Analysis of quorum sensing signal discrimination

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The system of differential equations describing the kinetic proofreading in quorum sensing signal discrimination model are entered below:

```
QSSF = {R'[t] == k_r + k_{1_r} * γ * R1[t] - k_1 * A_1 * R[t] - d_r * R[t],
   R1'[t] == k_1 * A_1 * R[t] + 2 * k_{2_r} * β * D1[t] - k_{1_r} * γ * R1[t] - α_1 * d_r * R1[t] -
     2 * k_2 * R1[t]^2,
   D1'[t] == k_2 * R1[t]^2 - k_{2_r} * β * D1[t] - α_2 * d_r * D1[t]
  };
TableForm[QSSF]
```

$R'[t] == -R[t]\, d_r - R[t]\, A_1\, k_1 + k_r + \gamma\, R1[t]\, k_{1_r}$

$R1'[t] == R[t]\, A_1\, k_1 - 2\, R1[t]^2\, k_2 - \gamma\, R1[t]\, k_{1_r} + 2\, \beta\, D1[t]\, k_{2_r} - R1[t]\, d_r\, \alpha_1$

$D1'[t] == R1[t]^2\, k_2 - \beta\, D1[t]\, k_{2_r} - D1[t]\, d_r\, \alpha_2$

A base parameter set is defined (see table S1 for an explanation of each parameter value)

```
baseparam = {k_r -> 20, k_1 -> .1, k_{1_r} -> 1, k_2 -> .1, k_{2_r} -> 1, d_{r_1} -> .023,
  β -> 1, α_1 → 1 , α_2 → 1, γ → 1, A_1 → 100, A_2 → 100}
```

$\{k_r \to 20,\ k_1 \to 0.1,\ k_{1_r} \to 1,\ k_2 \to 0.1,\ k_{2_r} \to 1,\ d_{r_1} \to 0.023,\ \beta \to 1,\ \alpha_1 \to 1,\ \alpha_2 \to 1,\ \gamma \to 1,\ A_1 \to 100,\ A_2 \to 100\}$

Solve the system at steady state

```
QSSFFixedPoints =
 Simplify[Solve[{QSSF[[1, 2]] == 0, QSSF[[2, 2]] == 0, QSSF[[3, 2]] == 0},
   {R[t], R1[t], D1[t]}]]
```

$$\Big\{\Big\{R[t] \to -\frac{1}{4\, d_r\, (d_r + A_1 k_1)^2\, k_2\, \alpha_2} \Big(\beta \gamma^2 d_r k_{1_r}^2 k_{2_r} + \beta \gamma d_r^2 k_{1_r} k_{2_r} \alpha_1 + \beta \gamma A_1 d_r k_1 k_{1_r} k_{2_r} \alpha_1 - 4 d_r^2 k_2 k_r \alpha_2 - 4 A_1 d_r k_1 k_2 k_r \alpha_2 + \gamma^2 d_r^2 k_{1_r}^2 \alpha_2 + \gamma d_r^3 k_{1_r} \alpha_1 \alpha_2 + \gamma A_1 d_r^2 k_1 k_{1_r} \alpha_1 \alpha_2 + \gamma k_{1_r} \sqrt{\Big(d_r (\beta k_{2_r} + d_r \alpha_2) \Big(8 A_1 k_1 (d_r + A_1 k_1) k_2 k_r \alpha_2 + d_r (\gamma k_{1_r} + (d_r + A_1 k_1) \alpha_1)^2 (\beta k_{2_r} + d_r \alpha_2)\Big)\Big)}\Big),$$

