

Supplementary Information for: Systematic comparison of the response properties of protein and RNA mediated gene regulatory motifs.

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1 Equations and parameters

1.1 Functions F and G used in the ODEs

	$F_{r_{out}}$	$G_{r_{out}}$	$F_{p_{out}}$	$F_{r_{ctrl}}$
unreg	$\frac{[I]}{K_{I_{r_{out}}} + [I]}$	1	1	1
tcFFLp	$\frac{[I]}{K_{I_{r_{out}}} + [I]} \times \frac{K_{p_{ctrl}^{r_{out}}}}{[p_{ctrl}] + K_{p_{ctrl}^{r_{out}}}}$	1	1	$\frac{[I]}{K_{I_{r_{ctrl}}} + [I]}$
ptFFLp	$\frac{[I]}{K_{I_{r_{out}}} + [I]}$	$1 + \lambda_{p_{ctrl}^{r_{out}}} [p_{ctrl}]$	1	$\frac{[I]}{K_{I_{r_{ctrl}}} + [I]}$
tlFFLp	$\frac{[I]}{K_{I_{r_{out}}} + [I]}$	1	$\frac{K_{p_{out}^{p_{ctrl}}}}{[p_{ctrl}] + K_{p_{out}^{p_{ctrl}}}}$	$\frac{[I]}{K_{I_{r_{ctrl}}} + [I]}$
tcFFLr	$\frac{[I]}{K_{I_{r_{out}}} + [I]} \times \frac{K_{r_{ctrl}^{r_{out}}}}{[r_{ctrl}] + K_{r_{ctrl}^{r_{out}}}}$	1	1	$\frac{[I]}{K_{I_{r_{ctrl}}} + [I]}$
ptFFLr	$\frac{[I]}{K_{I_{r_{out}}} + [I]}$	$1 + \lambda_{r_{ctrl}^{r_{out}}} [r_{ctrl}]$	1	$\frac{[I]}{K_{I_{r_{ctrl}}} + [I]}$
tlFFLr	$\frac{[I]}{K_{I_{r_{out}}} + [I]}$	1	$\frac{K_{r_{out}^{r_{ctrl}}}}{[r_{ctrl}] + K_{r_{out}^{r_{ctrl}}}}$	$\frac{[I]}{K_{I_{r_{ctrl}}} + [I]}$
tcFBLp	$\frac{[I]}{K_{I_{r_{out}}} + [I]} \times \frac{K_{p_{ctrl}^{r_{out}}}}{[p_{ctrl}] + K_{p_{ctrl}^{r_{out}}}}$	1	1	$\frac{[p_{out}]}{K_{p_{out}^{r_{ctrl}}} + [p_{out}]}$
ptFBLp	$\frac{[I]}{K_{I_{r_{out}}} + [I]}$	$1 + \lambda_{p_{ctrl}^{r_{out}}} [p_{ctrl}]$	1	$\frac{[p_{out}]}{K_{p_{out}^{r_{ctrl}}} + [p_{out}]}$
tlFBLp	$\frac{[I]}{K_{I_{r_{out}}} + [I]}$	1	$\frac{K_{p_{out}^{p_{ctrl}}}}{[p_{ctrl}] + K_{p_{out}^{p_{ctrl}}}}$	$\frac{[p_{out}]}{K_{p_{out}^{r_{ctrl}}} + [p_{out}]}$
tcFBLr	$\frac{[I]}{K_{I_{r_{out}}} + [I]} \times \frac{K_{r_{ctrl}^{r_{out}}}}{[r_{ctrl}] + K_{r_{ctrl}^{r_{out}}}}$	1	1	$\frac{[p_{out}]}{K_{p_{out}^{r_{ctrl}}} + [p_{out}]}$
ptFBLr	$\frac{[I]}{K_{I_{r_{out}}} + [I]}$	$1 + \lambda_{r_{ctrl}^{r_{out}}} [r_{ctrl}]$	1	$\frac{[p_{out}]}{K_{p_{out}^{r_{ctrl}}} + [p_{out}]}$
tlFBLr	$\frac{[I]}{K_{I_{r_{out}}} + [I]}$	1	$\frac{K_{r_{out}^{r_{ctrl}}}}{[r_{ctrl}] + K_{r_{out}^{r_{ctrl}}}}$	$\frac{[p_{out}]}{K_{p_{out}^{r_{ctrl}}} + [p_{out}]}$

Tab. S1: Mathematical functions used in the ODEs for different motifs

1.2 Parameter Values

Parameter	Value	Reference & Remarks
$\mathbf{b}_{r_{out}}$	$1.5 \times 10^{-3} \text{ nM.min}^{-1}$	Approx transcription rate of 1.2kb/min for a 1.2kb long RNA in a diploid cell ^[1]
$\mathbf{b}_{r_{ctrl}}$	$1.5 \times 10^{-3} \text{ nM.min}^{-1}$	Same as $\mathbf{b}_{r_{out}}$
$\mathbf{b}_{p_{out}}$	1.65 min^{-1}	Translation rate of 400 amino acid (aa) protein at 11 aa/sec ^[2]
$\mathbf{b}_{p_{ctrl}}$	1.65 min^{-1}	Same as $\mathbf{b}_{p_{out}}$
$\mathbf{d}_{r_{out}}$	$1.2 \times 10^{-3} \text{ min}^{-1}$	Average human mRNA degradation rate ^[3]
$\mathbf{d}_{r_{ctrl}}$	$1.2 \times 10^{-3} \text{ min}^{-1}$	Same as $\mathbf{d}_{r_{out}}$
$\mathbf{d}_{p_{out}}$	$1.3 \times 10^{-4} \text{ min}^{-1}$	Average human protein degradation rate ^[3]
$\mathbf{d}_{p_{ctrl}}$	$1.3 \times 10^{-4} \text{ min}^{-1}$	Same as $\mathbf{d}_{p_{out}}$
$K_{I_{r_{out}}}$	100 nM	Arbitrary
$K_{p_{ctrl}_{r_{out}}}$	30 nM	Average Hill constant ^[4]
$K_{r_{ctrl}_{r_{out}}}$	1 nM	Arbitrary (assumed to be an order of magnitude less than $K_{p_{ctrl}_{r_{out}}}$)
$K_{I_{r_{ctrl}}}$	100 nM	Same as $K_{I_{r_{out}}}$
$K_{p_{out}_{r_{ctrl}}}$	30 nM	Average Hill constant ^[4]
$K_{p_{ctrl}_{p_{out}}}$	50 nM	Similar to $K_{p_{ctrl}_{r_{out}}}$
$K_{r_{ctrl}_{p_{out}}}$	5 nM	Similar to $K_{r_{ctrl}_{r_{out}}}$
$\lambda_{p_{ctrl}_{r_{out}}}$	$1/K_{p_{ctrl}_{r_{out}}}$	-
$\lambda_{r_{ctrl}_{r_{out}}}$	$1/K_{r_{ctrl}_{r_{out}}}$	-
Input	$4000 - 8000 \text{ molecules/cell}$ $\approx 3 - 6 \text{ nM}$	Assumed
Volume of the cell	$2.25 \times 10^{-12} \text{ l}$	Volume of Hela cell ^[5]

Tab. S2: Default parameter values

1.3 Analytical expressions for steady state

1.3.1 Feedforward Motifs

The function relating the output steady state to the input, was similar in structure for all feedforward motifs. It has the basic form of:

$$p_o^* = \frac{K_{I_{r_{ctrl}}} + I}{K_{I_{r_{out}}} + I} \times R \frac{I}{I + Q}$$

A part of this expression resembles a saturating function, which may be responsible for the shapes of the curves seen in Fig. 4B. The expressions for R and Q for different feedforward motifs are listed in the table below:

Motif	R	Q
tcFFLp	$\frac{\mathcal{S}_o K_{r_{out}}^{p_{ctrl}}}{\mathcal{S}_c + K_{r_{out}}^{p_{ctrl}}}$	$\frac{K_{r_{out}}^{p_{ctrl}} K_{r_{ctrl}}}{\mathcal{S}_c + K_{r_{out}}^{p_{ctrl}}}$
ptFFLp	$\frac{\mathcal{S}_o}{1 + (\mathcal{S}_c / K_{r_{out}}^{p_{ctrl}})}$	$\frac{K_{r_{ctrl}}}{1 + \mathcal{S}_c \lambda_{r_{out}}^{p_{ctrl}}}$
tlFFLp	$\frac{\mathcal{S}_o K_{p_{out}}^{p_{ctrl}}}{\mathcal{S}_c + K_{p_{out}}^{p_{ctrl}}}$	$\frac{K_{p_{out}}^{p_{ctrl}} K_{r_{ctrl}}}{\mathcal{S}_c + K_{p_{out}}^{p_{ctrl}}}$
tcFFLr	$\frac{\mathcal{S}_o K_{r_{out}}^{r_{ctrl}}}{s_{r_c} + K_{r_{out}}^{r_{ctrl}}}$	$\frac{K_{r_{out}}^{r_{ctrl}} K_{r_{ctrl}}}{s_{r_c} + K_{r_{out}}^{r_{ctrl}}}$
ptFFLr	$\frac{\mathcal{S}_o}{1 + (s_{r_c} / K_{r_{out}}^{r_{ctrl}})}$	$\frac{K_{r_{ctrl}}}{1 + s_{r_c} \lambda_{r_{out}}^{r_{ctrl}}}$
tlFFLr	$\frac{\mathcal{S}_o K_{p_{out}}^{r_{ctrl}}}{s_{r_c} + K_{p_{out}}^{r_{ctrl}}}$	$\frac{K_{p_{out}}^{r_{ctrl}} K_{r_{ctrl}}}{s_{r_c} + K_{p_{out}}^{r_{ctrl}}}$

Tab. S3: Expressions for Q and R for different feedforward motifs. s_{r_c} denotes the ratio of formation and degradation rate constants for controller RNA ($b_{r_{ctrl}}/d_{r_{ctrl}}$). $\mathcal{S}_o$ denotes the ratio of formation and degradation rate constants for output mRNA times the ratio of formation and degradation rate constants for the output protein (similarly for $\mathcal{S}_c$). $\mathcal{S}_o = b_{r_{out}}/d_{r_{out}} \times b_{p_{out}}/d_{p_{out}}$

1.3.2 Feedback Motifs

For the feedback motifs, the output steady state was the positive root of a quadratic function of the input. The expression for output steady state for different feedback motifs is shown in the following table.

