Binding Assays

• Non-equilibrium assays that separate complexes
  – Filter binding
  – Pull-down
  – Gel shift

• Equilibrium assays
  – Fluorescence
    • Changes in quantum yield
    • Changes in wavelength maxima
    • Changes in anisotropy
  – Protection assays (quantitative footprinting, etc)
Equilibrium Math

$A + B \xrightleftharpoons[^{K_d}]{\text{←}} AB$
Equilibrium Math

\[ A + B \xleftrightarrow{K_d} AB \]  

Knowns
Equilibrium Math

\[ A + B \xrightleftharpoons[K_d]{.} AB \]

Knowns

\[ K_d = \frac{[A][B]}{[AB]} \]
Equilibrium Math

\[ A + B \xrightleftharpoons{K_d} AB \]

**Knowns**

\[
K_d = \frac{[A][B]}{[AB]}
\]

\[
A_T = [A] + [AB]
\]

\[
B_T = [B] + [AB]
\]
Equilibrium Math

\[ A + B \xrightleftharpoons{K_d} AB \]

\[
\begin{align*}
[A] &= A_T - [AB] \\
[B] &= B_T - [AB]
\end{align*}
\]

Knowns

\[ K_d = \frac{[A][B]}{[AB]} \]

\[
\begin{align*}
A_T &= [A] + [AB] \\
B_T &= [B] + [AB]
\end{align*}
\]
Equilibrium Math

\[ A + B \overset{K_d}{\rightleftharpoons} AB \]

**Knowns**

\[ K_d = \frac{[A][B]}{[AB]} \]

\[ A_T = [A] + [AB] \]

\[ B_T = [B] + [AB] \]

\[ K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB]) \]
**Equilibrium Math**

\[ A + B \xrightleftharpoons[K_d]{\leftrightarrow} AB \]

**Knowns**

\[ K_d = \frac{[A][B]}{[AB]} \]

\[ A_T = [A] + [AB] \]

\[ B_T = [B] + [AB] \]

\[ K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB]) \]

\[ K_d x = (A_T - x)(B_T - x) \]
Equilibrium Math

\[ A + B \xrightleftharpoons{K_d} AB \]

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\begin{align*}
[A] &= A_T - [AB] \\
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K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB])
\]

\[
K_d x = (A_T - x)(B_T - x)
\]

Knowns

\[
K_d = \frac{[A][B]}{[AB]}
\]

\[
A_T = [A] + [AB]
\]

\[
B_T = [B] + [AB]
\]

Assume \( B_T >> x \)
Equilibrium Math

\[ A + B \xrightleftharpoons[K_d]{\text{Kd}} AB \]

**Knowns**

\[ K_d = \frac{[A][B]}{[AB]} \]

\[ A_T = [A] + [AB] \]

\[ B_T = [B] + [AB] \]

\[ K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB]) \]

\[ K_d x = (A_T - x)(B_T - x) \]

Assume \( B_T \gg x \)

\[ K_d x \approx (A_T - x) B_T \]
Equilibrium Math

\[ A + B \xleftrightarrow{K_d} AB \]

**Knowns**

\[ K_d = \frac{[A][B]}{[AB]} \]

\[ A_T = [A] + [AB] \]

\[ B_T = [B] + [AB] \]

\[
\begin{align*}
[A] &= A_T - [AB] \\
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\[
K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB])
\]

\[
K_d x = (A_T - x)(B_T - x)
\]

**Assume** \( B_T \gg x \)

\[
K_d x \approx (A_T - x)B_T
\]

\[
(B_T + K_d) x \approx A_T B_T
\]
Equilibrium Math

\[
A + B \xrightleftharpoons{K_d} AB
\]

\[
[A] = A_T - [AB]
\]

\[
[B] = B_T - [AB]
\]

\[
K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB])
\]

\[
K_d x = (A_T - x)(B_T - x)
\]

Assume \(B_T \gg x\)

\[
K_d x \approx (A_T - x)B_T \quad x = [AB] \approx \frac{A_T B_T}{B_T + K_d}
\]

\[
(B_T + K_d)x \approx A_T B_T
\]
Equilibrium Math

\[ A + B \xrightleftharpoons{K_d} AB \]

**Knowns**

\[ K_d = \frac{[A][B]}{[AB]} \]

\[ A_T = [A] + [AB] \]

\[ B_T = [B] + [AB] \]

\[
\begin{align*}
[A] &= A_T - [AB] \\
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\]

\[
K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB])
\]

\[
K_d x = (A_T - x)(B_T - x)
\]