$$D1[t] \to \frac{1}{8\, d_r\, (d_r + A_1 k_1)^2\, k_2\, \alpha_2^2} \Big(\beta \gamma^2 d_r k_{1_r}^2 k_{2_r} + 2 \beta \gamma d_r^2 k_{1_r} k_{2_r} \alpha_1 + 2 \beta \gamma A_1 d_r k_1 k_{1_r} k_{2_r} \alpha_1 + \beta d_r^3 k_{2_r} \alpha_1^2 + 2 \beta A_1 d_r^2 k_1 k_{2_r} \alpha_1^2 + \beta A_1^2 d_r k_1^2 k_{2_r} \alpha_1^2 + 4 A_1 d_r k_1 k_2 k_r \alpha_2 + 4 A_1^2 k_1^2 k_2 k_r \alpha_2 + \gamma^2 d_r^2 k_{1_r}^2 \alpha_2 + 2 \gamma d_r^3 k_{1_r} \alpha_1 \alpha_2 + 2 \gamma A_1 d_r^2 k_1 k_{1_r} \alpha_1 \alpha_2 + d_r^4 \alpha_1^2 \alpha_2 + 2 A_1 d_r^3 k_1 \alpha_1^2 \alpha_2 + A_1^2 d_r^2 k_1^2 \alpha_1^2 \alpha_2 + \gamma k_{1_r} \sqrt{\Big(d_r (\beta k_{2_r} + d_r \alpha_2) \Big(8 A_1 k_1 (d_r + A_1 k_1) k_2 k_r \alpha_2 + d_r (\gamma k_{1_r} + (d_r + A_1 k_1) \alpha_1)^2 (\beta k_{2_r} + d_r \alpha_2)\Big)\Big)} + d_r \alpha_1 \sqrt{\Big(d_r (\beta k_{2_r} + d_r \alpha_2) \Big(8 A_1 k_1 (d_r + A_1 k_1) k_2 k_r \alpha_2 + d_r (\gamma k_{1_r} + (d_r + A_1 k_1) \alpha_1)^2 (\beta k_{2_r} + d_r \alpha_2)\Big)\Big)} + A_1 k_1 \alpha_1 \sqrt{\Big(d_r (\beta k_{2_r} + d_r \alpha_2) \Big(8 A_1 k_1 (d_r + A_1 k_1) k_2 k_r \alpha_2 + d_r (\gamma k_{1_r} + (d_r + A_1 k_1) \alpha_1)^2 (\beta k_{2_r} + d_r \alpha_2)\Big)\Big)}\Big),$$

$$R1[t] \to -\Big(\beta \gamma d_r k_{1_r} k_{2_r} + \beta d_r^2 k_{2_r} \alpha_1 + \beta A_1 d_r k_1 k_{2_r} \alpha_1 + \gamma d_r^2 k_{1_r} \alpha_2 + d_r^3 \alpha_1 \alpha_2 + A_1 d_r^2 k_1 \alpha_1 \alpha_2 + \sqrt{\Big(d_r (\beta k_{2_r} + d_r \alpha_2) \Big(8 A_1 k_1 (d_r + A_1 k_1) k_2 k_r \alpha_2 + d_r (\gamma k_{1_r} + (d_r + A_1 k_1) \alpha_1)^2 (\beta k_{2_r} + d_r \alpha_2)\Big)\Big)}\Big) \Big/ \big(4\, d_r\, (d_r + A_1 k_1)\, k_2\, \alpha_2\big)\Big\},$$

$$\Big\{R[t] \to -\frac{1}{4\, d_r\, (d_r + A_1 k_1)^2\, k_2\, \alpha_2} \Big(\beta \gamma^2 d_r k_{1_r}^2 k_{2_r} + \beta \gamma d_r^2 k_{1_r} k_{2_r} \alpha_1 + \beta \gamma A_1 d_r k_1 k_{1_r} k_{2_r} \alpha_1 - 4 d_r^2 k_2 k_r \alpha_2 - 4 A_1 d_r k_1 k_2 k_r \alpha_2 + \gamma^2 d_r^2 k_{1_r}^2 \alpha_2 + \gamma d_r^3 k_{1_r} \alpha_1 \alpha_2 + \gamma A_1 d_r^2 k_1 k_{1_r} \alpha_1 \alpha_2 - \gamma k_{1_r} \sqrt{\Big(d_r (\beta k_{2_r} + d_r \alpha_2) \Big(8 A_1 k_1 (d_r + A_1 k_1) k_2 k_r \alpha_2 + d_r (\gamma k_{1_r} + (d_r + A_1 k_1) \alpha_1)^2 (\beta k_{2_r} + d_r \alpha_2)\Big)\Big)}\Big),$$