Motif	p_o^*
tcFBLp	$\frac{K_{r_{out}}^{p_{ctrl}} (\mathcal{S}_o \mathcal{H} - K_{r_{ctrl}}^{p_{out}}) + \sqrt{4\mathcal{S}_o \mathcal{S}_c K_{r_{out}}^{p_{ctrl}} K_{r_{ctrl}}^{p_{out}} \mathcal{H} + (K_{r_{out}}^{p_{ctrl}} K_{r_{ctrl}}^{p_{out}} + \mathcal{S}_o \mathcal{H} K_{r_{out}}^{p_{ctrl}})^2}}{2(\mathcal{S}_c + K_{r_{out}}^{p_{ctrl}})}$
ptFBLp	$\frac{\mathcal{S}_o \mathcal{H} - K_{r_{ctrl}}^{p_{out}} + \sqrt{(\mathcal{S}_o \mathcal{H} + K_{r_{ctrl}}^{p_{out}})^2 + 4K_{r_{ctrl}}^{p_{out}} \mathcal{H} \mathcal{S}_o \mathcal{S}_c \lambda_{r_{out}}^{p_{ctrl}}}}{2 + 2\lambda_{r_{out}}^{p_{ctrl}} \mathcal{S}_c}$
tlFBLp	$\frac{K_{p_{out}}^{p_{ctrl}} (\mathcal{S}_o \mathcal{H} - K_{r_{ctrl}}^{p_{out}}) + \sqrt{4\mathcal{S}_o \mathcal{S}_c K_{p_{out}}^{p_{ctrl}} K_{r_{ctrl}}^{p_{out}} \mathcal{H} + (K_{p_{out}}^{p_{ctrl}} K_{r_{ctrl}}^{p_{out}} + \mathcal{S}_o \mathcal{H} K_{p_{out}}^{p_{ctrl}})^2}}{2(\mathcal{S}_c + K_{p_{out}}^{p_{ctrl}})}$
tcFBLr	$\frac{K_{r_{out}}^{r_{ctrl}} (\mathcal{S}_o \mathcal{H} - K_{r_{ctrl}}^{p_{out}}) + \sqrt{4\mathcal{S}_o s_{r_c} K_{r_{out}}^{r_{ctrl}} K_{r_{ctrl}}^{p_{out}} \mathcal{H} + (K_{r_{out}}^{r_{ctrl}} K_{r_{ctrl}}^{p_{out}} + \mathcal{S}_o \mathcal{H} K_{r_{out}}^{r_{ctrl}})^2}}{2(s_{r_c} + K_{r_{out}}^{r_{ctrl}})}$
ptFBLr	$\frac{\mathcal{S}_o \mathcal{H} - K_{r_{ctrl}}^{p_{out}} + \sqrt{(\mathcal{S}_o \mathcal{H} + K_{r_{ctrl}}^{p_{out}})^2 + 4K_{r_{ctrl}}^{p_{out}} \mathcal{H} \mathcal{S}_o s_{r_c} \lambda_{r_{out}}^{r_{ctrl}}}}{2 + 2\lambda_{r_{out}}^{r_{ctrl}} s_{r_c}}$
tlFBLr	$\frac{K_{p_{out}}^{r_{ctrl}} (\mathcal{S}_o \mathcal{H} - K_{r_{ctrl}}^{p_{out}}) + \sqrt{4\mathcal{S}_o s_{r_c} K_{p_{out}}^{r_{ctrl}} K_{r_{ctrl}}^{p_{out}} \mathcal{H} + (K_{p_{out}}^{r_{ctrl}} K_{r_{ctrl}}^{p_{out}} + \mathcal{S}_o \mathcal{H} K_{p_{out}}^{r_{ctrl}})^2}}{2(s_{r_c} + K_{p_{out}}^{r_{ctrl}})}$

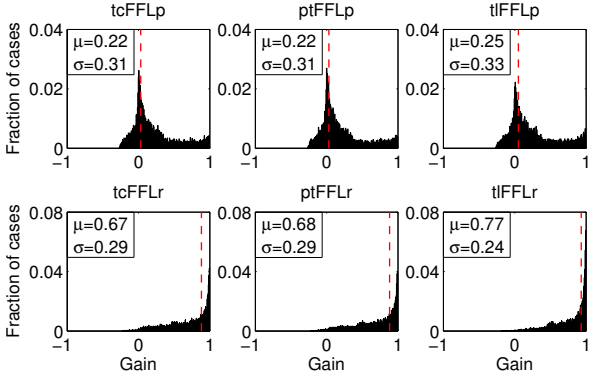
Tab. S4: Expressions for output steady state for different feedback motifs. The symbols s_{r_c} , $\mathcal{S}_o$ and $\mathcal{S}_c$ are as described in Tab. S 3. $\mathcal{H} = \frac{K_{r_{out}}}{[I] + K_{r_{out}}}$, $\lambda_{r_{out}}^{p_{ctrl}} = \frac{1}{K_{r_{out}}^{p_{ctrl}}}$, $\lambda_{r_{out}}^{r_{ctrl}} = \frac{1}{K_{r_{out}}^{r_{ctrl}}}$

2 Figures

2.1 Steady state properties of feedback and feedforward motifs for the broad range of parameter variation

Following figures show the distribution of steady state gain and input-output characteristics for different motifs for parameter sets sampled from the broad range i.e. $1/20$ to 20 times the default values described in Tab. S 2.

(A) FFL



(B) FBL

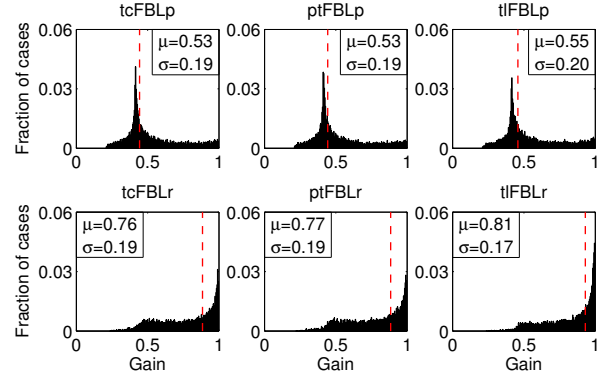
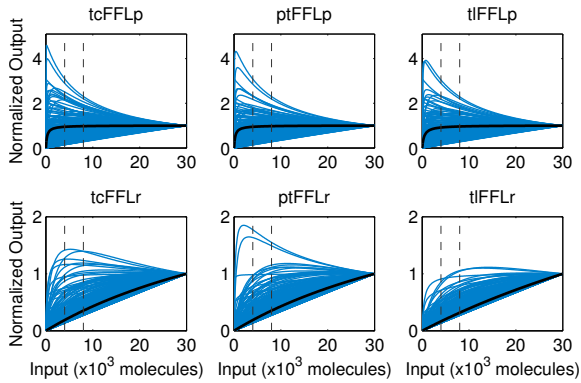


Fig. S1: Steady state gain distributions for (A) feedforward motifs and (B) feedback motifs. Distribution of steady state gain to input step change from 4000 to 8000 molecules, with randomly sampled parameter sets. The dashed red vertical lines denote the gain values corresponding to the default parameter set.

(A) FFL



(B) FBL

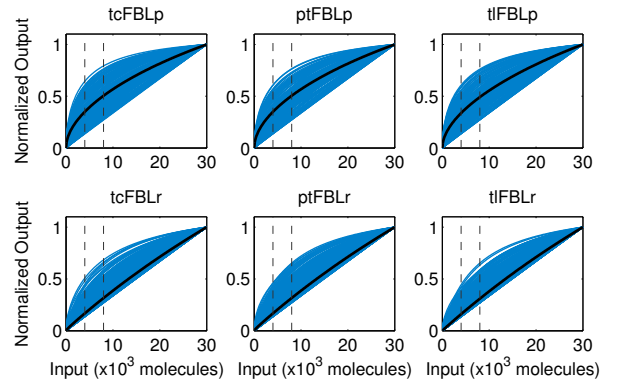
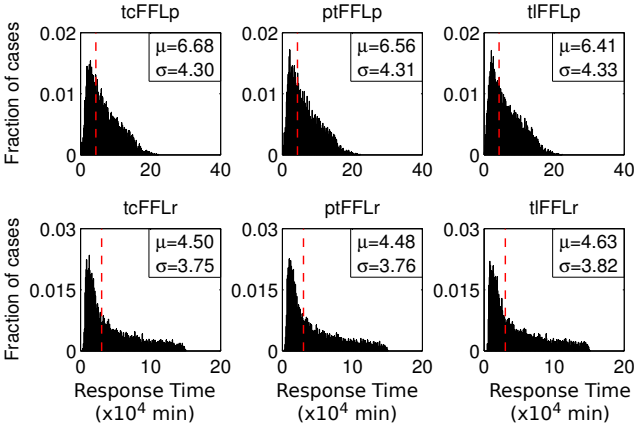


Fig. S2: Input-output characteristics for 200 randomly selected cases of (A) feedforward and (B) feedback motifs. The steady state values at each value of input, are normalized with the value at the maximum value of input (30×10^3). Black line represents the characteristics of the default parameter set. Dashed vertical lines (from left to right) denote input corresponding to 4000 and 8000 molecules respectively.

2.2 Response times

(A) FFL



(B) FBL

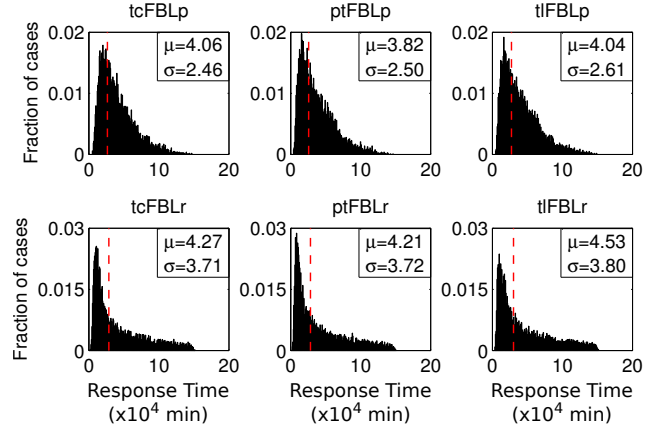
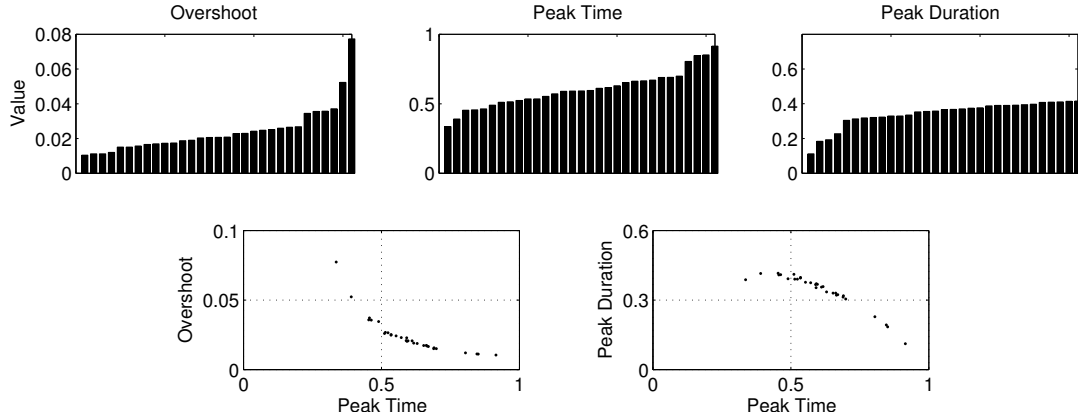


