Supplemental Information

Derivation of dynamic noise equations

We defined two-node regulatory systems in which protein A constitutively expressed at rate $R_a^+ = \alpha_a$, either activates: $R_b^+ = \frac{\alpha_b a^n}{a^n+K}$, or represses: $R_b^+ = \frac{\alpha_b}{a^n+K}$, synthesis of its downstream target gene, B. We assume that expression of A is independent of B and that degradation of both, A and B, is a first-order, linear process dependent only on the concentration of the respective protein: $R_a^- = \gamma_a a$, and $R_b^- = \gamma_b b$. In our model, each reaction produces or degrades a single molecule at a time.

Based on previously published work by Engblom (Engblom, 2006 and equation 4 in main text), the following equations can be derived for the systems described above:

$$\frac{dC_{aa}}{dt} = -2\frac{\partial R_a^-}{\partial a}C_{aa} + R_a^+ + R_a^- \quad (1)$$

$$\frac{dC_{ab}}{dt} = -\frac{\partial R_a^-}{\partial a}C_{ab} + \frac{\partial R_b^+}{\partial a}C_{aa} - \frac{\partial R_b^-}{\partial b}C_{ab} \quad (2)$$

$$\frac{dC_{bb}}{dt} = 2\frac{\partial R_b^+}{\partial a}C_{ab} - 2\frac{\partial R_b^-}{\partial b}C_{bb} + R_b^+ + R_b^- \quad (3)$$

In our derivations of covariance, we assumed that a linearized model constitutes an adequate sufficient approximation of the dynamics of these systems and, hence, that contributions of higher moments beyond covariance are minor and can be ignored. To verify numerically when this approximation holds, we investigated how the addition of the second term ($s_2 = -\eta_{aa}^2 H_{ba}\frac{R_b^+}{2b^2}\frac{K-nK+a^n+na^n}{a^n+K}$) to the downstream noise equation (Equation 5 below) impacted predictions in our method. We compared the equations with and without this added term in terms

of their ability to quantitatively predict dynamic noise values obtained from SSA simulations[2]. We quantified these contributions of higher moments as the difference of the noise values generated by the equations with and without the additional term, normalized by the total noise of B. We repeated this procedure for 70 randomly chosen parameter sets for both inhibitory and activation regulatory motifs. We found that the inclusion of the second moment changed the computed noise values only marginally (by less 2% and in most cases by less than 0.5% of total noise of B (Figure S6).

Finally, we define noise of A (η_{aa}^2), B (η_{bb}^2), and of their shared noise (η_{ab}^2), as the squared coefficients of variation: $\eta_{aa}^2 = \frac{C_{aa}^2}{a^2}$, $\eta_{bb}^2 = \frac{C_{bb}^2}{b^2}$, and $\eta_{ab}^2 = \frac{C_{ab}^2}{ab}$, respectively. These quantities are described by the following equations:

$$\frac{d\eta_{aa}^2}{dt} = \frac{d}{dt}\left(\frac{C_{aa}}{a^2}\right) = -2\eta_{aa}^2\frac{R_a^+}{a} + \frac{R_a^+}{a^2} + \frac{R_a^-}{a^2} \tag{4}$$

$$\frac{d\eta_{bb}^2}{dt} = \frac{d}{dt}\left(\frac{C_{bb}}{b^2}\right) = -2\frac{R_b^+}{b}(\eta_{bb}^2 - H_{ba}^*\eta_{ab}^2) + \frac{R_b^+}{b^2} + \frac{R_b^-}{b^2} \tag{5}$$

$$\frac{d\eta_{ab}^2}{dt} = \frac{d}{dt}\left(\frac{C_{ab}}{ab}\right) = -\eta_{ab}^2\left(\frac{R_a^+}{a} + \frac{R_b^+}{b}\right) + \eta_{aa}^2 H_{ba}^*\frac{R_b^+}{b} \tag{6}$$

For the activation system, the resulting equations are:

$$\frac{d\eta_{aa}^2}{dt} = -2\eta_{aa}^2\frac{\alpha_a}{a} + \frac{\alpha_a}{a^2} + \frac{\gamma_a}{a} \tag{7}$$

$$\frac{d\eta_{bb}^2}{dt} = -2\frac{\alpha_b a^n}{b(a^n+K)}\left(\eta_{bb}^2 - \frac{nK}{a^n+K}\eta_{ab}^2\right) + \frac{\alpha_b a^n}{b^2(a^n+K)} + \frac{\gamma_b}{b} \tag{8}$$

$$\frac{d\eta_{ab}^2}{dt} = -\eta_{ab}^2\left(\frac{\alpha_a}{a} + \frac{\alpha_b a^n}{b(a^n+K)}\right) + \eta_{aa}^2\frac{\alpha_b a^n nK}{b(a^n+K)^2} \tag{9}$$