$$D1[t] \to \frac{1}{8\, d_r\, (d_r + A_1 k_1)^2\, k_2\, \alpha_2^2} \Big(\beta \gamma^2 d_r k_{1_r}^2 k_{2_r} + 2 \beta \gamma d_r^2 k_{1_r} k_{2_r} \alpha_1 + 2 \beta \gamma A_1 d_r k_1 k_{1_r} k_{2_r} \alpha_1 + \beta d_r^3 k_{2_r} \alpha_1^2 + 2 \beta A_1 d_r^2 k_1 k_{2_r} \alpha_1^2 + \beta A_1^2 d_r k_1^2 k_{2_r} \alpha_1^2 + 4 A_1 d_r k_1 k_2 k_r \alpha_2 + 4 A_1^2 k_1^2 k_2 k_r \alpha_2 + \gamma^2 d_r^2 k_{1_r}^2 \alpha_2 + 2 \gamma d_r^3 k_{1_r} \alpha_1 \alpha_2 + 2 \gamma A_1 d_r^2 k_1 k_{1_r} \alpha_1 \alpha_2 + d_r^4 \alpha_1^2 \alpha_2 + 2 A_1 d_r^3 k_1 \alpha_1^2 \alpha_2 + A_1^2 d_r^2 k_1^2 \alpha_1^2 \alpha_2 - \gamma k_{1_r} \sqrt{\Big(d_r (\beta k_{2_r} + d_r \alpha_2) \Big(8 A_1 k_1 (d_r + A_1 k_1) k_2 k_r \alpha_2 + d_r (\gamma k_{1_r} + (d_r + A_1 k_1) \alpha_1)^2 (\beta k_{2_r} + d_r \alpha_2)\Big)\Big)} -$$

$$d_r\,\alpha_1\sqrt{\left(d_r\,(\beta\,k_{2_r}+d_r\,\alpha_2)\,\left(8\,A_1\,k_1\,(d_r+A_1\,k_1)\,k_2\,k_r\,\alpha_2+d_r\,(\gamma\,k_{1_r}+(d_r+A_1\,k_1)\,\alpha_1)^2\,(\beta\,k_{2_r}+d_r\,\alpha_2)\right)\right)}-A_1\,k_1\,\alpha_1\sqrt{\left(d_r\,(\beta\,k_{2_r}+d_r\,\alpha_2)\,\left(8\,A_1\,k_1\,(d_r+A_1\,k_1)\,k_2\,k_r\,\alpha_2+d_r\,(\gamma\,k_{1_r}+(d_r+A_1\,k_1)\,\alpha_1)^2\,(\beta\,k_{2_r}+d_r\,\alpha_2)\right)\right)}\Big),$$

$$\mathrm{R1}[t]\to-\Big(\beta\,\gamma\,d_r\,k_{1_r}\,k_{2_r}+\beta\,d_r^2\,k_{2_r}\,\alpha_1+\beta\,A_1\,d_r\,k_1\,k_{2_r}\,\alpha_1+\gamma\,d_r^2\,k_{1_r}\,\alpha_2+d_r^3\,\alpha_1\,\alpha_2+A_1\,d_r^2\,k_1\,\alpha_1\,\alpha_2-\sqrt{\left(d_r\,(\beta\,k_{2_r}+d_r\,\alpha_2)\,\left(8\,A_1\,k_1\,(d_r+A_1\,k_1)\,k_2\,k_r\,\alpha_2+d_r\,(\gamma\,k_{1_r}+(d_r+A_1\,k_1)\,\alpha_1)^2\,(\beta\,k_{2_r}+d_r\,\alpha_2)\right)\right)}\Big)\Big/\left(4\,d_r\,(d_r+A_1\,k_1)\,k_2\,\alpha_2\right)\}\}$$

Check the fixed points for the base parameter values to determine which solution provides the biologically relevant one (i.e. product concentrations are not less than 0)

```
QSSFFixedPoints[[1, 1, 2]] /. baseparam /. dr → 10
QSSFFixedPoints[[2, 1, 2]] /. baseparam /. dr → 10
QSSFFixedPoints[[1, 2, 2]] /. baseparam /. dr → 10
QSSFFixedPoints[[2, 2, 2]] /. baseparam /. dr → 10
QSSFFixedPoints[[1, 3, 2]] /. baseparam /. dr → 10
QSSFFixedPoints[[2, 3, 2]] /. baseparam /. dr → 10
```

```
-1.93436
```

```
1.04686
```

```
31.3108
```

```
0.00798447
```

```
-58.6872
```

```
0.937172
```