Fig. S3: Response time distributions for (A) feedforward and (B) feedback motifs to step change in the input for the normal range of parameter variation. Dashed vertical red lines denote values corresponding to the default parameter set. Protein mediated FFLs have a higher mean response time, compared to the RNA mediated FFLs. This difference was not seen in case of FBLs

2.3 Dynamic metrics of tIFFLr and tIFBLr

(A) tIFFLr



(B) tIFBLr

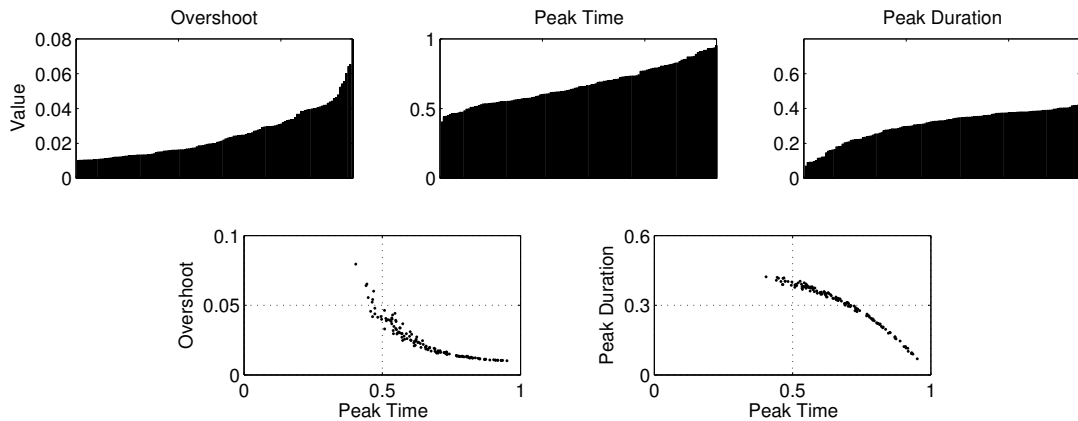


Fig. S4: Dynamic metrics of (A) tIFFLr and (B) tIFBLr excluded from the main text. The top three subplots in each subfigure are **bar graphs** of all the metric values sorted in an ascending order.

2.4 Motif properties at different degradation rates of p_{ctrl} and p_{out}

The following section shows the properties/distributions of different metrics at different degradation rate constants for p_{ctrl} and p_{out} (both are assumed to be the same). The cases chosen were $0.1\times$, $10\times$, $100\times$ and $1000\times$ of the original value.

Summary

	$1 \times d_p$	$0.1 \times d_p$	$10 \times d_p$	$100 \times d_p$	$1000 \times d_p$
Gain	Narrow distribution with peak at 0 for protein mediated FFLs. High (near 1) gain for all RNA mediated motifs.	Narrower distribution for protein mediated FFL (wrt $1 \times d_p$)	Broader distribution (all motifs); Higher mean for all protein mediated motifs; Lower mean for RNA mediated FFLs (wrt $1 \times d_p$)	Similar trend as $10\times d_p$ but enhanced	Very mild regulation by all motifs (peak at 1)
i/o char.	Saturating and in certain cases non-monotonic i/o characteristics for protein mediated FFLs	No significant change (wrt $1 \times d_p$)	Saturation point higher (wrt $1 \times d_p$)	Most cases of do not saturate and appear linear	Linear response like unregulated
Peaking tendency	Protein mediated motifs show more number of peaks compared to RNA mediated motifs	Same trend as $1 \times d_p$ but no peaks for RNA mediated motifs	Same trend as $1 \times d_p$ but lower for protein mediated motifs and higher for RNA mediated	Similar trend as $10\times d_p$ but enhanced	%peaks of RNA and protein mediated motifs similar
Overshoot	Higher mean and broader distribution for protein mediated motifs	No significant change (wrt $1 \times d_p$)	Lower mean overshoot (wrt $1 \times d_p$)	Similar trend as $10\times d_p$ but enhanced	near 0 mean overshoot
Peak time	Narrower distribution for protein mediated FFLs (wrt $1 \times d_p$)	No significant change (wrt $1 \times d_p$)	Broader distribution (wrt $1 \times d_p$)	Similar trend as $10\times d_p$ but enhanced	Similar trend as $100\times d_p$ but enhanced
Peak duration	Apparently bimodal distribution for protein mediated FFLs	Unimodal	Apparently bimodal	Unimodal	Unimodal
Peak time vs Overshoot	Overshoot decreases with peak time	No change (wrt $1 \times d_p$)	No change (wrt $1 \times d_p$)	No change (wrt $1 \times d_p$)	No change (wrt $1 \times d_p$)
Peak time vs Peak duration	Peak duration first increases and then decreases with peak time	No change (wrt $1 \times d_p$)	No change (wrt $1 \times d_p$)	No change (wrt $1 \times d_p$)	No change (wrt $1 \times d_p$)

Tab. S5: Summary of the changes in the metric trends because of changes in protein abundance.

2.4.1 Steady state levels of the controller

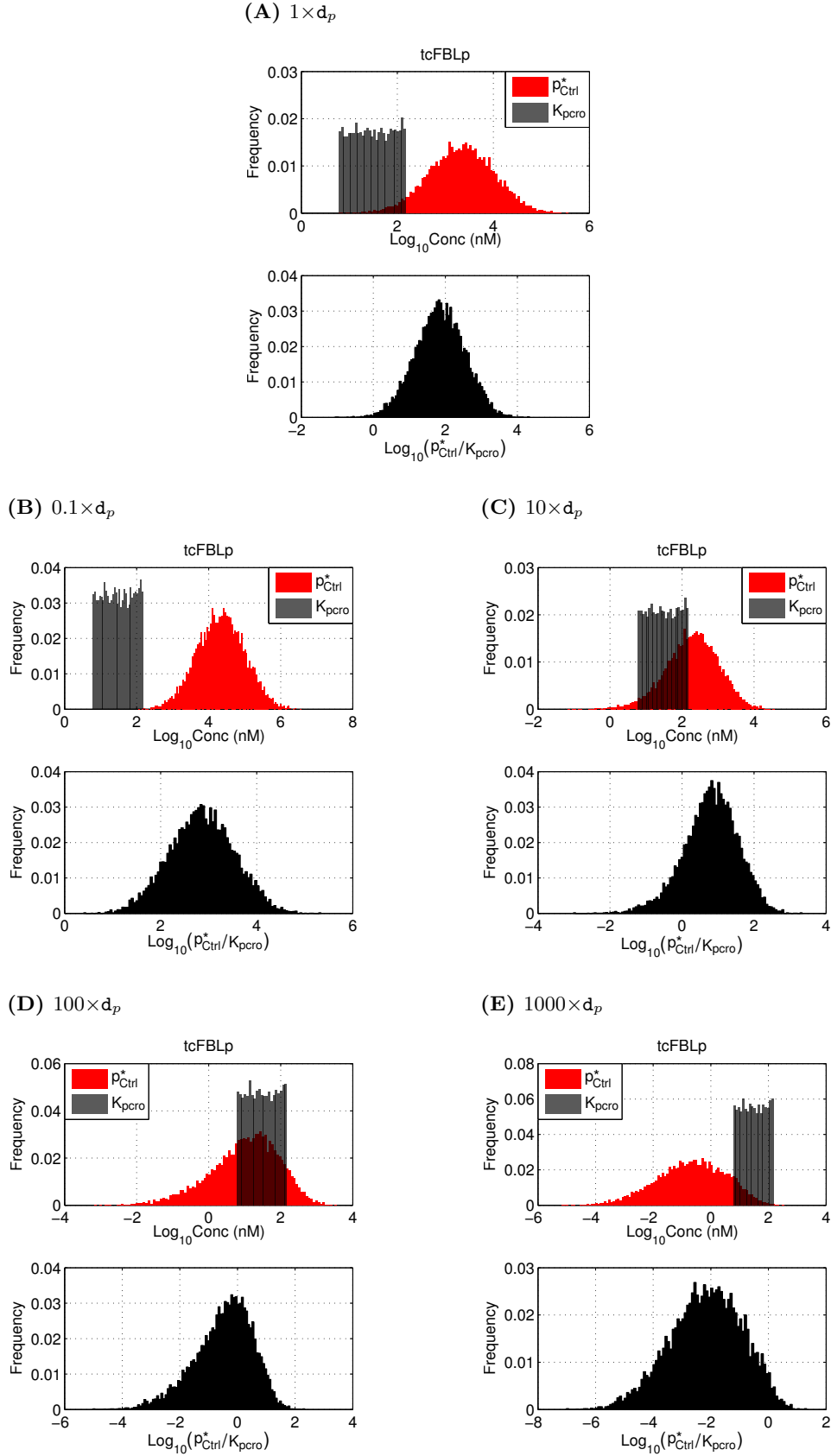
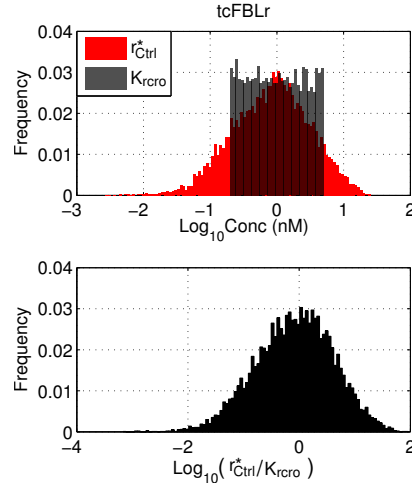
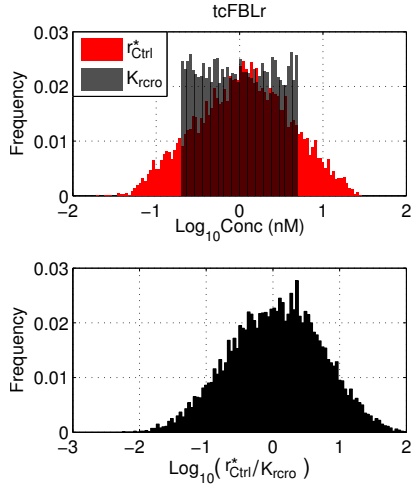


Fig. S5: Steady state levels of the controller protein with respect to $K_{p_{ctrl}}$ for the tcFBLp motif, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. In each subfigure, the upper panel shows the distribution of steady state levels of the controller protein, p_{ctrl}^* (red bars) and the distribution of $K_{p_{ctrl}}$ ($K_{p_{ctrl}}$, grey bars), for the 10000 parameter sets. Lower panel (black bars) shows the distribution of the ratio of p_{ctrl}^* and $K_{p_{ctrl}}$. All values are Log_{10} transformed.

