Simple Binding Curve Mathematics:

Consider the equilibrium among free protein *P*, free DNA *D*, and the bound complex *P•D*, with equilibrium dissociation constant K_d:

$$P\bullet D \overset{K_d}{\rightleftharpoons} P + D \quad \text{, where } K_d = \frac{[P][D]}{[P\bullet D]}.$$

To measure K_d we want to express it in terms of the following known or measurable quantities:

Total (input) active protein concentration: $P_T = [P] + [P\bullet D]$

Total (input) active DNA concentration: $D_T = [D] + [P\bullet D]$

Observed bound fraction of DNA: $\Theta = \dfrac{[P\bullet D]}{D_T}$

So we have:

$$K_d = \frac{[P][D]}{[P\bullet D]} = \frac{\left(P_T - [P\bullet D]\right)\left(D_T - [P\bullet D]\right)}{[P\bullet D]}$$

Solving for [P•D] we get:

$$[P\bullet D]^2 - D_T[P\bullet D] - P_T[P\bullet D] + P_T D_T = [P\bullet D] \times K_d$$

$$[P\bullet D]^2 - [P\bullet D]\left(P_T + D_T + K_d\right) + P_T D_T = 0$$

$$[P\bullet D] = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} = \frac{\left(P_T + D_T + K_d\right) \pm \sqrt{\left(P_T + D_T + K_d\right)^2 - 4P_T D_T}}{2}$$

The quadratic equation must have one physically realizable root. If the complex is infinitely loose ($K_d = \infty$), then the expression $\left(P_T + D_T + K_d\right)$ tends to ∞ but [P•D] must be zero, so we must choose the minus sign. For $K_d = 0$, an infinitely tight complex, the expression inside the square root reduces to $\left|P_T - D_T\right|$, so [P•D] reduces to $\min(P_T, D_T)$ as it must. Establishing the sign allows us to provide an expression for the fraction bound:

$$\Theta = \frac{[P\bullet D]}{D_T} = \frac{\left(P_T + D_T + K_d\right) - \sqrt{\left(P_T + D_T + K_d\right)^2 - 4P_T D_T}}{2D_T}$$

It is typical and desirable to perform experiments under conditions where D_T is negligible, with the definition of negligible being $D_T \ll K_d$. This is why we often use radiolabel! Under these conditions we Taylor expand the square root:

$$\sqrt{A^2 - \varepsilon} \approx A - \frac{1}{2}\left(A^2 - \varepsilon\right)^{-1/2}\Big|_{\varepsilon=0} \varepsilon = A - \frac{\varepsilon}{2A}$$

$$\sqrt{\left(P_T + D_T + K_d\right)^2 - 4P_T D_T} \approx P_T + D_T + K_d - \frac{2P_T D_T}{P_T + D_T + K_d}$$

Substituting into the equation for the fraction bound, we have

$$\Theta = \frac{[P \bullet D]}{D_T} \approx \frac{2P_T D_T / \left(P_T + D_T + K_d\right)}{2D_T} = \frac{P_T}{P_T + D_T + K_d} \approx \frac{P_T}{P_T + K_d}$$

which is the desired result, the classic expression for a hyperbolic binding curve.

We could have short-circuited some of the math by assuming at the outset that the amount of DNA is negligible, such that the concentration of free protein is always approximately equal to the total protein concentration. This would have given us:

$$K_d = \frac{[P][D]}{[P \bullet D]} \approx \frac{P_T \left(D_T - [P \bullet D]\right)}{[P \bullet D]} = \frac{P_T D_T}{[P \bullet D]} - P_T = \frac{P_T}{\Theta} - P_T$$

which upon rearrangement is the same hyperbolic binding curve:

$$\boxed{\Theta = \frac{[P \bullet D]}{D_T} = \frac{P_T}{P_T + K_d}}$$

The advantage of going through all the math is that we have an understanding of what "negligible" means operationally: the total DNA concentration must be much less than the dissociation constant. This can be challenging if the protein binds its target with high affinity, because it requires very low DNA concentration and hence very sensitive detection or very large volumes.

So, the way we determine K_d is by measuring the fraction bound as a function of protein concentration, and curve fitting to obtain K_d. The physical meaning of K_d is that it is the protein concentration at which half of the DNA will be bound.