Supporting Information for the World Wide Web Edition

A General Framework for Development and Data Analysis of Competitive High-Throughput Screens for Small-Molecule Inhibitors of Protein-Protein Interactions by Fluorescence Polarization

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The derivation of equations in this paper can be readily accomplished with Mathematica (Wolfram Research, Champaign, IL). The following shows input and raw output from Mathematica 4.1.

Equation [5]

Input:

```
Simplify[Solve[{KD1 == R * LS / RLS, RT == R + RLS, LST == LS + RLS, FSB == 1 - LS / LST}, RT, {R, LS, RLS}]]
```

Output:

$$\left\{RT \to \frac{FSB\ (-KD1 - LST + FSB\ LST)}{-1 + FSB}\right\}$$

Equation [6]

Input:

```
Simplify[Solve[{KD1 == R * LS / RLS, RT == R + RLS, LST == LS + RLS, FSB == 1 - LS / LST}, FSB, {R, LS, RLS}]]
```

Output:

$$\left\{FSB \to \frac{KD1 + LST + RT - \sqrt{-4\,LST\,RT + (KD1 + LST + RT)^2}}{2\,LST}\right\},$$

$$\left\{FSB \to \frac{KD1 + LST + RT + \sqrt{-4\,LST\,RT + (KD1 + LST + RT)^2}}{2\,LST}\right\}$$

(Note that only the first root has physical meaning.)

Equation [8]

Input:

```
Simplify[Solve[{KD1 == R * LS / RLS, RT == R + RLS, LST == LS + RLS + NS * LS, FSB == 1 - LS / LST}, RT, {R, LS, RLS}]]
```

Output:

$$\left\{RT \to -\frac{(KD1 + LST - FSB\,LST)\ (FSB - NS + FSB\,NS)}{-1 + FSB}\right\}$$

Equation [9]

Input:

```
Simplify[Solve[{KD1 == R * LS / RLS, RT == R + RLS, LST == LS + RLS + NS * LS, FSB == 1 - LS / LST}, FSB, {R, LS, RLS}]]
```

Output:

$$\left\{FSB \to \frac{KD1 + LST + KD1\,NS + 2\,LST\,NS + RT + \sqrt{KD1^2\ (1 + NS)^2 + (LST - RT)^2 + 2\,KD1\ (1 + NS)\ (LST + RT)}}{2\,LST\ (1 + NS)}\right\},$$

$$\left\{FSB \to \frac{KD1 + LST + KD1\,NS + 2\,LST\,NS + RT - \sqrt{KD1^2\ (1 + NS)^2 + (LST - RT)^2 + 2\,KD1\ (1 + NS)\ (LST + RT)}}{2\,LST\ (1 + NS)}\right\}$$

(Note that only the second root has physical meaning.)

Equation [12]

Input:

```
Simplify[Solve[{KD1A == R * LS / RLS, RT == R + RLS, LST == LS + RLS, FSBA == 1 - LS / LST, FSBA == (1 + NS) * FSB - NS},
  RT, {FSBA, R, LS, RLS}]]
```

Output:

$$\left\{RT \to \frac{(FSB - NS + FSB\,NS)\ (-KD1A + (-1 + FSB)\ LST\ (1 + NS))}{(-1 + FSB)\ (1 + NS)}\right\}$$

Equation [16]

Input:

```
Simplify[Solve[{KD1 == R * LS / RLS, KD2 == R * L / RL, RT == R + RLS + RL, LST == LS + RLS, LT == L + RL, FSB == 1 - LS / LST}, LT,
  {R, LS, RLS, L, RL}]]
```

Output:

$$\left\{LT \to -\frac{(FSB\ (KD1 - KD2) + KD2)\ (FSB^2\ LST + RT - FSB\ (KD1 + LST + RT))}{(-1 + FSB)\ FSB\ KD1}\right\}$$

Equation [19]

Input:

```
Simplify[Solve[{KD1 == R * LS / RLS, KD2 == R * L / RL, RT == R + RLS + RL, LST == LS + RLS, LT == L + RL + N * L,
   FSB == 1 - LS / LST}, LT, {R, LS, RLS, L, RL}]]
```

Output:

$$\left\{LT \to -\frac{(KD2\ (1 + N) + FSB\ (KD1 - KD2\ (1 + N)))\ (FSB^2\ LST + RT - FSB\ (KD1 + LST + RT))}{(-1 + FSB)\ FSB\ KD1}\right\}$$

Equation [27]