For inhibition system, the equations are:

$$\frac{d\eta_{aa}^2}{dt} = -2\eta_{aa}^2 \frac{\alpha_a}{a} + \frac{\alpha_a}{a^2} + \frac{\gamma_a}{a} \tag{10}$$

$$\frac{d\eta_{bb}^2}{dt} = -2\frac{\alpha_b}{b(a^n+K)}\left(\eta_{bb}^2 + \frac{na^n}{a^n+K}\eta_{ab}^2\right) + \frac{\alpha_b}{b^2(a^n+K)} + \frac{\gamma_b}{b} \tag{11}$$

$$\frac{d\eta_{ab}^2}{dt} = -\eta_{ab}^2\left(\frac{\alpha_a}{a} + \frac{\alpha_b}{b(a^n+K)}\right) + \eta_{aa}^2 \frac{\alpha_b n a^n}{b^2(a^n+K)^2} \tag{12}$$

For both activation and inhibition, the total noise of B can be decomposed into intrinsic and propagated components. For activation, the resulting propagated noise equations, which we used to infer regulatory relationships in networks, are:

$$\frac{d\eta_{bb_{prp}}^2}{dt} = -2\frac{\alpha_b a^n}{b(a^n+K)}\left(\eta_{bb_{ext}}^2 - \frac{nK}{a^n+K}\eta_{ab}^2\right) \tag{13}$$

and for inhibition:

$$\frac{d\eta_{bb_{prp}}^2}{dt} = -2\frac{\alpha_b}{b(a^n+K)}\left(\eta_{bb_{ext}}^2 + \frac{na^n}{a^n+K}\eta_{ab}^2\right) \tag{14}$$

Measuring propagated noise from *in silico* data

Intrinsic and propagated noise sum up to the total noise: $\eta_{bb}^2 = \eta_{bb_{int}}^2 + \eta_{bb_{prp}}^2$,[3-5] we can, therefore, estimate the propagated component, $\eta_{bb_{prp}}^2$, from measurements of the total and intrinsic noise. We measure the total expression noise of a protein, *B*, as the squared coefficient of variation, $\eta_{bb}^2 = \frac{C_{bb}^2}{b^2}$, where b denotes the mean and C_{bb}^2 variance of the population. For our model system, in which intrinsic noise stems just from the random birth and death events of individual proteins, intrinsic noise is defined as: $\eta_{bb_{int}}^2 = \frac{1}{b}$.[3,4] This can be verified numerically by replicating *in silico* the classical two-color experiment.[5] Specifically, we used stochastic simulations[1] of three node circuits in which one node regulates two identical downstream nodes,

A and B, and where the reaction propensities are as described in our models above. We obtained time-varying measurements of A and B's distributions and covariance, allowing us to compute their total and shared noise. For either node, measured intrinsic noise was defined as the uncorrelated (not shared) portion of the total noise.[5] We compared the measured intrinsic noise of B to $\eta^2_{bb_{int}} = \frac{1}{b}$, as well as the dynamic equation of $\eta^2_{bb_{int}}$ (as described in main text). For all parameter sets we tested, $\eta^2_{bb_{int}}$ was well represented by $\frac{1}{b}$ even under non-steady state conditions (Figure S7).

Estimation of intrinsic noise in *in vivo* data

In our experimental data, we assume intrinsic noise also scales with protein copy number and can be approximated by $\eta^2_{bb_{int}} = \frac{C}{b}$, where C is a scalability constant similar for all genes in our networks.[6,7] We do not have a direct measure of protein copy number but rather a proportional quantity, fluorophore intensity f.[5] Therefore, to compute intrinsic noise, we used $\eta^2_{bb_{int}} = \frac{C_f}{f}$, where C_f is a fluorophore-specific scalability factor. We estimated C_{f_i} for each fluorophor, GFP and RFP, from experimental steady state data collected for a circuit in which the two reporters were driven by two copies of the pGAL1 promoter. Specifically, we estimated the scaling constants from the intrinsic noise equation: $\frac{C_{f_i}}{f_i} = \eta^2_{f_i} - \eta^2_{f_i f_j}$, where $\eta^2_{f_i}$ is the total noise of either GFP or RFP computed from measurements of their respective intensities, f_i and f_j, and $\eta^2_{f_i f_j}$ is the fluorophores' shared noise. Estimation of the fluorophore scaling constants was done at the same time as collection of all the other data to ensure identical calibration of the flow cytometer.