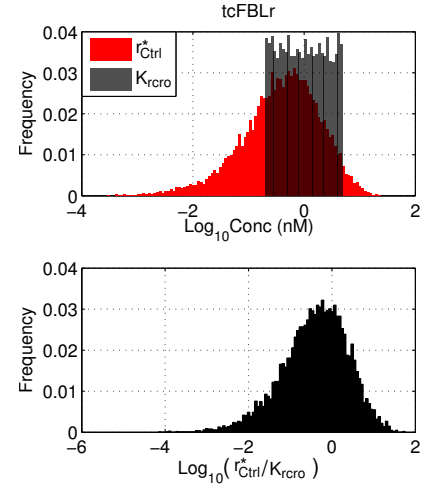
(A) $1 \times d_p$



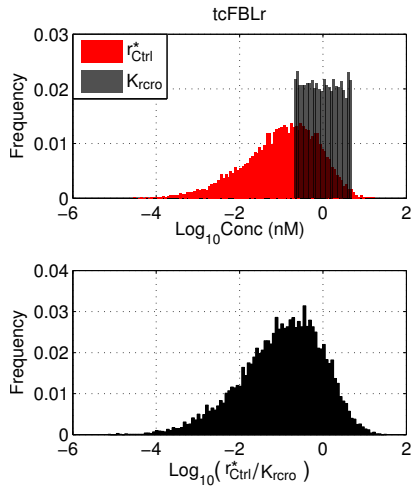
(B) $0.1 \times d_p$



(C) $10 \times d_p$



(D) $100 \times d_p$



(E) $1000 \times d_p$

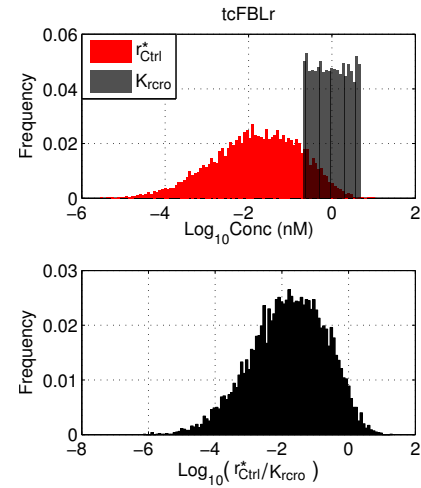
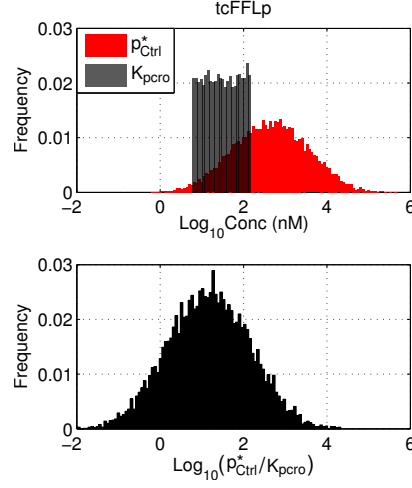
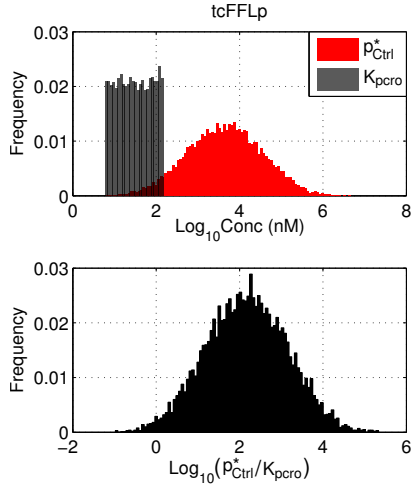


Fig. S6: Steady state levels of the controller protein with respect to $K_{r_{ctrl}^{out}}$ for the tcFBLr motif, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. In each subfigure, the upper panel shows the distribution of steady state levels of the controller RNA, r_{ctrl}^* (red bars) and the distribution of $K_{r_{ctrl}^{out}}$ (K_{rcro} , grey bars), for the 10000 parameter sets. Lower panel (black bars) shows the distribution of the ration of r_{ctrl}^* and $K_{r_{ctrl}^{out}}$. All values are Log_{10} transformed.

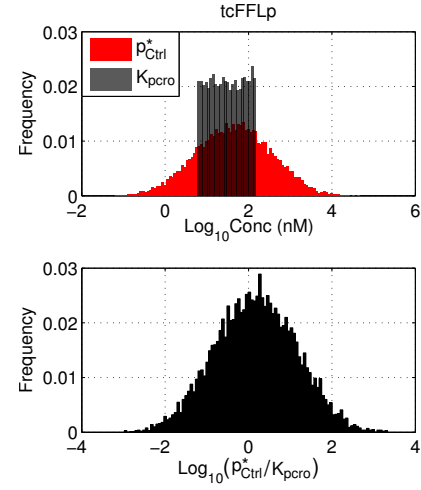
(A) $1 \times d_p$



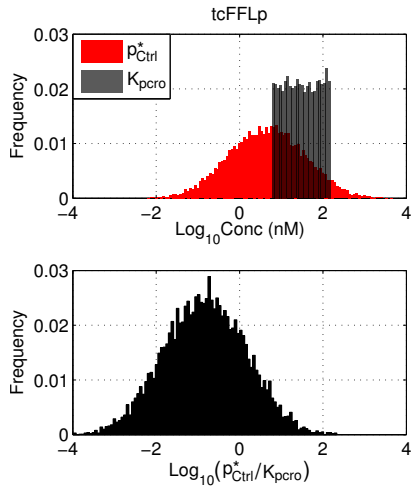
(B) $0.1 \times d_p$



(C) $10 \times d_p$



(D) $100 \times d_p$



(E) $1000 \times d_p$

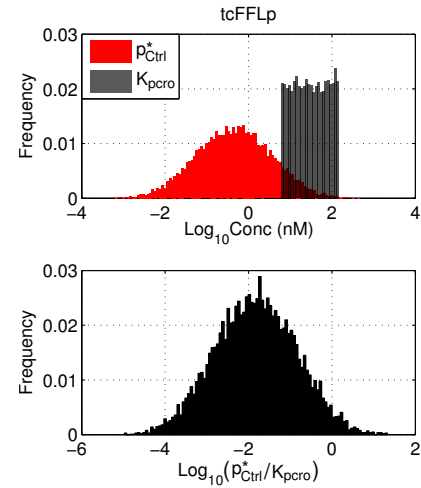
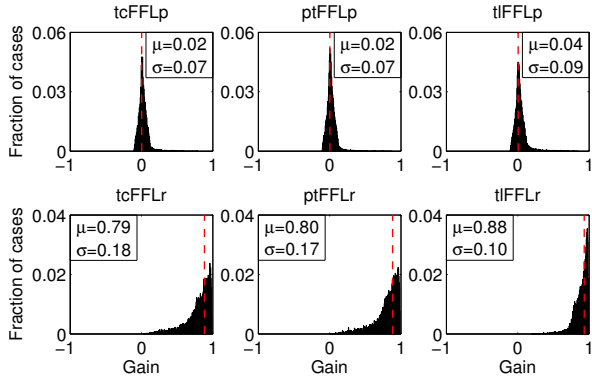


Fig. S7: Steady state levels of the controller protein with respect to $K_{p_{ctrl}}^{r_{out}}$ for the tcFFLp motif, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. In each subfigure, the upper panel shows the distribution of steady state levels of the controller protein, p_{ctrl}^* (red bars) and the distribution of $K_{p_{ctrl}}^{r_{out}}$ ($K_{p_{ctrl}}$, grey bars), for the 10000 parameter sets. Lower panel (black bars) shows the distribution of the ratio of p_{ctrl}^* and $K_{p_{ctrl}}^{r_{out}}$. All values are Log_{10} transformed.

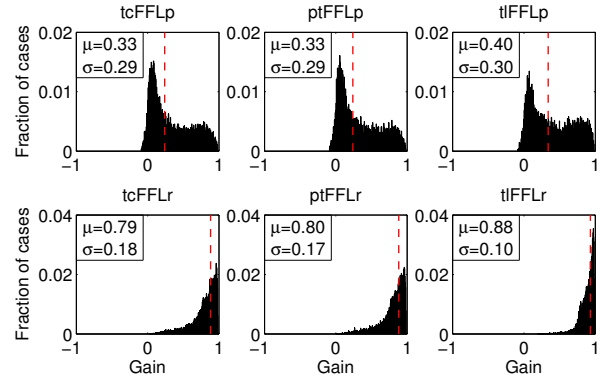
2.4.2 Steady State Gain

FFL

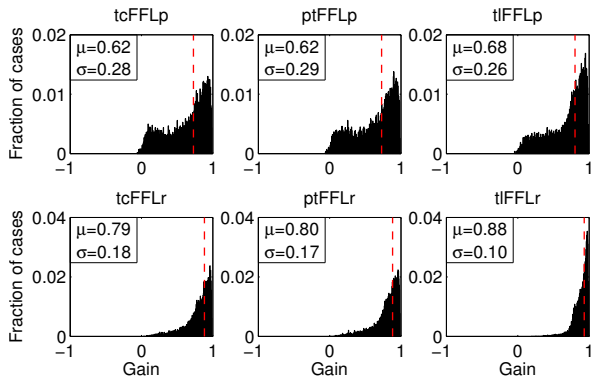
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