Assume \( B_T \gg x \)

\[
K_d x \approx (A_T - x)B_T \quad x = [AB] \approx \frac{A_T B_T}{B_T + K_d}
\]

**Fraction Bound**

\[
(B_T + K_d)x \approx A_T B_T
\]
Equilibrium Math

\[ A + B \xleftrightarrow{K_d} AB \]

\[ [A] = A_T - [AB] \]
\[ [B] = B_T - [AB] \]

\[ K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB]) \]
\[ K_d x = (A_T - x)(B_T - x) \]

Assume \( B_T >> x \)
\[ K_d x \approx (A_T - x)B_T \]
\[ (B_T + K_d)x \approx A_T B_T \]

Knowns
\[ K_d = \frac{[A][B]}{[AB]} \]
\[ A_T = [A] + [AB] \]
\[ B_T = [B] + [AB] \]

Fraction Bound
\[ \frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d} \]
Equilibrium Math

\[ A + B \xrightleftharpoons[K_d]{\rightleftharpoons} AB \]
Equilibrium Math

\[ A + B \overset{K_d}{\underset{\text{Fraction Bound}}{\leftrightarrow}} AB \]
Equilibrium Math

\[ A + B \xrightleftharpoons[K_d]{\quad} AB \]

Fraction Bound

\[
\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d}
\]
Equilibrium Math

\[ A + B \xrightleftharpoons[\kappa_d]{\kappa_d} AB \]

Fraction Bound

\[
\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d}
\]

\[
\frac{[PL]}{P_T} \approx \frac{L_T}{L_T + K_d}
\]
Equilibrium Math

\[ A + B \xrightleftharpoons[^{K_d}]{\text{↔}} AB \]

Fraction Bound

\[
\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d}
\]

\[
\frac{[PL]}{P_T} \approx \frac{L_T}{L_T + K_d} = f = \frac{1}{1 + \frac{K_d}{L_T}}
\]
Equilibrium Math

\[ A + B \overset{K_d}{\rightleftharpoons} AB \]

Fraction Bound

\[
\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d}
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\[
\frac{[PL]}{P_T} \approx \frac{L_T}{L_T + K_d} = f = \frac{1}{1 + \frac{K_d}{L_T}}
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Equilibrium Math

\[ A + B \overset{K_d}{\rightleftharpoons} AB \]

**Fraction Bound**

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\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d}
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\[
\frac{[PL]}{P_T} \approx \frac{L_T}{L_T + K_d} = f = \frac{1}{1 + \frac{K_d}{L_T}}
\]

\[ f = 1 - K_d \frac{f}{L} \]
Equilibrium Math

\[ A + B \xrightleftharpoons[K_d]{\leftarrow} AB \]

Fraction Bound

\[
\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d}
\]

\[
\frac{[PL]}{P_T} \approx \frac{L_T}{L_T + K_d} = f = \frac{1}{1 + \frac{K_d}{L_T}}
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\[ f = 1 - K_d \frac{f}{L} \]
Equilibrium Math

\[ A + B \xrightleftharpoons[K_d]{\text{[AB]}} AB \]

Fraction Bound

\[
\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d}
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f = 1 - K_d \frac{f}{L}
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Equilibrium Math

\[ A + B \overset{K_d}{\rightleftharpoons} AB \]

Fraction Bound

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\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d}
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Equilibrium Math

\[ A + B \xrightleftharpoons[K_d]{\ \ } AB \]

Fraction Bound

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\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d}
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\]

At half-saturation, \( f = 0.5 \)

\[
f = 0.5 = \frac{1}{1 + \frac{K_d}{L_T}}
\]
Equilibrium Math

\[ A + B \xrightleftharpoons[K_d]{\text{\(\rightleftharpoons\)}} AB \]

Fraction Bound

\[
\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d} \\
\frac{[PL]}{P_T} \approx \frac{L_T}{L_T + K_d} = f = \frac{1}{1 + \frac{K_d}{L_T}}
\]

At half-saturation, \( f = 0.5 \)

\[
f = 0.5 = \frac{1}{1 + \frac{K_d}{L_T}}
\]

\[
0.5 + 0.5 \frac{K_d}{L_T} = 1
\]
Equilibrium Math

\[ A + B \xleftrightarrow{K_d} AB \]