Introduce the dissociation constant, $K=k_{1r}/k_1=k_{2r}/k_2$ and then allow k_1, k_{1r}, k_2, and k_{2r} to approach infinity as a mathematical representation of infinitely fast binding kinetics, note that this procedure only requires taking the limits as k1 and k2 approach infinity following substitution for k_{1r}

```
fastbindR = QSSFFixedPoints[[2, 1, 2]] /. {k1r → (K k1), k2r → (K k2)};
fastbindR1 = QSSFFixedPoints[[2, 3, 2]] /. {k1r → (K k1), k2r → (K k2)};
fastbindD1 = QSSFFixedPoints[[2, 2, 2]] /. {k1r → (K k1), k2r → (K k2)};
Limit[Limit[fastbindR, k2 → Infinity], k1 → Infinity]
Limit[Limit[fastbindR1, k2 → Infinity], k1 → Infinity]
Limit[Limit[fastbindD1, k2 → Infinity], k1 → Infinity]
```

$$-\frac{K\gamma\left(K\beta d_r\,(K\gamma+A_1\alpha_1)-\sqrt{K\beta d_r\left(K\beta d_r\,(K\gamma+A_1\alpha_1)^2+8A_1^2k_r\alpha_2\right)}\right)}{4A_1^2d_r\alpha_2}$$

$$\frac{-K\beta d_r\,(K\gamma+A_1\alpha_1)+\sqrt{K\beta d_r\left(K\beta d_r\,(K\gamma+A_1\alpha_1)^2+8A_1^2k_r\alpha_2\right)}}{4A_1d_r\alpha_2}$$

$$\frac{1}{8A_1^2d_r\alpha_2^2}\left(K\beta d_r\,(K\gamma+A_1\alpha_1)^2+4A_1^2k_r\alpha_2-K\gamma\sqrt{K\beta d_r\left(K\beta d_r\,(K\gamma+A_1\alpha_1)^2+8A_1^2k_r\alpha_2\right)}-A_1\alpha_1\sqrt{K\beta d_r\left(K\beta d_r\,(K\gamma+A_1\alpha_1)^2+8A_1^2k_r\alpha_2\right)}\right)$$

Compute the steady-state concentrations of D1 and D2 and then take their ratio to determine f

```
symbD1 =
 Simplify[QSSFFixedPoints[[2, 2, 2]] /.
   {α1 → dr1/dr, α2 → dr1/dr, β → 1, γ → 1}]
symbD2 = Simplify[QSSFFixedPoints[[2, 2, 2]] /. A1 → A2]
symbf = Simplify[symbD1 / symbD2]
```

$$\begin{aligned}
&\frac{1}{8\, d_{r_1}^2\, (d_r + A_1 k_1)^2\, k_2} \\
&d_r \Bigg(d_r\, d_{r_1}^3 + 2 A_1 d_{r_1}^3 k_1 + \frac{A_1^2 d_{r_1}^3 k_1^2}{d_r} + 4 A_1 d_{r_1} k_1 k_2 k_r + \frac{4 A_1^2 d_{r_1} k_1^2 k_2 k_r}{d_r} + 2 d_r d_{r_1}^2 k_{1_r} + \\
&\quad 2 A_1 d_{r_1}^2 k_1 k_{1_r} + d_r d_{r_1} k_{1_r}^2 + d_r d_{r_1}^2 k_{2_r} + 2 A_1 d_{r_1}^2 k_1 k_{2_r} + \frac{A_1^2 d_{r_1}^2 k_1^2 k_{2_r}}{d_r} + \\
&\quad 2 d_r d_{r_1} k_{1_r} k_{2_r} + 2 A_1 d_{r_1} k_1 k_{1_r} k_{2_r} + d_r k_{1_r}^2 k_{2_r} - d_{r_1} \sqrt{\Big(d_r \big(d_{r_1} + k_{2_r}\big)} \\
&\qquad \Big(\frac{8 A_1 d_{r_1} k_1 (d_r + A_1 k_1) k_2 k_r}{d_r} + d_r \Big(d_{r_1} \Big(1 + \frac{A_1 k_1}{d_r}\Big) + k_{1_r}\Big)^2 \big(d_{r_1} + k_{2_r}\big)\Big)\Big) - \\
&\quad \frac{1}{d_r} A_1 d_{r_1} k_1 \sqrt{\Big(d_r \big(d_{r_1} + k_{2_r}\big)} \Big(\frac{8 A_1 d_{r_1} k_1 (d_r + A_1 k_1) k_2 k_r}{d_r} + \\
&\qquad d_r \Big(d_{r_1} \Big(1 + \frac{A_1 k_1}{d_r}\Big) + k_{1_r}\Big)^2 \big(d_{r_1} + k_{2_r}\big)\Big)\Big) - k_{1_r} \sqrt{\Big(d_r \big(d_{r_1} + k_{2_r}\big)} \\
&\qquad \Big(\frac{8 A_1 d_{r_1} k_1 (d_r + A_1 k_1) k_2 k_r}{d_r} + d_r \Big(d_{r_1} \Big(1 + \frac{A_1 k_1}{d_r}\Big) + k_{1_r}\Big)^2 \big(d_{r_1} + k_{2_r}\big)\Big)\Big)\Bigg)
\end{aligned}$$

