

Appendix

Scatchard analysis

For the reaction: $\text{Cell} + \text{Ab} \rightleftharpoons \text{Ab-Cell}$

$$K_a = [\text{Ab-Cell}] / ([\text{Ab}][\text{Cell}])$$

Or

$$[\text{Ab-Cell}] / [\text{Ab}] = K_a [\text{Cell}]$$

in addition:

$$[\text{Cell}]_{\text{total}} = [\text{Cell}] + [\text{Ab-Cell}]$$

So

$$[\text{Ab-Cell}] / [\text{Ab}] = K_a [\text{Cell}]_{\text{total}} - K_a [\text{Ab-Cell}]$$

If this is differentiated as a function of $[\text{Ab-Cell}]$:

$$[\text{Ab-Cell}] / [\text{Ab}]'([\text{Ab-Cell}]) = -K_a$$

Because the volume and the amount of cells are constant

Furthermore, the extrapolation of the curve to $[\text{Ab-Cell}] / [\text{Ab}] = 0$ gives a value for the amount of bindingsites.

Dissociation

Reaction: $\text{Ab}_{\text{bound}} \rightarrow \text{Ab}_{\text{Free}}$ (first order kinetics)

$$d[\text{Ab}_{\text{bound}}] / dt = -k [\text{Ab}_{\text{bound}}]$$

$$d[\text{Ab}_{\text{bound}}] / [\text{Ab}_{\text{bound}}] = -k dt$$

Integration gives:

$$\ln([\text{Ab}_{\text{bound}}]_0 / [\text{Ab}_{\text{bound}}]) = k t$$

Half-life is calculated from

$$\ln(1/\{1/2\}) = k t_{1/2}$$

$$t_{1/2} = \ln(2) / k = 0.693 / k$$

Lindmo

Reaction: $\text{Cell} + \text{Ab} \rightleftharpoons \text{Ab-Cell}$

$$K_a = [\text{Ab-Cell}] / ([\text{Ab}][\text{Cell}])$$

$$[\text{Ab}_{\text{Total}}] = [\text{Ab}] + [\text{Ab-Cell}]$$

so...

$$K_a = [\text{Ab-Cell}] / \{([\text{Ab}_{\text{Total}}] - [\text{Ab-Cell}])[\text{Cell}]\}$$

With $r :=$ Active fraction

$$K_a = [\text{Ab-Cell}] / \{r[\text{Ab}_{\text{Total}}] - [\text{Ab-Cell}]\}[\text{Cell}]$$

Or

$$K_a[\text{Cell}](r[\text{Ab}_{\text{Total}}] - [\text{Ab-Cell}]) = [\text{Ab-Cell}]$$

Or

$$K_a[\text{Cell}]r[\text{Ab}_{\text{Total}}] / [\text{Ab-Cell}] - k_a[\text{Cell}] = 1$$

Or

$$[\text{Ab}_{\text{Total}}] / [\text{Ab-Cell}] = 1/r + 1/(k_a[\text{Cell}]r)$$

Thus plotting the left side of the equation against the inverse cell concentration should yield a straight trendline, and the interception with the ordinate equals $1/r$, whereby the immunoreactive fraction can be found.