Fraction Bound

\[ \frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d} \]

\[ \frac{[PL]}{P_T} \approx \frac{L_T}{L_T + K_d} = f = \frac{1}{1 + \frac{K_d}{L_T}} \]

At half-saturation, \( f = 0.5 \)

\[ f = 0.5 = \frac{1}{1 + \frac{K_d}{L_T}} \quad 0.5 \frac{K_d}{L_T} = 0.5 \]

\[ 0.5 + 0.5 \frac{K_d}{L_T} = 1 \]
Equilibrium Math

\[ A + B \overset{K_d}{\leftrightarrow} AB \]

Fraction Bound

\[
\frac{[AB]}{A_T} \approx \frac{B_T}{B_T + K_d}
\]

\[
\frac{[PL]}{P_T} \approx \frac{L_T}{L_T + K_d} = f = \frac{1}{1 + \frac{K_d}{L_T}}
\]

At half-saturation, \( f = 0.5 \)

\[
f = 0.5 = \frac{1}{1 + \frac{K_d}{L_T}} \quad 0.5 \frac{K_d}{L_T} = 0.5
\]

\[
0.5 + 0.5 \frac{K_d}{L_T} = 1 \quad L_T = K_d
\]
Ligand binding titration

$[P]_T = 0.1 \mu M$
Ligand binding titration

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Ligand binding titration

\[ [P]_T = 0.1 \mu M \]
Fluorescence Anisotropy Titration

\[ [P] = 0.1 \, \mu M \]
Fluorescence Anisotropy Titration

- Scatchard Analysis
  - Pick beginning and end values
  - Calculate \( v \) & \( v/\left[ L \right] \)
  - Plot \( v/\left[ L \right] \) vs \( v \)

\[ [P] = 0.1 \mu M \]
Fluorescence Anisotropy Titration

- Scatchard Analysis
  - Pick beginning and end values
  - Calculate \( v \) & \( v/[L] \)
  - Plot \( v/[L] \) vs \( v \)

\( [P] = 0.1 \mu M \)
Scatchard Analysis

Slope = -0.27 ± 0.05
Intercept = 0.41 ± 0.04
Correlation Coefficient = -0.74

leads to

K = 0.27 ± 0.05 µM
n = 1.5

How many binding sites?
Direct Fit Gives a Better Result

\[ [P] = 0.1 \, \mu M \]

\[ K = 0.27 \pm 0.05 \, \mu M \]

\[ A_{\text{bound}} = 0.28 \]

\[ A_{\text{unbound}} = 0.12 \]
Direct Fit Gives a Better Result

\[ K = 1.35 \pm 0.66 \mu M \]

\[ A_{\text{bound}} = 0.312 \pm 0.012 \]
\[ A_{\text{unbound}} = 0.087 \pm 0.040 \]

\[ [P] = 0.1 \mu M \]

\[ K = 0.27 \pm 0.05 \mu M \]

\[ A_{\text{bound}} = 0.28 \]
\[ A_{\text{unbound}} = 0.12 \]
Direct Fit Gives a Better Result

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Direct Fit Gives a Better Result

$K = 1.35 \pm 0.66 \mu M$

$A_{\text{bound}} = 0.312 \pm 0.012$

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$K = 0.27 \pm 0.05 \mu M$

$A_{\text{bound}} = 0.28$

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$[P] = 0.1 \mu M$
Direct Fit Gives a Better Result

\[ K = 1.35 \pm 0.66 \, \mu M \]
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\[ K = 0.27 \pm 0.05 \, \mu M \]
\[ A_{\text{bound}} = 0.28 \]
\[ A_{\text{unbound}} = 0.12 \]

[\[P\] = 0.1 \, \mu M]
Direct fit
$K = 1.35 \pm 0.66 \, \mu M$

$A_{\text{bound}} = 0.312 \pm 0.012$
$A_{\text{unbound}} = 0.087 \pm 0.040$

Weighted fit
$K = 0.75 \pm 0.20 \, \mu M$
$n = 0.99$

Fixed
$A_{\text{bound}} = 0.312$
$A_{\text{unbound}} = 0.087$

Unweighted fit
$K = 0.57 \pm 0.07 \, \mu M$
$n = 1.06$
Direct fit

\[ K = 1.35 \pm 0.66 \, \mu M \]

\[ A_{\text{bound}} = 0.312 \pm 0.012 \]
\[ A_{\text{unbound}} = 0.087 \pm 0.040 \]

Weighted fit

\[ K = 0.75 \pm 0.20 \, \mu M \]
\[ n = 0.99 \]

