**Supplementary File 2**

**Model 1: Berkeley Madonna model for XLX variants (nvar >= 3)**

METHOD RK4

STARTTIME = 0

STOPTIME = 10

DT = 0.001

{\*\*\* Initial parameters \*\*\*}

{rate constants (in µM and min, as appropriate)}

k\_on = 0.96

k\_off = 0.085

k\_step\_wt = 4.27359

k\_init = k\_step\_wt

k\_block\_wt = 0.31

k\_fail\_wt = 0.24189

k\_step\_var = 5

k\_block\_var = 0.1

k\_fail\_var = 0.1

{supplied concentrations (µM)}

Lg = 0.005 {11S concentration (final)}

A0 = 0.004 {starting SecYEG concentration}

B0 = 1 {starting pre-protein concentration}

{additional parameters}

n\_wt = 5

n\_var = 5

brightness = 471.082 {how much signal is produced per nLuc}

{\*\*\* Reaction setup \*\*\*}

{initiate concentrations}

b\_quad = A0 + B0 + (k\_off / k\_on)

INIT C = (b\_quad - SQRT((b\_quad \* b\_quad) - (4 \* A0 \* B0))) / 2

INIT A = A0 - C

INIT B = B0 - C

INIT D[0..n\_wt] = 0

INIT E[1..n\_var] = 0

{\*\*\* Differential equations \*\*\*}

d/dt (A) = (C \* k\_off) - (A \* B \* k\_on) + (ARRAYSUM(D[\*]) \* k\_fail\_wt) + ((ARRAYSUM(E[\*]) - E[n\_var]) \* k\_fail\_var)

d/dt (B) = (C \* k\_off) - (A \* B \* k\_on) + (ARRAYSUM(D[\*]) \* k\_fail\_wt) + ((ARRAYSUM(E[\*]) - E[n\_var]) \* k\_fail\_var)

d/dt (C) = (A \* B \* k\_on) - (C \* k\_off) - (C \* k\_init)

d/dt (D[0]) = (C \* k\_init) - (D[0] \* k\_step\_wt) - (D[0] \* k\_block\_wt) - (D[0] \* k\_fail\_wt)

d/dt (D[1..n\_wt-1]) = (D[i-1] \* k\_step\_wt) - (D[i] \* k\_step\_wt) - (D[i] \* k\_block\_wt) - (D[i] \* k\_fail\_wt)

d/dt (D[n\_wt]) = (D[n\_wt-1] \* k\_step\_wt) - (D[n\_wt] \* k\_step\_var) - (D[n\_wt] \* k\_block\_var) - (D[n\_wt] \* k\_fail\_var)

d/dt (E[1]) = (D[n\_wt] \* k\_step\_var) - (E[1] \* k\_step\_var) - (E[1] \* k\_block\_var) - (E[1] \* k\_fail\_var)

d/dt (E[2..n\_var-1]) = (E[i-1] \* k\_step\_var) - (E[i] \* k\_step\_var) - (E[i] \* k\_block\_var) - (E[i] \* k\_fail\_var)

d/dt (E[n\_var]) = (E[n\_var-1] \* k\_step\_var)

{\*\*\* Output \*\*\*}

signal = min(E[n\_var], Lg) \* brightness

**Model 2: Berkeley Madonna model for XLX variants (nvar = 2)**

METHOD RK4

STARTTIME = 0

STOPTIME = 10

DT = 0.001

{\*\*\* Initial parameters \*\*\*}

{rate constants (in µM and min, as appropriate)}

k\_on = 0.96

k\_off = 0.085

k\_step\_wt = 4.27359

k\_init = k\_step\_wt

k\_block\_wt = 0.31

k\_fail\_wt = 0.24189

k\_step\_var = 5

k\_block\_var = 0.1

k\_fail\_var = 0.1

{supplied concentrations (µM)}

Lg = 0.005 {11S concentration (final)}

A0 = 0.004 {starting SecYEG concentration}

B0 = 1 {starting pre-protein concentration}

{additional parameters}

n\_wt = 5

n\_var = 2

brightness = 471.082 {how much signal is produced per nLuc}

{\*\*\* Reaction setup \*\*\*}

{initiate concentrations}

b\_quad = A0 + B0 + (k\_off / k\_on)

INIT C = (b\_quad - SQRT((b\_quad \* b\_quad) - (4 \* A0 \* B0))) / 2

INIT A = A0 - C

INIT B = B0 - C

INIT D[0..n\_wt] = 0

INIT E[1..n\_var] = 0

{\*\*\* Differential equations \*\*\*}

d/dt (A) = (C \* k\_off) - (A \* B \* k\_on) + (ARRAYSUM(D[\*]) \* k\_fail\_wt) + ((ARRAYSUM(E[\*]) - E[n\_var]) \* k\_fail\_var)

d/dt (B) = (C \* k\_off) - (A \* B \* k\_on) + (ARRAYSUM(D[\*]) \* k\_fail\_wt) + ((ARRAYSUM(E[\*]) - E[n\_var]) \* k\_fail\_var)

d/dt (C) = (A \* B \* k\_on) - (C \* k\_off) - (C \* k\_init)

d/dt (D[0]) = (C \* k\_init) - (D[0] \* k\_step\_wt) - (D[0] \* k\_block\_wt) - (D[0] \* k\_fail\_wt)

d/dt (D[1..n\_wt-1]) = (D[i-1] \* k\_step\_wt) - (D[i] \* k\_step\_wt) - (D[i] \* k\_block\_wt) - (D[i] \* k\_fail\_wt)

d/dt (D[n\_wt]) = (D[n\_wt-1] \* k\_step\_wt) - (D[n\_wt] \* k\_step\_var) - (D[n\_wt] \* k\_block\_var) - (D[n\_wt] \* k\_fail\_var)

d/dt (E[1]) = (D[n\_wt] \* k\_step\_var) - (E[1] \* k\_step\_var) - (E[1] \* k\_block\_var) - (E[1] \* k\_fail\_var)

d/dt (E[2]) = (E[1] \* k\_step\_var)

{\*\*\* Output \*\*\*}

signal = min(E[2], Lg) \* brightness