Supplementary Text S1. Computational Modeling

All computational models were created using Matlab. For all GRN models in this study (except for AC-DC motif; see below), the activity of an enhancer ***E*** of gene ***X*** to which N_a activators ($\boldsymbol{A}_i$, i=1 to N_a) and N_r repressors ($\boldsymbol{R}_j$, j=1 to N_r) bind is modelled using AND gated Hill functions of individual binding proteins:

$$E = \prod_{i=1}^{Na} \frac{(A_i/K_i)^{na_i}}{1 + (A_i/K_i)^{na_i}} \prod_{j=1}^{Nr} \frac{1}{1 + (R_j/K_j')^{nr_j}}$$

where E is the activity of enhancer ***E***, A_i is the concentration of activator $\boldsymbol{A}_i$, R_j is the concentration of repressor $\boldsymbol{R}_j$, na_i is the cooperativity of $\boldsymbol{A}_i$ binding to the enhancer **E**, nr_j is the cooperativity of $\boldsymbol{R}_j$ binding to the enhancer ***E***, K_i is the dissociation constant of activator $\boldsymbol{A}_i$, and K'_j is the dissociation constant of repressor $\boldsymbol{R}_j$.

Below are complete differential equations for GRN models used in this study.

French Flag GRN 1

The following are the equations used for modeling 5-genes French Flag GRN 1 (5-genes version of the GRN in Figure 1A''). $\boldsymbol{X}_i$ is the mRNA concentration transcribed by gene $\boldsymbol{X}_i$. G is the concentration of the gradient ***G***. Maximum concentration of ***G*** is 1.5 (see Gradient Setup section below). Autorepression links are added for steady-state level control.

$$\frac{dX_1}{dt} = \frac{(\frac{G}{0.2})^3}{1 + (\frac{G}{0.2})^3} \times \frac{1}{1 + \left(\frac{X_2}{2.5}\right)^5} \times \frac{1}{1 + \left(\frac{X_3}{0.2}\right)^5} \times \frac{1}{1 + \left(\frac{X_4}{0.2}\right)^5} \times \frac{1}{1 + \left(\frac{X_5}{0.2}\right)^5} \times \frac{1}{1 + \left(\frac{X_1}{0.8}\right)^5} - X_1$$

$$\frac{dX_2}{dt} = \frac{(\frac{G}{0.6})^3}{1 + (\frac{G}{0.6})^3} \times \frac{1}{1 + \left(\frac{X_3}{2.5}\right)^5} \times \frac{1}{1 + \left(\frac{X_4}{0.2}\right)^5} \times \frac{1}{1 + \left(\frac{X_5}{0.2}\right)^5} \times \frac{1}{1 + \left(\frac{X_2}{0.8}\right)^5} - X_2$$

$$\frac{dX_3}{dt} = \frac{(\frac{G}{1})^3}{1 + (\frac{G}{1})^3} \times \frac{1}{1 + \left(\frac{X_4}{2.5}\right)^5} \times \frac{1}{1 + \left(\frac{X_5}{0.2}\right)^5} \times \frac{1}{1 + \left(\frac{X_3}{0.8}\right)^5} - X_3$$

$$\frac{dX_4}{dt} = \frac{(\frac{G}{1.4})^3}{1 + (\frac{G}{1.4})^3} \times \frac{1}{1 + \left(\frac{X_5}{0.4}\right)^5} \times \frac{1}{1 + \left(\frac{X_4}{0.8}\right)^5} - X_4$$

$$\frac{dX_5}{dt} = \frac{(\frac{G}{1.8})^3}{1 + (\frac{G}{1.8})^3} \times \frac{1}{1 + \left(\frac{X_5}{0.8}\right)^5} - X_5$$

<u>Initial conditions:</u> X_1=X_2=X_3=X_4=X_5=0.