Unweighted fit

\[ K = 0.57 \pm 0.07 \, \mu M \]
\[ n = 1.06 \]

Fixed

\[ K = 1.0 \]
\[ A_{\text{bound}} = 0.312 \]
\[ A_{\text{unbound}} = 0.087 \]
Tight binding - requires exact solution

\[ [P]_T = 1.0 \, \mu M \]
Equilibrium Math

\[ A + B \xrightleftharpoons{K_d} AB \]

\[
\begin{align*}
[A] &= A_T - [AB] \\
[B] &= B_T - [AB]
\end{align*}
\]

**Knowns**

\[
K_d = \frac{[A][B]}{[AB]}
\]

\[
\begin{align*}
A_T &= [A] + [AB] \\
B_T &= [B] + [AB]
\end{align*}
\]
Equilibrium Math

\[ A + B \xrightleftharpoons{K_d} AB \]

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\begin{align*}
[A] &= A_T - [AB] \\
[B] &= B_T - [AB]
\end{align*}
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\[
K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB])
\]

Knowns

\[
K_d = \frac{[A][B]}{[AB]}
\]
\[
A_T = [A] + [AB]
\]
\[
B_T = [B] + [AB]
\]
Equilibrium Math

\[ A + B \overset{K_d}{\longleftrightarrow} AB \]

\[
\begin{align*}
[A] &= A_T - [AB] \\
[B] &= B_T - [AB]
\end{align*}
\]

\[
K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB])
\]

\[
K_d x = (A_T - x)(B_T - x)
\]

Knowns

\[
K_d = \frac{[A][B]}{[AB]}
\]

\[
A_T = [A] + [AB]
\]

\[
B_T = [B] + [AB]
\]
Equilibrium Math

\[ A + B \xrightleftharpoons[K_d]{\longrightarrow} AB \]

\[
\begin{align*}
[A] &= A_T - [AB] \\
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\end{align*}
\]

\[
K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB])
\]

\[
K_d x = (A_T - x)(B_T - x)
\]

Assume \( B_T \gg x \)
Equilibrium Math

$A + B \xrightarrow{K_d} AB$

$[A] = A_T - [AB]$
$[B] = B_T - [AB]$

$K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB])$

$K_d x = (A_T - x)(B_T - x)$

Assume $B_T >> x$

$x^2 - (A_T + B_T + K_d)x + A_T B_T = 0$
Equilibrium Math

\[ A + B \xleftrightarrow{K_d} AB \]

Knowns

\[ K_d = \frac{[A][B]}{[AB]} \]
\[ A_T = [A] + [AB] \]
\[ B_T = [B] + [AB] \]

\[ K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB]) \]

\[ K_d x = (A_T - x)(B_T - x) \]

Assume \( B_T \gg x \)

\[ x^2 - (A_T + B_T + K_d)x + A_T B_T = 0 \]

\[ ax^2 + bx + c = 0 \quad x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \]
Equilibrium Math

\[ A + B \xrightleftharpoons{K_d} AB \]

\[
\begin{align*}
[A] &= A_T - [AB] \\
[B] &= B_T - [AB]
\end{align*}
\]

\[ K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB]) \]

\[ K_d x = (A_T - x)(B_T - x) \]

Assume \(B_T >> x\)

\[ x^2 - (A_T + B_T + K_d)x + A_T B_T = 0 \]

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Equilibrium Math

\[
A + B \overset{K_d}{\rightleftharpoons} AB
\]

\[
[A] = A_T - [AB]
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[B] = B_T - [AB]
\]

\[
K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB])
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K_d x = (A_T - x)(B_T - x)
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Assume \(B_T \gg x\)

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x^2 - (A_T + B_T + K_d)x + A_T B_T = 0
\]

\[
ax^2 + bx + c = 0 \quad x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}
\]

\[
x = \frac{(A_T + B_T + K_d) - \sqrt{(A_T + B_T + K_d)^2 - 4A_T B_T}}{2}
\]
**Equilibrium Math**

\[ A + B \xrightleftharpoons{K_d} AB \]

\[
[A] = A_T - [AB] \\
[B] = B_T - [AB]
\]

\[ K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB]) \]

\[ K_d x = (A_T - x)(B_T - x) \]