$$\begin{aligned}
&\frac{1}{8\, d_r\, (d_r + A_2 k_1)^2\, k_2\, \alpha_2^2} \\
&\Big(\beta \gamma^2 d_r k_{1_r}^2 k_{2_r} + 2 \beta \gamma d_r^2 k_{1_r} k_{2_r} \alpha_1 + 2 \beta \gamma A_2 d_r k_1 k_{1_r} k_{2_r} \alpha_1 + \beta d_r^3 k_{2_r} \alpha_1^2 + \\
&\quad 2 \beta A_2 d_r^2 k_1 k_{2_r} \alpha_1^2 + \beta A_2^2 d_r k_1^2 k_{2_r} \alpha_1^2 + 4 A_2 d_r k_1 k_2 k_r \alpha_2 + \\
&\quad 4 A_2^2 k_1^2 k_2 k_r \alpha_2 + \gamma^2 d_r^2 k_{1_r}^2 \alpha_2 + 2 \gamma d_r^3 k_{1_r} \alpha_1 \alpha_2 + 2 \gamma A_2 d_r^2 k_1 k_{1_r} \alpha_1 \alpha_2 + \\
&\quad d_r^4 \alpha_1^2 \alpha_2 + 2 A_2 d_r^3 k_1 \alpha_1^2 \alpha_2 + A_2^2 d_r^2 k_1^2 \alpha_1^2 \alpha_2 - \gamma k_{1_r} \sqrt{\Big(d_r \big(\beta k_{2_r} + d_r \alpha_2\big)} \\
&\qquad \Big(8 A_2 k_1 (d_r + A_2 k_1) k_2 k_r \alpha_2 + d_r \big(\gamma k_{1_r} + (d_r + A_2 k_1) \alpha_1\big)^2 \big(\beta k_{2_r} + d_r \alpha_2\big)\Big)\Big) - \\
&\quad d_r \alpha_1 \sqrt{\Big(d_r \big(\beta k_{2_r} + d_r \alpha_2\big)} \Big(8 A_2 k_1 (d_r + A_2 k_1) k_2 k_r \alpha_2 + \\
&\qquad d_r \big(\gamma k_{1_r} + (d_r + A_2 k_1) \alpha_1\big)^2 \big(\beta k_{2_r} + d_r \alpha_2\big)\Big)\Big) - A_2 k_1 \alpha_1 \sqrt{\Big(d_r \big(\beta k_{2_r} + d_r \alpha_2\big)} \\
&\qquad \Big(8 A_2 k_1 (d_r + A_2 k_1) k_2 k_r \alpha_2 + d_r \big(\gamma k_{1_r} + (d_r + A_2 k_1) \alpha_1\big)^2 \big(\beta k_{2_r} + d_r \alpha_2\big)\Big)\Big)\Big)
\end{aligned}$$