Speed Regulation GRN (Module Switching model)

The transcription rate of gene ***X*** (*X*) regulated by two separate enhancers (***E***$_1$ and ***E***$_2$) is modeled as a weighted sum of the activity of the two enhancers: $= c_1E_1 + c_2E_2$. The following are the equations used for modeling the 5-genes gradual enhancer switching model (5-genes version of the GRN in Figure 1B''). X_{Di} is the activity of the dynamic enhancer of gene ***X****$_i$*. X_{Si} is the activity of the static enhancer of gene ***X****$_i$*. ***X****$_i$* is the mRNA concentration transcribed by gene ***X****$_i$*. *G* is the concentration of the (speed regulator) gradient ***G***.

<u>Dynamic Enhancers</u>:

$$\frac{dX_{D1}}{dt} = \frac{G}{1+G} \times \frac{1}{1 + \left(\frac{X_2}{0.4}\right)^5} \times \frac{1}{1 + \left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1 + \left(\frac{X_4}{0.4}\right)^5} \times \frac{1}{1 + \left(\frac{X_5}{0.4}\right)^5}$$

$$\frac{dX_{D2}}{dt} = \frac{G}{1+G} \times \frac{1}{1 + \left(\frac{X_1}{2.5}\right)^5} \times \frac{1}{1 + \left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1 + \left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1 + \left(\frac{X_5}{0.4}\right)^5}$$

$$\frac{dX_{D3}}{dt} = \frac{G}{1+G} \times \frac{1}{1 + \left(\frac{X_1}{0.4}\right)^5} \times \frac{1}{1 + \left(\frac{X_2}{2.5}\right)^5} \times \frac{1}{1 + \left(\frac{X_4}{0.4}\right)^5} \times \frac{1}{1 + \left(\frac{X_5}{0.4}\right)^5}$$

$$\frac{dX_{D4}}{dt} = \frac{G}{1+G} \times \frac{1}{1 + \left(\frac{X_1}{0.4}\right)^5} \times \frac{1}{1 + \left(\frac{X_2}{0.4}\right)^5} \times \frac{1}{1 + \left(\frac{X_3}{2.5}\right)^5} \times \frac{1}{1 + \left(\frac{X_5}{0.4}\right)^5}$$

$$\frac{dX_{D5}}{dt} = \frac{G}{1+G} \times \frac{1}{1+\left(\frac{X_1}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_2}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_4}{2.5}\right)^5}$$

Static Enhancers:

$$\frac{dX_{S1}}{dt} = \frac{1}{1+G} \times \frac{1}{1+\left(\frac{X_2}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_4}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_5}{0.4}\right)^5}$$

$$\frac{dX_{S2}}{dt} = \frac{1}{1+G} \times \frac{1}{1+\left(\frac{X_1}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_4}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_5}{0.4}\right)^5}$$

$$\frac{dX_{S3}}{dt} = \frac{1}{1+G} \times \frac{1}{1+\left(\frac{X_1}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_2}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_4}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_5}{0.4}\right)^5}$$

$$\frac{dX_{S4}}{dt} = \frac{1}{1+G} \times \frac{1}{1+\left(\frac{X_1}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_2}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_5}{0.4}\right)^5}$$

$$\frac{dX_{S5}}{dt} = \frac{1}{1+G} \times \frac{1}{1+\left(\frac{X_1}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_2}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_4}{0.4}\right)^5}$$

Combining the activities of Dynamic and Static Enhancers:

$$\frac{dX_1}{dt} = 3\frac{dX_{D1}}{dt} + 2\frac{dX_{S1}}{dt} - X_1$$

$$\frac{dX_2}{dt} = 3\frac{dX_{D2}}{dt} + 2\frac{dX_{S2}}{dt} - X_2$$

$$\frac{dX_3}{dt} = 3\frac{dX_{D3}}{dt} + 2\frac{dX_{S3}}{dt} - X_3$$

$$\frac{dX_4}{dt} = 3\frac{dX_{D4}}{dt} + 2\frac{dX_{S4}}{dt} - X_4$$

$$\frac{dX_5}{dt} = 3\frac{dX_{D5}}{dt} + 2\frac{dX_{S5}}{dt} - X_5$$

Initial conditions: $X_1 = 0.1$, $X_2=X_3=X_4=X_5=0$.