Assume \( B_T \gg x \)

\[ x^2 - (A_T + B_T + K_d)x + A_T B_T = 0 \]

\[ a x^2 + b x + c = 0 \]

\[ x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \]

\[ x = \frac{(A_T + B_T + K_d) - \sqrt{(A_T + B_T + K_d)^2 - 4A_T B_T}}{2} = [AB] \]

**Knowns**

\[ K_d = \frac{[A][B]}{[AB]} \]

\[ A_T = [A] + [AB] \]

\[ B_T = [B] + [AB] \]
Equilibrium Math

\[ A + B \xrightleftharpoons{K_d} AB \]

\[ [A] = A_T - [AB] \]
\[ [B] = B_T - [AB] \]

\[ K_d [AB] = [A][B] = (A_T - [AB])(B_T - [AB]) \]

\[ K_d x = (A_T - x)(B_T - x) \]

Assume \( B_T \gg x \)

\[ x^2 - (A_T + B_T + K_d) x + A_T B_T = 0 \]

\[ a x^2 + b x + c = 0 \quad x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \]

\[ x = \frac{(A_T + B_T + K_d) - \sqrt{(A_T + B_T + K_d)^2 - 4A_T B_T}}{2} \]

Fraction Bound

\[ \frac{[AB]}{A_T} = [AB] \]
Equilibrium Math

\[ A + B \xrightleftharpoons[K_d]{\rightleftharpoons} AB \]

\[ x = \frac{(A_T + B_T + K_d)^2 - 4A_T B_T}{2} = [AB] \]
Equilibrium Math

\[ A + B \xrightleftharpoons{K_d} AB \]

\[ x = \frac{(A_T + B_T + K_d) - \sqrt{\left( A_T + B_T + K_d \right)^2 - 4A_T B_T}}{2} = [AB] \]

Fraction Bound

\[ f = \frac{[AB]}{A_T} = \frac{(A_T + B_T + K_d) - \sqrt{\left( A_T + B_T + K_d \right)^2 - 4A_T B_T}}{2A_T} \]

At half-saturation, \( f=0.5 \)

\[ 0.5 = \frac{[AB]}{A_T} = \frac{(A_T + B_T + K_d) - \sqrt{\left( A_T + B_T + K_d \right)^2 - 4A_T B_T}}{2A_T} \]

\[ A_T = (A_T + B_T + K_d) - \sqrt{\left( A_T + B_T + K_d \right)^2 - 4A_T B_T} \]

\[ B_T + K_d = \sqrt{\left( A_T + B_T + K_d \right)^2 - 4A_T B_T} \quad 0 = A_T^2 + 2A_T K_d - 2A_T B_T \]

\[ (B_T + K_d)^2 = (A_T + B_T + K_d)^2 - 4A_T B_T \quad B_T = \frac{1}{2} A_T + K_d \]
Equilibrium Math

\[ P + L \overset{K_d}{\rightleftharpoons} PL \]

**Assume** $L >> P$

**Fraction Bound**

\[ f \approx \frac{1}{1 + \frac{K_d}{L_T}} \]

At half-saturation, $f=0.5$

\[ L_T = K_d \]
Equilibrium Math

\[ P + L \rightleftharpoons ^{K_d} PL \]

Assume \( L >> P \)

Fraction Bound

\[ f \approx \frac{1}{1 + \frac{K_d}{L_T}} \]

At half-saturation, \( f = 0.5 \)

\[ L_T = K_d \]

No assumptions

Fraction Bound

\[ f = \frac{[PL]}{P_T} = \frac{\left( P_T + L_T + K_d \right)}{2P_T} - \sqrt{\left( P_T + L_T + K_d \right)^2 - 4P_T L_T} \]
**Equilibrium Math**

\[ P + L \xleftrightarrow{K_d} PL \]

**Assume L >> P**

**Fraction Bound**

\[ f \approx \frac{1}{1 + \frac{K_d}{L_T}} \]

At half-saturation, \( f=0.5 \)

\[ L_T = K_d \]

**No assumptions**

**Fraction Bound**

\[ f = \frac{[PL]}{P_T} = \frac{(P_T + L_T + K_d) - \sqrt{(P_T + L_T + K_d)^2 - 4P_TL_T}}{2P_T} \]

At half-saturation, \( f=0.5 \)

\[ L_T = \frac{1}{2} P_T + K_d \]
Inhibition

Assume $L \gg K_d$

Fix $L \gg P$

Titrate in $I$

Fraction Bound

\[ f \approx 1 - \frac{[I]}{[I] + IC_{50}} \]

Looks similar to the binding equation

...BUT beware the assumptions!