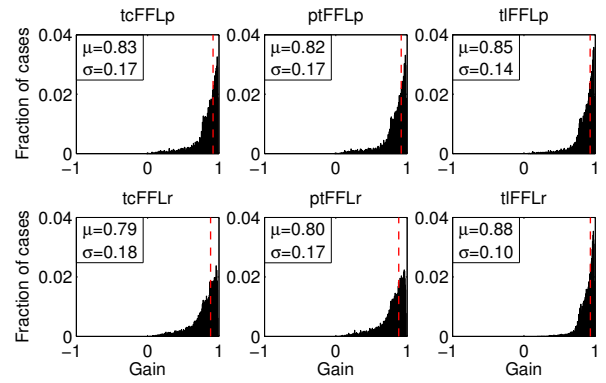
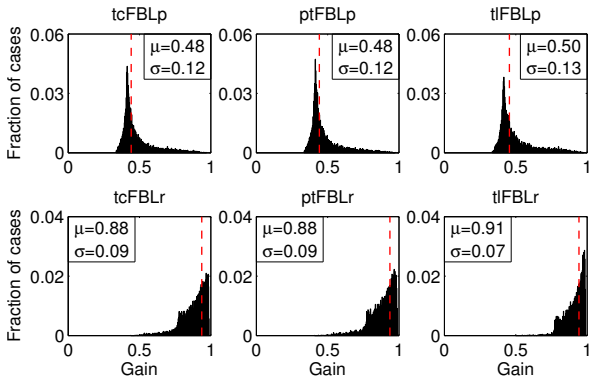


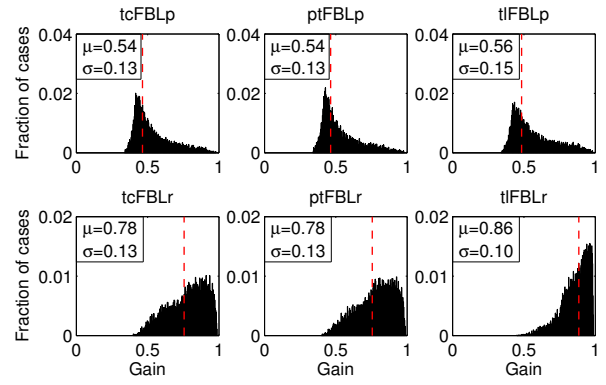
Fig. S8: Steady state gain distributions for the FFL motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) $0.1 \times d_p$, (B) $10 \times d_p$, (C) $100 \times d_p$ and (D) $1000 \times d_p$. The dashed red vertical lines denote the gain values corresponding to the default parameter set.

FBL

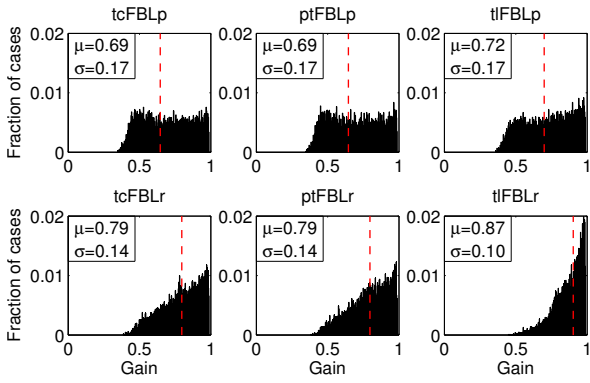
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

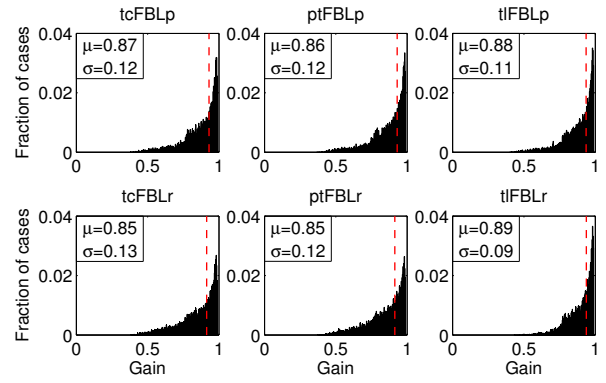
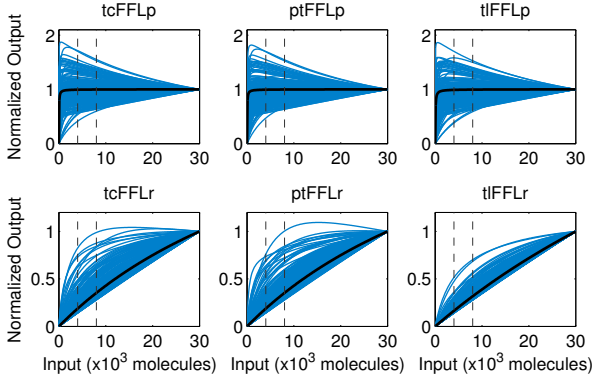


Fig. S9: Steady state gain distributions for the FBL motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) $0.1 \times d_p$, (B) $10 \times d_p$, (C) $100 \times d_p$ and (D) $1000 \times d_p$. The dashed red vertical lines denote the gain values corresponding to the default parameter set.

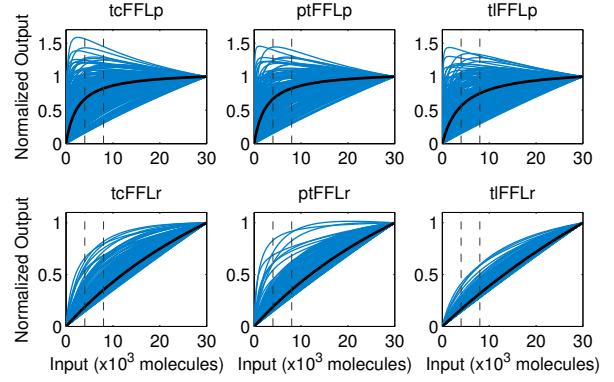
2.4.3 Input-Output characteristics

FFL

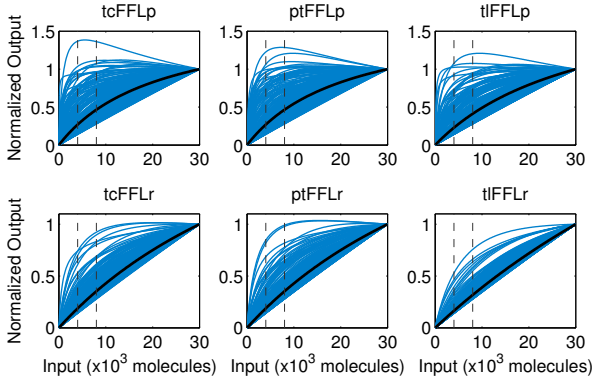
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

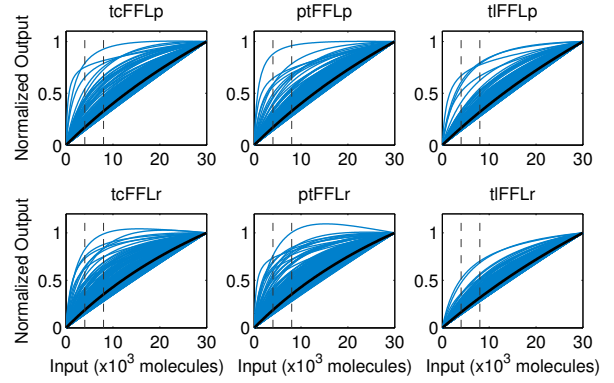
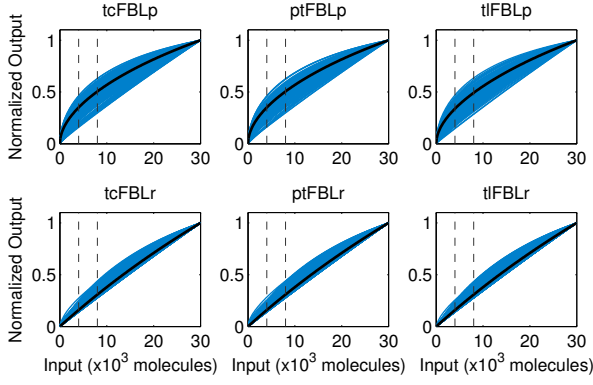


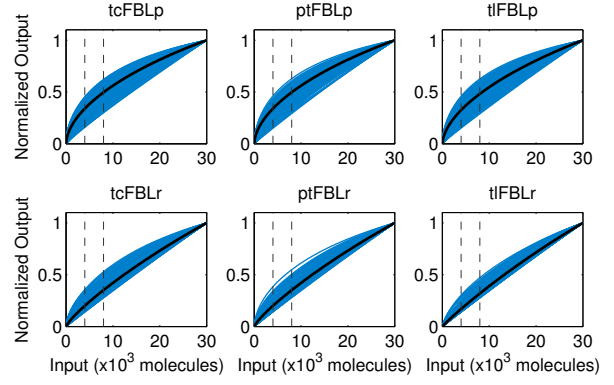
Fig. S10: Input-output characteristics for 200 randomly selected cases of the **FFL** motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) $0.1 \times d_p$, (B) $10 \times d_p$, (C) $100 \times d_p$ and (D) $1000 \times d_p$. The steady state values at each value of input, are normalized with the value at the maximum value of input (30×10^3). Black line represents the characteristics of the default parameter set. Dashed vertical lines (from left to right) denote input corresponding to 4000 and 8000 molecules respectively.

FBL

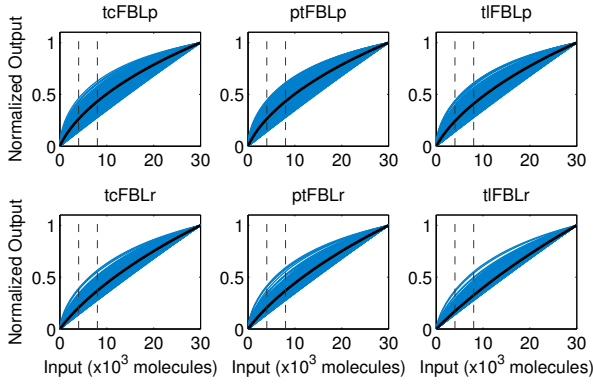
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

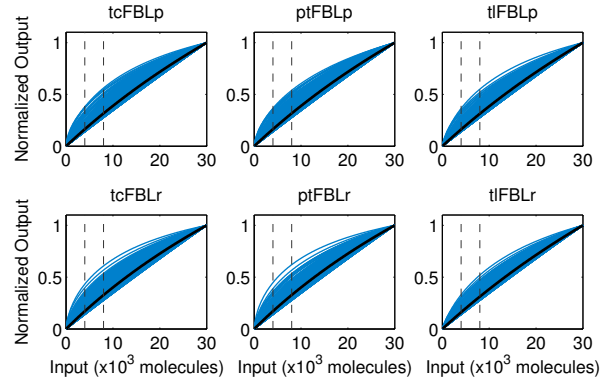


Fig. S11: Input-output characteristics for 200 randomly selected cases of the **FBL** motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) $0.1 \times d_p$, (B) $10 \times d_p$, (C) $100 \times d_p$ and (D) $1000 \times d_p$. The steady state values at each value of input, are normalized with the value at the maximum value of input (30×10^3). Black line represents the characteristics of the default parameter set. Dashed vertical lines (from left to right) denote input corresponding to 4000 and 8000 molecules respectively.