3-Genes French Flag GRN 2 (AC-DC circuit motif)

Here repressors are assumed to have overlapping recognition sequences and repression strengths are controlled by the number of binding sites (cooperativity). Below is the differential equations of a 3-Genes French Flag GRN 2 (Figure S1 A). Maximum concentration of the gradient *G* is 2.

$$\frac{dX_1}{dt} = 3 \times \frac{1}{1 + {X_2}^2 + {X_3}^6} - X_1$$

$$\frac{dX_2}{dt} = 5 \times \frac{G}{1+G} \times \frac{1}{1 + {X_3}^5} - X_2$$

$$\frac{dX_3}{dt} = 5 \times \frac{G}{1+G} \times \frac{1}{1 + X_1 + X_2} - X_3$$

<u>Initial conditions:</u> $X_1 = 5$, $X_2 = X_3 = 0$.

4-Genes French Flag GRN 2 (AC-DC circuit motif)

Repressors are assumed to have overlapping recognition sequences and repression strengths are controlled by the number of binding sites (cooperativity). Below is the differential equations of a 4-Genes French Flag GRN 2 (Figure S1 B). Maximum concentration of the gradient *G* is 2.

$$\frac{dX_1}{dt} = 3 \times \frac{1}{1 + X_2 + {X_3}^6 + {X_4}^6} - X_1$$

$$\frac{dX_2}{dt} = 5 \times \frac{G}{1+G} \times \frac{1}{1 + {X_3}^6 + {X_4}^6} - X_2$$

$$\frac{dX_3}{dt} = 8 \times \frac{G}{1+G} \times \frac{1}{1 + X_1 + X_2 + {X_4}^6} - X_3$$

$$\frac{dX_4}{dt} = 5 \times \frac{G}{1+G} \times \frac{1}{1 + X_1 + X_2 + X_3} - X_4$$

<u>Initial conditions:</u> $X_1 = 5$, $X_2 = X_3 = X_4 = 0$.

French Flag with a Timer Gene GRN 1

Same as French Flag GRN 1 but with gradient *G* replaced with a Timer Gene *TG* as activator of genes X_i, i=1 to 5. *TG* is activated by the gradient *G* and has zero decay rate:

$$\frac{dTG}{dt} = 0.05 \times G$$

Speed Regulation model realization by jointly modulating gene activation and gene product decay rates

Another realization of the Speed Regulation model is for the morphogen gradient to activate the constituent genes of a genetic cascade (or oscillator) and at the same time positively regulate their decay rates. The following is a set of differential equations realizing this concept. $\boldsymbol{X_i}$ is the mRNA concentration transcribed by gene $\boldsymbol{X_i}$. G is the concentration of the (speed regulator) gradient $\boldsymbol{G}$.

$$\frac{dX_1}{dt} = \frac{G}{1+G}\left(\frac{1}{1+\left(\frac{X_2}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_4}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_5}{0.4}\right)^5} - 3X_1\right)$$

$$\frac{dX_2}{dt} = \frac{G}{1+G}\left(\frac{1}{1+\left(\frac{X_1}{2.5}\right)^5} \times \frac{1}{1+\left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_5}{0.4}\right)^5} - 3X_2\right)$$

$$\frac{dX_3}{dt} = 2 \times \frac{G}{1+G}\left(\frac{1}{1+\left(\frac{X_1}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_2}{2.5}\right)^5} \times \frac{1}{1+\left(\frac{X_4}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_5}{0.4}\right)^5} - 3X_3\right)$$

$$\frac{dX_4}{dt} = 2 \times \frac{G}{1+G}\left(\frac{1}{1+\left(\frac{X_1}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_2}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_3}{2.5}\right)^5} \times \frac{1}{1+\left(\frac{X_5}{0.4}\right)^5} - 3X_4\right)$$

$$\frac{dX_5}{dt} = 2 \times \frac{G}{1+G}\left(\frac{1}{1+\left(\frac{X_1}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_2}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_3}{0.4}\right)^5} \times \frac{1}{1+\left(\frac{X_4}{2.5}\right)^5} - 3X_5\right)$$