(B^3*KD3^2*(4*KD3*(9*B*KD2 + 9*KD1*KD2 - 4*B*KD3)^3 + \\ & B*(2*B*(27*KD2 - 8*KD3)*KD3 + 27*KD1*KD2*(2*KD3 + 9*A_{tot}))^2)))^{1/3} + \\ & (2^{2/3}) * (2*B^3*(27*KD2 - 8*KD3)*KD3^2 + 27*B^2*KD1*KD2*KD3*(2*KD3 + 9*A_{tot}) + \\ & \text{sqrt}(B^3*KD3^2*(4*KD3*(9*B*KD2 + 9*KD1*KD2 - 4*B*KD3)^3 + \\ & B*(2*B*(27*KD2 - 8*KD3)*KD3 + 27*KD1*KD2*(2*KD3 + 9*A_{tot}))^2)))^{1/3}) \\ & / B)^2) / (324 * KD1 * KD2)) / 3 \end{aligned}$$

R_{eq} = measured equilibrium response in resonance units (RU).

A_{tot} = total concentration of ligand (M) (proportional to maximum response, R_{max}).

B = concentration of free analyte (M).

Scale = conversion factor (RU/M) = $100 \times M_r(\text{analyte})$ or $\sim 2.6 \times 10^7$ for HD exon 1.

KD1, KD2, KD3 = affinity constants.

1. West, A. P. & Bjorkman, P. J. (2000) *Biochemistry* **39**, 9698–9708.
2. West, A. P., Giannetti, A. M., Herr, A. B., Bennett, M. J., Nangiana, J. S., Pierce, J. R., Weiner, L. P., Snow, P. M. & Bjorkman, P. J. (2001) *J. Mol. Biol.* **313**, 385–397.