2.4.4 Peaking tendency

FFL

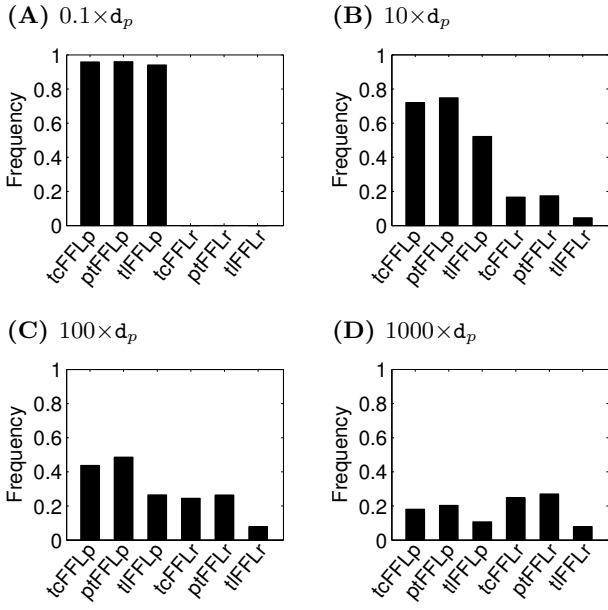


Fig. S12: Peaking tendencies for the **FFL** motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) $0.1 \times d_p$, (B) $10 \times d_p$, (C) $100 \times d_p$ and (D) $1000 \times d_p$.

FBL

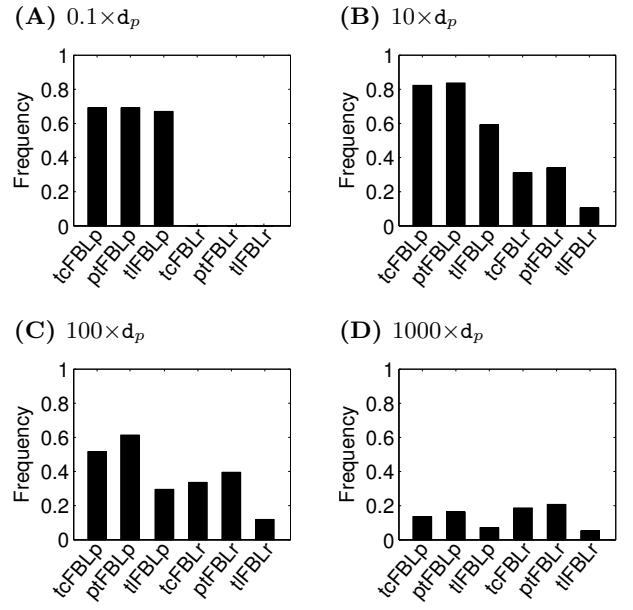
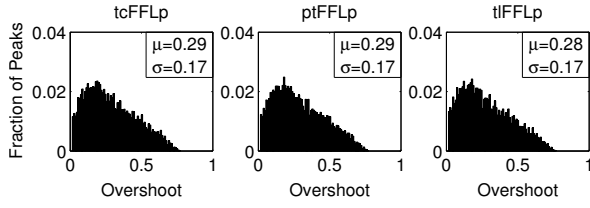


Fig. S13: peaking tendencies for the **FBL** motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) $0.1 \times d_p$, (B) $10 \times d_p$, (C) $100 \times d_p$ and (D) $1000 \times d_p$.

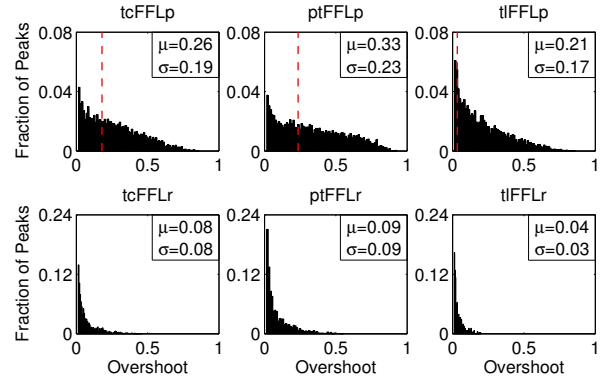
2.4.5 Overshoot

FFL

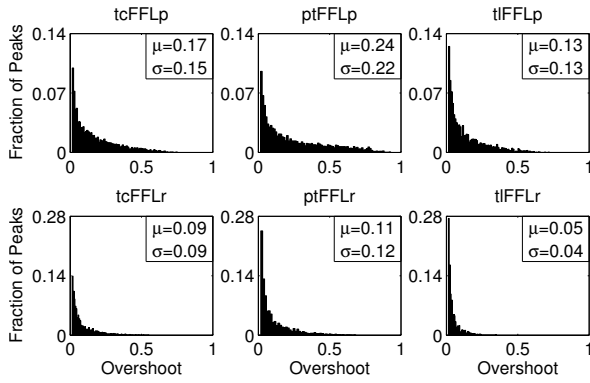
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

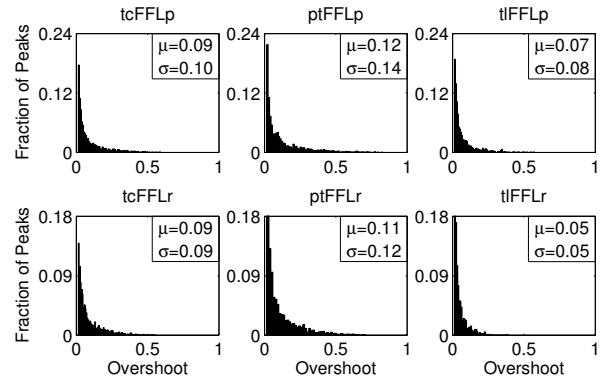
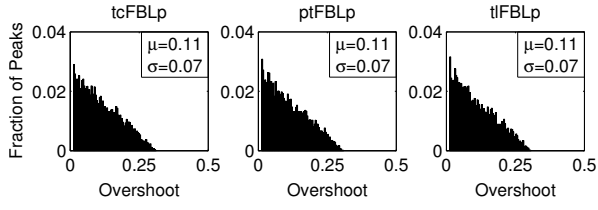


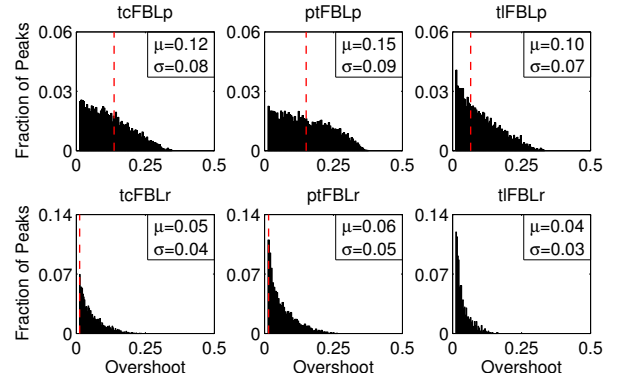
Fig. S14: Overshoot distributions for the FFL motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. The dashed red vertical lines denote the gain values corresponding to the default parameter set.

FBL

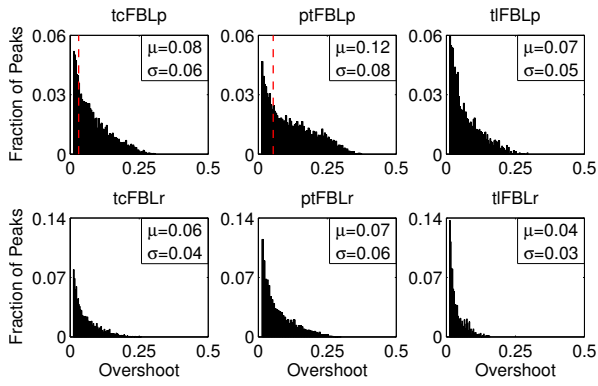
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

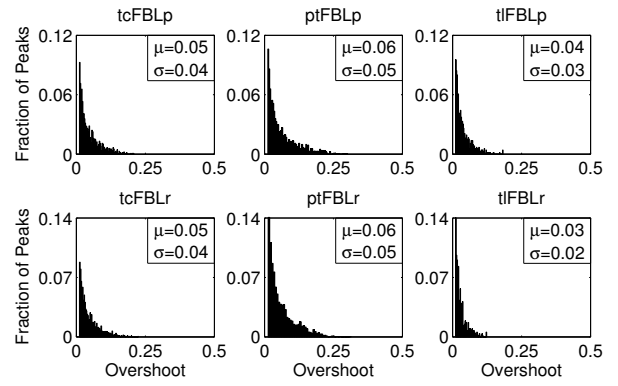
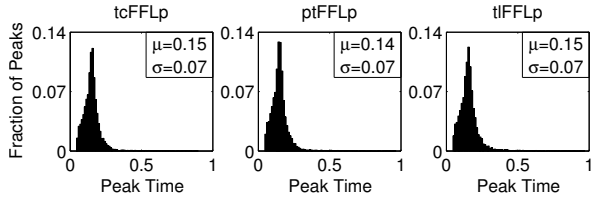


Fig. S15: Overshoot distributions for the FFL motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. The dashed red vertical lines denote the gain values corresponding to the default parameter set.