Input:

```
Simplify[Solve[{KD1 == R * LS / RLS, KD2 == R * L / RL, KD3 == RL * LS / RLLS, KD2 * KD3 / KD1 == RLS * L / RLLS,
   RT == R + RLS + RL + RLLS, LST == LS + RLS + RLLS, LT == L + RL + RLLS, FSB == 1 - LS / LST}, LT, {R, LS, RLS, L, RL, RLLS}]]
```

Output:

$$\{LT \to -((FSB^2\ LST + RT - FSB\ (KD1 + LST + RT))\ (-(-1 + FSB)\ KD2\ KD3^2 + KD1\ ((-1 + FSB)\ KD2\ KD3 + (KD3 + LST - FSB\ LST)\ (-FSB^2\ LST - RT + FSB\ (KD3 + LST + RT)))))\ /\ ((-1 + FSB)\ KD1\ (KD1 - KD3)\ (FSB^2\ LST + RT - FSB\ (KD3 + LST + RT)))\}$$

Equation [30]

Input:

```
Simplify[Solve[{KD1 == R * LS / RLS, KD2 == R * L / RL, KD3 == RL * LS / RLLS, KD2 * KD3 / KD1 == RLS * L / RLLS,
   RT == R + RLS + RL + RLLS, LST == LS + RLS + RLLS, LT == L + RL + RLLS + N * L, FSB == 1 - LS / LST}, LT, {R, LS, RLS, L, RL, RLLS}]]
```

Output:

$$\{LT \to -((FSB^2\ LST + RT - FSB\ (KD1 + LST + RT))\ (-(-1 + FSB)\ KD2\ KD3^2\ (1 + N) + KD1\ ((-1 + FSB)\ KD2\ KD3\ (1 + N) + (KD3 + LST - FSB\ LST)\ (-FSB^2\ LST - RT + FSB\ (KD3 + LST + RT)))))\ /\ ((-1 + FSB)\ KD1\ (KD1 - KD3)\ (FSB^2\ LST + RT - FSB\ (KD3 + LST + RT)))\}$$

Equation [43]

Input:

```
Simplify[Solve[((KD1 - KD2) * FSB + KD2) * (LST * FSB^2 - (KD1 + LST + RT) * FSB + RT) / ((1 - FSB) * FSB * KD1) == LT, RT]]
```

Output:

$$\{RT \to (FSB\ (FSB^2\ (KD1 - KD2)\ LST - KD2\ LST - KD1\ (KD2 + LT) + FSB\ (-KD1^2 + 2\ KD2\ LST + KD1\ (KD2 - LST + LT))))\ /\ ((-1 + FSB)\ (FSB\ (KD1 - KD2) + KD2))\}$$

Equation [44]

Input:

```
Simplify[D[((KD1 - KD2) * FSB + KD2) * (LST * FSB^2 - (KD1 + LST + RT) * FSB + RT) / ((1 - FSB) * FSB * KD1), FSB]]
```

Output:

$$\frac{1}{(-1+FSB)^2\,FSB^2\,KD1}(2\,FSB^3\,(KD1-KD2)\,LST+$$
$$FSB^4\,(-KD1+KD2)\,LST-KD2\,RT+2\,FSB\,KD2\,RT-FSB^2\,(KD1^2+KD1\,LST+KD2\,(-LST+RT)))$$

Input:

```
RT = (FSB (FSB^2 (KD1 - KD2) LST - KD2 LST - KD1 (KD2 + LT) + FSB (-KD1^2 + 2 KD2 LST + KD1 (KD2 - LST + LT)))) /
  ((-1 + FSB) (FSB (KD1 - KD2) + KD2))

Simplify[1/((-1 + FSB)^2 FSB^2 KD1)
 (2 FSB^3 (KD1 - KD2) LST + FSB^4 (-KD1 + KD2) LST - KD2 RT + 2 FSB KD2 RT -
   FSB^2 (KD1^2 + KD1 LST + KD2 (-LST + RT)))]
```

Output:

$$-(FSB^4\,(KD1-KD2)^2\,LST-2\,FSB^3\,(KD1^2-3\,KD1\,KD2+2\,KD2^2)\,LST+KD2\,(KD2\,LST+KD1\,(KD2+LT))+$$
$$FSB^2\,(KD1^3+6\,KD2^2\,LST+KD1^2\,(-2\,KD2+LST)+KD1\,KD2\,(KD2-6\,LST+LT))+$$
$$2\,FSB\,KD2\,(KD1^2-2\,KD2\,LST-KD1\,(KD2-LST+LT)))\Big/$$
$$((-1+FSB)^2\,FSB\,KD1\,(FSB\,(KD1-KD2)+KD2))$$