$$
\begin{aligned}
&\Bigg(d_r^2\,(d_r + A_2 k_1)^2 \\
&\quad\Bigg(d_r\, d_{r_1}^3 + 2 A_1 d_{r_1}^3 k_1 + \frac{A_1^2 d_{r_1}^3 k_1^2}{d_r} + 4 A_1 d_{r_1} k_1 k_2 k_r + \frac{4 A_1^2 d_{r_1} k_1^2 k_2 k_r}{d_r} + 2 d_r d_{r_1}^2 k_{1_r} + \\
&\quad 2 A_1 d_{r_1}^2 k_1 k_{1_r} + d_r d_{r_1} k_{1_r}^2 + d_r d_{r_1}^2 k_{2_r} + 2 A_1 d_{r_1}^2 k_1 k_{2_r} + \frac{A_1^2 d_{r_1}^2 k_1^2 k_{2_r}}{d_r} + \\
&\quad 2 d_r d_{r_1} k_{1_r} k_{2_r} + 2 A_1 d_{r_1} k_1 k_{1_r} k_{2_r} + d_r k_{1_r}^2 k_{2_r} - d_{r_1} \sqrt{\Bigg(d_r\,\big(d_{r_1} + k_{2_r}\big)} \\
&\qquad\overline{\left(\frac{8 A_1 d_{r_1} k_1 (d_r + A_1 k_1)\, k_2 k_r}{d_r} + d_r \left(d_{r_1}\left(1 + \frac{A_1 k_1}{d_r}\right) + k_{1_r}\right)^2 \big(d_{r_1} + k_{2_r}\big)\right)\Bigg)} - \\
&\quad \frac{1}{d_r} A_1 d_{r_1} k_1 \sqrt{\Bigg(d_r\,\big(d_{r_1} + k_{2_r}\big)\,\Bigg(\frac{8 A_1 d_{r_1} k_1 (d_r + A_1 k_1)\, k_2 k_r}{d_r} +} \\
&\qquad\overline{d_r \left(d_{r_1}\left(1 + \frac{A_1 k_1}{d_r}\right) + k_{1_r}\right)^2 \big(d_{r_1} + k_{2_r}\big)\Bigg)\Bigg)} - k_{1_r} \sqrt{\Bigg(d_r\,\big(d_{r_1} + k_{2_r}\big)} \\
&\qquad\overline{\left(\frac{8 A_1 d_{r_1} k_1 (d_r + A_1 k_1)\, k_2 k_r}{d_r} + d_r \left(d_{r_1}\left(1 + \frac{A_1 k_1}{d_r}\right) + k_{1_r}\right)^2 \big(d_{r_1} + k_{2_r}\big)\right)\Bigg)}\Bigg) \\
&\quad \alpha_2^2\Bigg) \Bigg/ \Big(d_{r_1}^2\,(d_r + A_1 k_1)^2\,\Big(\beta \gamma^2 d_r k_{1_r}^2 k_{2_r} + 2 \beta \gamma d_r^2 k_{1_r} k_{2_r} \alpha_1 + \\
&\quad 2 \beta \gamma A_2 d_r k_1 k_{1_r} k_{2_r} \alpha_1 + \beta d_r^3 k_{2_r} \alpha_1^2 + 2 \beta A_2 d_r^2 k_1 k_{2_r} \alpha_1^2 + \\
&\quad \beta A_2^2 d_r k_1^2 k_{2_r} \alpha_1^2 + 4 A_2 d_r k_1 k_2 k_r \alpha_2 + 4 A_2^2 k_1^2 k_2 k_r \alpha_2 + \\
&\quad \gamma^2 d_r^2 k_{1_r}^2 \alpha_2 + 2 \gamma d_r^3 k_{1_r} \alpha_1 \alpha_2 + 2 \gamma A_2 d_r^2 k_1 k_{1_r} \alpha_1 \alpha_2 + d_r^4 \alpha_1^2 \alpha_2 + \\
&\quad 2 A_2 d_r^3 k_1 \alpha_1^2 \alpha_2 + A_2^2 d_r^2 k_1^2 \alpha_1^2 \alpha_2 - \gamma k_{1_r} \sqrt{\Big(d_r\,(\beta k_{2_r} + d_r \alpha_2)} \\
&\qquad\overline{\Big(8 A_2 k_1 (d_r + A_2 k_1)\, k_2 k_r \alpha_2 + d_r\,(\gamma k_{1_r} + (d_r + A_2 k_1)\,\alpha_1)^2\,(\beta k_{2_r} + d_r \alpha_2)\Big)\Big)} - \\
&\quad d_r \alpha_1 \sqrt{\Big(d_r\,(\beta k_{2_r} + d_r \alpha_2)\,\Big(8 A_2 k_1 (d_r + A_2 k_1)\, k_2 k_r \alpha_2 + d_r} \\
&\qquad\quad\overline{(\gamma k_{1_r} + (d_r + A_2 k_1)\,\alpha_1)^2\,(\beta k_{2_r} + d_r \alpha_2)\Big)\Big)} - A_2 k_1 \alpha_1 \sqrt{\Big(d_r\,(\beta k_{2_r} + d_r \alpha_2)} \\
&\qquad\overline{\Big(8 A_2 k_1 (d_r + A_2 k_1)\, k_2 k_r \alpha_2 + d_r\,(\gamma k_{1_r} + (d_r + A_2 k_1)\,\alpha_1)^2\,(\beta k_{2_r} + d_r \alpha_2)\Big)\Big)}\Big)\Big)
\end{aligned}
$$