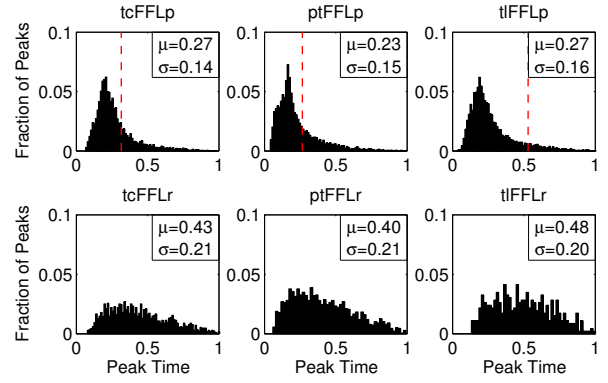
2.4.6 Peak Time

FFL

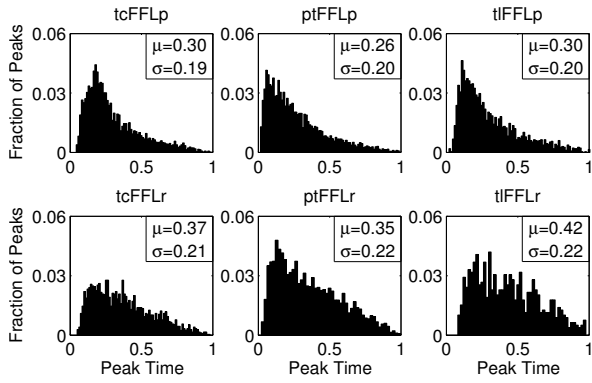
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

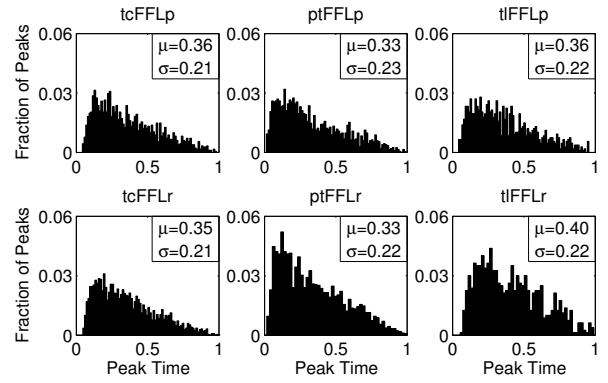
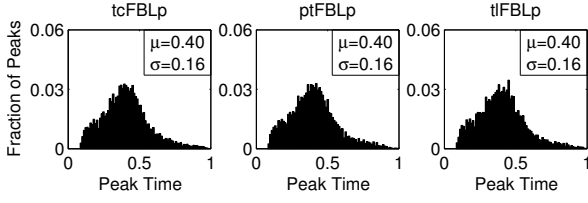


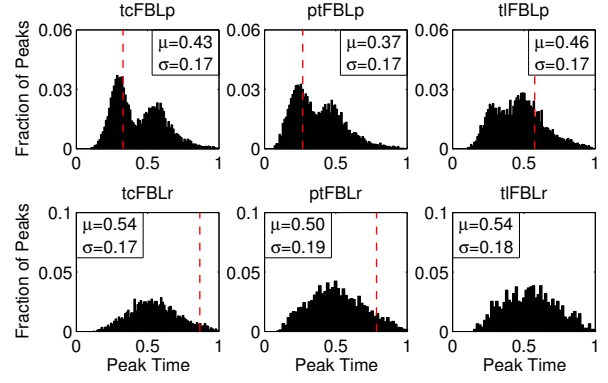
Fig. S16: Peak time distributions for the FFL motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. The dashed red vertical lines denote the gain values corresponding to the default parameter set.

FBL

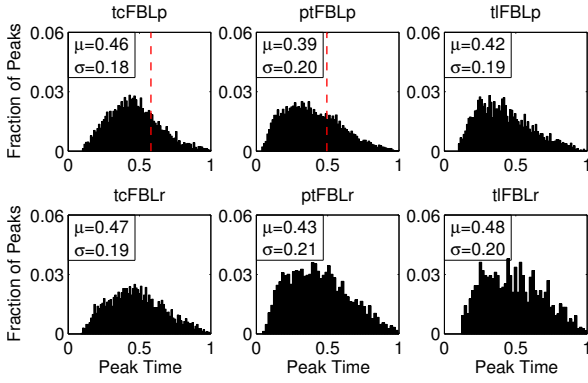
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

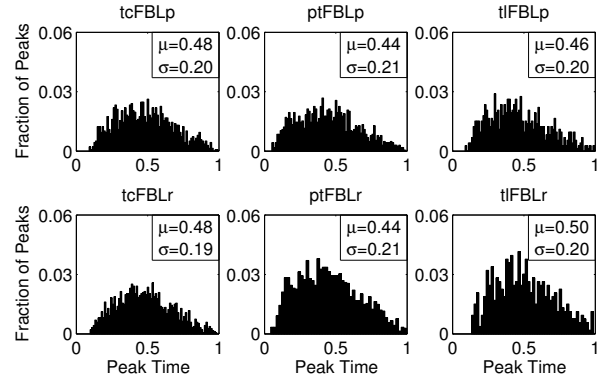
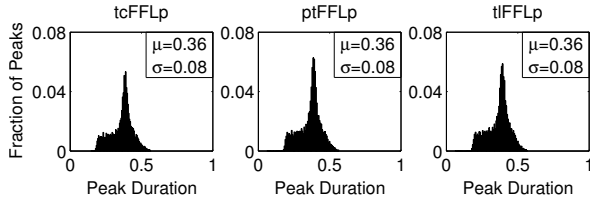


Fig. S17: Peak time distributions for the FFL motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. The dashed red vertical lines denote the gain values corresponding to the default parameter set.

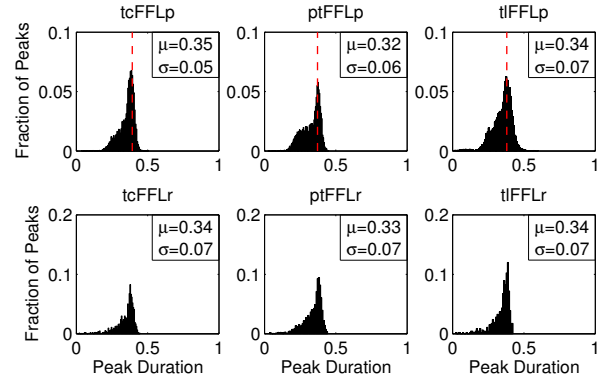
2.4.7 Peak Duration

FFL

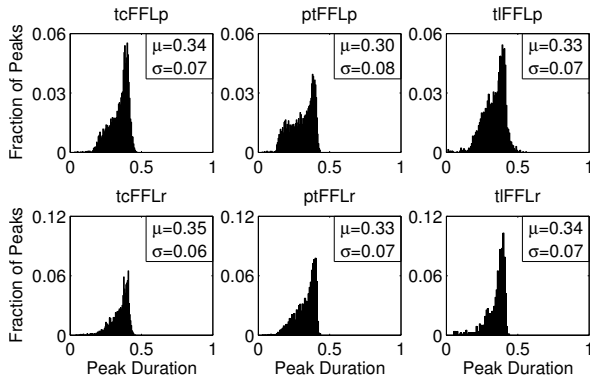
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

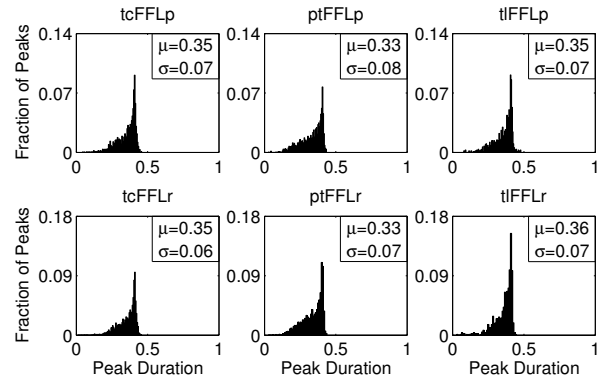
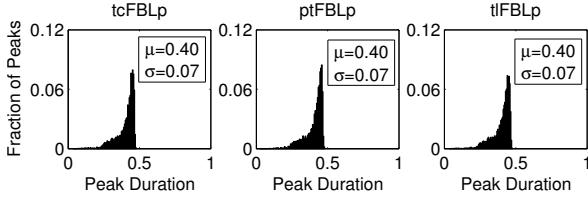


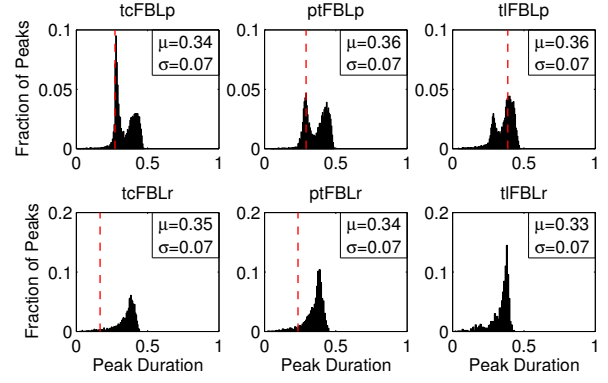
Fig. S18: Peak duration distributions for the FFL motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. The dashed red vertical lines denote the gain values corresponding to the default parameter set.

FBL

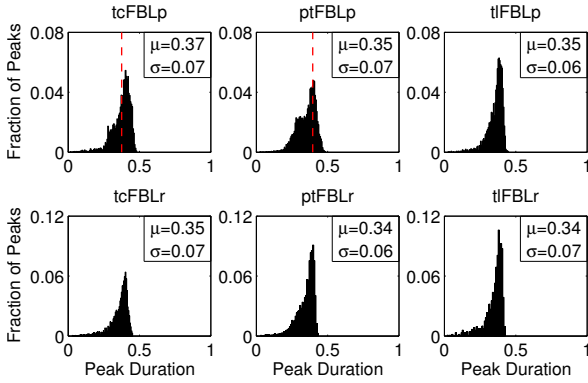
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

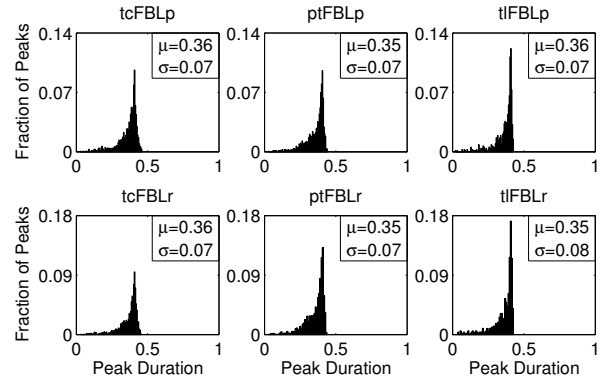
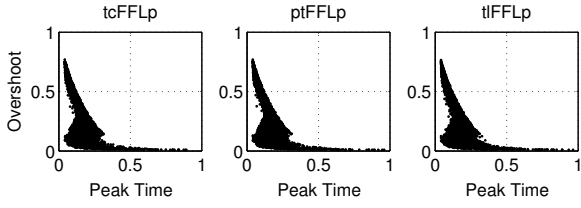


Fig. S19: Peak duration distributions for the FFL motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. The dashed red vertical lines denote the gain values corresponding to the default parameter set.