Equation [46]

Input:

```
FullSimplify[
 D[
  -(FSB^4 (KD1 - KD2)^2 LST - 2 FSB^3 (KD1^2 - 3 KD1 KD2 + 2 KD2^2) LST + KD2 (KD2 LST + KD1 (KD2 + LT)) +
     FSB^2 (KD1^3 + 6 KD2^2 LST + KD1^2 (-2 KD2 + LST) + KD1 KD2 (KD2 - 6 LST + LT)) +
     2 FSB KD2 (KD1^2 - 2 KD2 LST - KD1 (KD2 - LST + LT))) / ((-1 + FSB)^2 FSB KD1 (FSB (KD1 - KD2) + KD2)), FSB] == 0]
```

Output:

$$((FSB\,(KD1-KD2)+KD2)^2\,(KD1\,(2\,FSB^2\,(KD1-KD2)-KD2+3\,FSB\,KD2)+(-1+FSB)^3\,KD2\,LST)+$$
$$(-1+FSB)^3\,KD1\,KD2\,(2\,FSB\,(KD1-KD2)+KD2)\,LT)\Big/$$
$$((-1+FSB)^3\,FSB^2\,KD1\,(FSB\,(KD1-KD2)+KD2)^2)==0$$

(Note that a necessary condition is that the numerator equals zero. It can be shown that numerator and denominator cannot be simultaneously zero under the physical restraints imposed on the variables.)

Equation [51]

Input:

```
Simplify[Solve[{KD1 == R * LS / RLS, KD2 == R * L / RL, RT == R + RLS + RL, LST == LS + RLS, LT == L + RL, FSB == 1 - LS / LST}, KD2,
  {R, LS, RLS, L, RL}]]
```

Output:

$$\left\{KD2 \to \frac{FSB\ KD1\ (FSB^2\ LST - LT + RT - FSB\ (KD1 + LST - LT + RT))}{(-1 + FSB)\ (FSB^2\ LST + RT - FSB\ (KD1 + LST + RT))}\right\}$$

Input:

$$\text{Simplify}\left[\text{Solve}\left[\left\{\frac{KD1 + LST + RT - \sqrt{-4\ LST\ RT + (KD1 + LST + RT)^2}}{2\ LST} == (AU - AF) / ((AB - AU) * Q + AU - AF)\right\}, AB\right]\right]$$

Output:

$$\left\{AB \to -\left(AF\left(-KD1 + LST - RT + \sqrt{-4\ LST\ RT + (KD1 + LST + RT)^2}\right) + AU\left(KD1 - KD1\ Q - LST\ (1 + Q) + (-1 + Q)\left(-RT + \sqrt{-4\ LST\ RT + (KD1 + LST + RT)^2}\right)\right)\right) \Big/ \left(Q\left(KD1 + LST + RT - \sqrt{-4\ LST\ RT + (KD1 + LST + RT)^2}\right)\right)\right\}$$

Input:

```
AB =
 -(AF (-KD1 + LST - RT + √(-4 LST RT + (KD1 + LST + RT)^2)) +
     AU (KD1 - KD1 Q - LST (1 + Q) + (-1 + Q) (-RT + √(-4 LST RT + (KD1 + LST + RT)^2)))) /
   (Q (KD1 + LST + RT - √(-4 LST RT + (KD1 + LST + RT)^2)))

FullSimplify[(AOBS - AF) / ((AB - AOBS) * Q + AOBS - AF)]
```

Output:

$$(2\ (AF - AOBS)\ RT) \Big/ \left(2\ AOBS\ (-1 + Q)\ RT + AF\left(KD1 + LST + RT + \sqrt{-4\ LST\ RT + (KD1 + LST + RT)^2}\right) - AU\left(KD1 + LST - RT + 2\ Q\ RT + \sqrt{-4\ LST\ RT + (KD1 + LST + RT)^2}\right)\right)$$

Input:

```
Q = 1

FullSimplify[(AOBS - AF) / ((AB - AOBS) * Q + AOBS - AF)]
```

Output:

$$\frac{(\mathrm{AF}-\mathrm{AOBS})\left(\mathrm{KD1}+\mathrm{LST}+\mathrm{RT}-\sqrt{-4\,\mathrm{LST}\,\mathrm{RT}+(\mathrm{KD1}+\mathrm{LST}+\mathrm{RT})^{2}}\right)}{2\,(\mathrm{AF}\,\mathrm{LST}-\mathrm{AU}\,\mathrm{LST})}$$