Check once again that biologically relevant solutions have been achieved (i.e. concentrations not less than 0)

```
symbD1 /. baseparam /. d_r → 10
symbD2 /. baseparam /. d_r → 0.2
```

27.3195

37.3902

The expression denoted as f in equation (5) is determined, the additional assumptions are:

1)binding kinetics much faster than protein dynamics

2)Signal concentrations are equivalent ($A = A_1 = A_2$)

```
dissocf = symbf /. {k1_r → (K k1), k2_r → (K k2), A2 → A, A1 → A};
fastbindf = Simplify[Limit[Limit[dissocf, k2 → Infinity], k1 → Infinity]]
```

$$\left(d_r \left(K^3 d_r^2 + K d_r \left(2 A K d_{r_1} - \sqrt{K \left(K^3 d_r^2 + 2 A K^2 d_r d_{r_1} + A^2 d_{r_1} \left(K d_{r_1} + 8 k_r\right)\right)}\right) + A d_{r_1} \left(A K d_{r_1} + 4 A k_r - \sqrt{K \left(K^3 d_r^2 + 2 A K^2 d_r d_{r_1} + A^2 d_{r_1} \left(K d_{r_1} + 8 k_r\right)\right)}\right)\right) \alpha_2^2\right) \Big/ \left(d_{r_1}^2 \left(K \beta d_r (K\gamma + A \alpha_1)^2 + 4 A^2 k_r \alpha_2 - (K\gamma + A\alpha_1) \sqrt{K \beta d_r \left(K \beta d_r (K\gamma + A\alpha_1)^2 + 8 A^2 k_r \alpha_2\right)}\right)\right)$$

The maximum achievable limit of f occurs when $\alpha_1 = \alpha_2 = 1$

```
fmax = FullSimplify[fastbindf /. {α1 → 1, α2 → 1}]
```

$$-\left(d_r \left(K^3 d_r^2 + K d_r \left(2 A K d_{r_1} - \sqrt{K \left(K \left(K d_r + A d_{r_1}\right)^2 + 8 A^2 d_{r_1} k_r\right)}\right) + A d_{r_1} \left(A K d_{r_1} + 4 A k_r - \sqrt{K \left(K \left(K d_r + A d_{r_1}\right)^2 + 8 A^2 d_{r_1} k_r\right)}\right)\right)\right) \Big/ \left(d_{r_1}^2 \left(-K \beta (A + K\gamma)^2 d_r - 4 A^2 k_r + (A + K\gamma) \sqrt{K \beta d_r \left(K \beta (A + K\gamma)^2 d_r + 8 A^2 k_r\right)}\right)\right)$$

The minimum achievable limit of f occurs when $\alpha_1 = \alpha_2 = \frac{d_{r1}}{d_r}$

```
fmin = FullSimplify[fastbindf /. {α1 → dr1 / dr, α2 → dr1 / dr}]
```

$$\left(K^3 d_r^2 + K d_r \left(2 A K d_{r_1} - \sqrt{K \left(K \left(K d_r + A d_{r_1}\right)^2 + 8 A^2 d_{r_1} k_r\right)}\right) + A d_{r_1} \left(A K d_{r_1} + 4 A k_r - \sqrt{K \left(K \left(K d_r + A d_{r_1}\right)^2 + 8 A^2 d_{r_1} k_r\right)}\right)\right) \Big/ \left(K \beta \left(K \gamma d_r + A d_{r_1}\right)^2 + 4 A^2 d_{r_1} k_r - \left(K \gamma d_r + A d_{r_1}\right) \sqrt{K \beta \left(K \beta \left(K \gamma d_r + A d_{r_1}\right)^2 + 8 A^2 d_{r_1} k_r\right)}\right)$$

Compute f for irreversible (i.e. the dissociation constant, K=0) binding kinetics

```
Limit[fastbindf, K → 0]
```

$$\frac{d_r \alpha_2}{d_{r_1}}$$