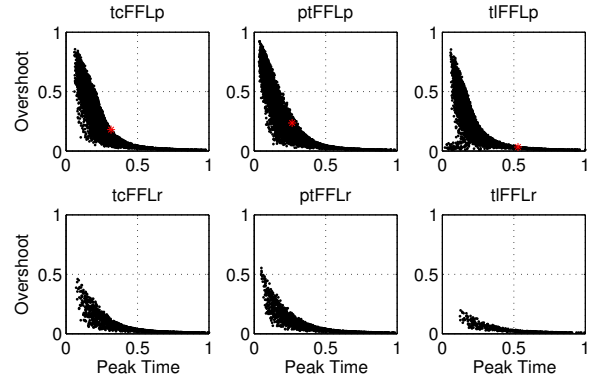
2.4.8 Overshoot vs Peak Time

FFL

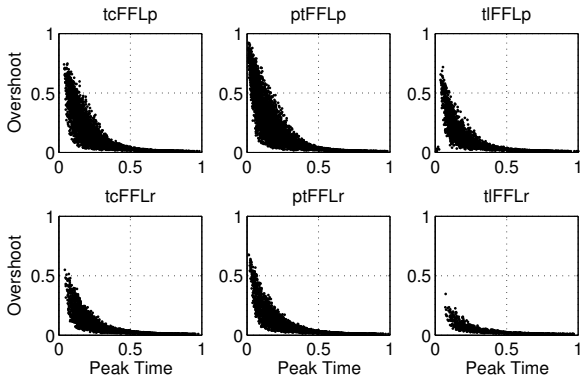
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

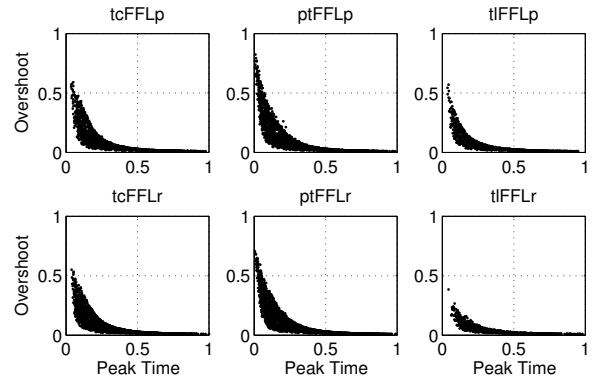
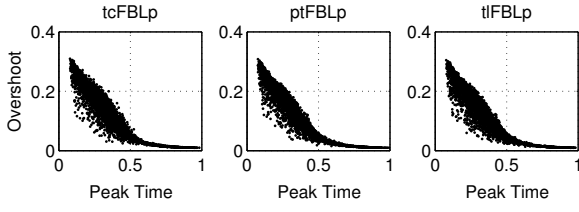


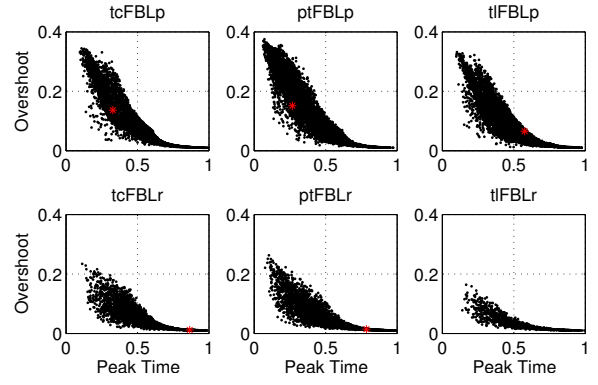
Fig. S20: Scatter plots between overshoot and peak duration for **FFL** motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. Asterisks denote the values corresponding to the default parameter set.

FBL

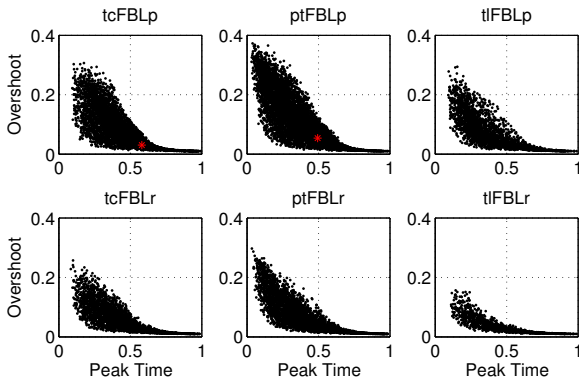
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

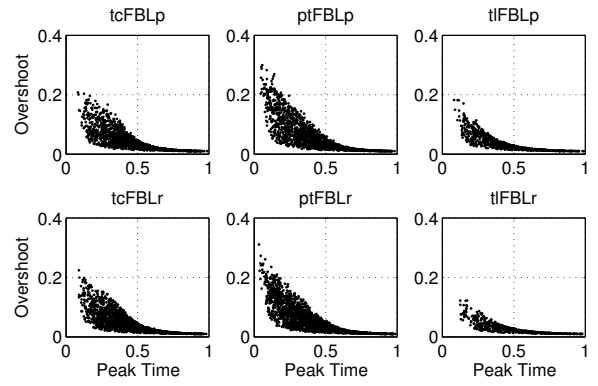
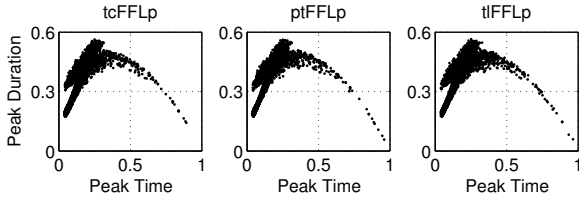


Fig. S21: Scatter plots between peak time and overshoot for FFL motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. Asterisks denote the values corresponding to the default parameter set.

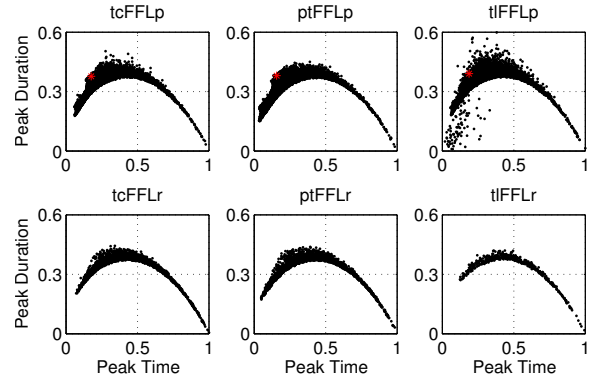
2.4.9 Peak Duration vs Peak Time

FFL

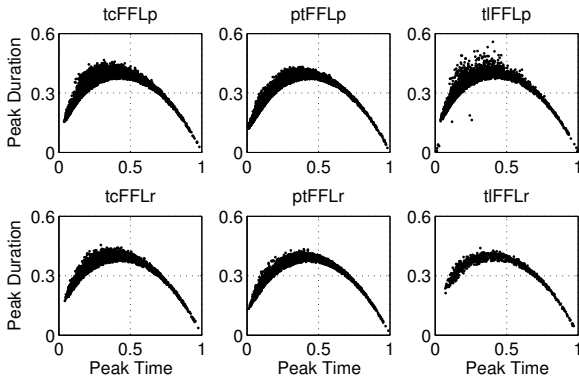
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

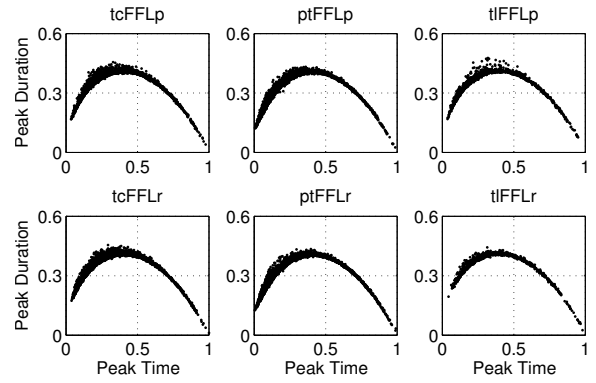
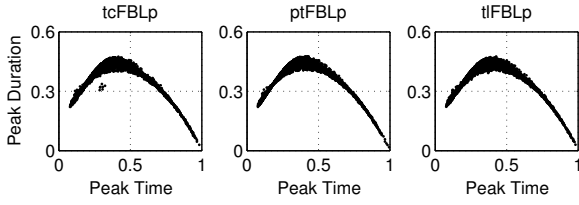


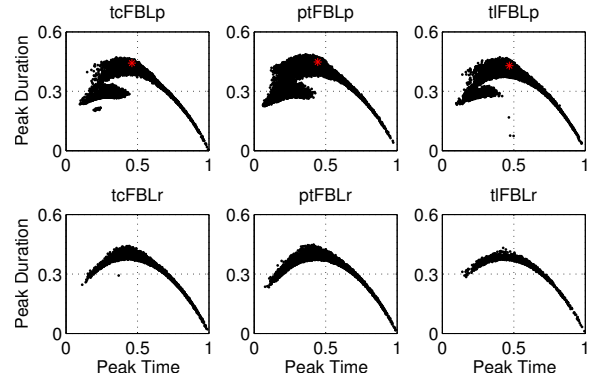
Fig. S22: Scatter plots between peak time and peak duration for **FFL** motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. Asterisks denote the values corresponding to the default parameter set.

FBL

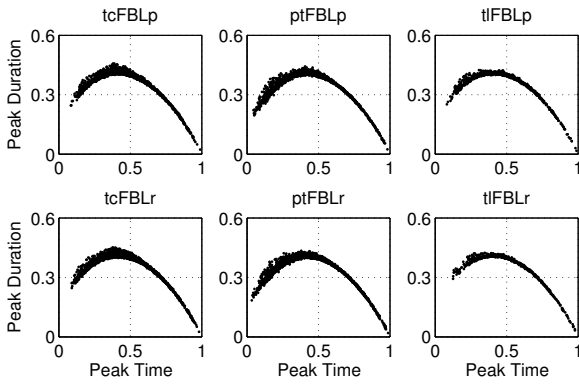
(A) $0.1 \times d_p$



(B) $10 \times d_p$



(C) $100 \times d_p$



(D) $1000 \times d_p$

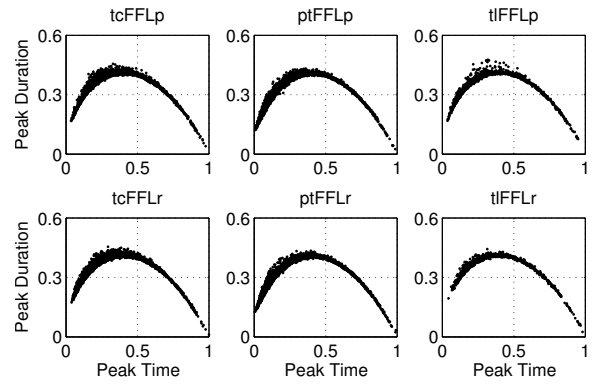


Fig. S23: Scatter plots between peak time and peak duration for **FBL** motifs, at different degradation rates of p_{ctrl} and p_{out} – (A) default, (B) $0.1 \times d_p$, (C) $10 \times d_p$, (D) $100 \times d_p$ and (E) $1000 \times d_p$. Asterisks denote the values corresponding to the default parameter set.

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