Determining the hill coefficient *n*

Experimental data was collected over a time-course, allowing the system to go from one steady state before the addition of an input to another steady-state after input addition. Measurements at the beginning and end of the time-course were used to compute *n*.

For our models, the steady-state mean of B is: $b = \frac{\alpha_b a^n}{\gamma_b(a^n+K)}$ and $b = \frac{\alpha_b}{\gamma_b(a^n+K)}$ for activation and inhibition, respectively. We represent these expressions as a function of H^*_{ba}. Specifically for activation:

$$b = \frac{\alpha_b a^n}{\gamma_b(a^n + K)}$$

$$\frac{\gamma_b}{\alpha_b} b = \frac{a^n}{a^n + K}$$

$$\frac{n\gamma_b}{\alpha_b} b = \frac{na^n}{(a^n + K)} \tag{15}$$

$$\frac{n\gamma_b}{\alpha_b} b = \frac{na^n + nK - nK}{(a^n + K)}$$

$$\frac{n\gamma_b}{\alpha_b} b = \frac{n(a^n + K)}{(a^n + K)} - \frac{nK}{(a^n + K)} = n - \frac{nK}{(a^n + K)}$$

Defining $\omega = \frac{n\gamma_b}{\alpha_b}$, we obtain the relationship between susceptibility and the mean:

$$\omega b = n - H^*_{ba} \tag{16}$$

Similarly for inhibition:

$$b = \frac{\alpha_b}{\gamma_b(a^n + K)}$$

$$\frac{n\gamma_b}{\alpha_b} b = \frac{n}{a^n + K} \tag{17}$$

$$\frac{n\gamma_b K}{\alpha_b} b = \frac{nK}{a^n + K}$$

$$\frac{n\gamma_b K}{\alpha_b} b = \frac{nK + na^n - na^n}{(a^n + K)}$$

$$\frac{n\gamma_b K}{\alpha_b} b = \frac{n(a^n + K)}{(a^n + K)} - \frac{na^n}{(a^n + K)} = n - \frac{na^n}{(a^n + K)}$$

If $\omega = \frac{nK\gamma_b}{\alpha_b}$,

$$\omega b = n + H^*_{ba} \tag{18}$$

These equations relating the mean and susceptibility have two unknown constants, *n* and *ω*, which can uniquely be identified from distribution information collected at the two different steady-states.

If a second steady state is not available, we can obtain a rough estimate of *n* from a single measure of susceptibility taken in the dynamic range. The definition of susceptibility, which for our activation model is: $H^*_{ba} = \frac{nK}{a^n+K}$ and for inhibition: $H^*_{ba} = \frac{-na^n}{a^n+K}$, sets the lower bound on the value of *n* at H^*_{ba} (since $\frac{K}{a^n+K} \leq 1$ & $\frac{a^n}{a^n+K} \leq 1$). This allows us obtain an estimate for $n = \left|\frac{\eta^2_{bb_{ext}}}{\eta^2_{ab}}\right|$. We can further constrain *n* by picking a value that guarantees a positive value for $K = \frac{a^n \eta^2_{bb_{prp}}}{n\eta^2_{ab} - \eta^2_{bb_{prp}}}$ or $K = -\frac{a^n\left(n\eta^2_{ab} + \eta^2_{bb_{prp}}\right)}{\eta^2_{bb_{prp}}}$ for activation or inhibition, respectively. To further fine-tune the value of *n,* we can compute the mean square error of the fit of prediction made using these parameters to a set of steady-state measurements for H^*_{ba}. We can also repeat this procedure for multiple values of *n* and pick the value one that minimizes the error of fit. We find

that in most cases, the estimate of $n = \left|\frac{\eta^2_{bb_{prp}}}{\eta^2_{ab}}\right|$ using these approximations did not deviate from the value computed using two steady states by more than 1.

Contribution of global noise

Global noise affects expression of all components of a circuit and, hence, might cause independent genes to appear to be co-regulated. We investigated whether this is a confounding factor for our method. We modeled global noise as zero-mean stochastic signal[8] with constant production, α_ε, and first order decay, $\gamma_\varepsilon\varepsilon$, rates. The chosen parameter values were such that $\alpha_\varepsilon/\gamma_\varepsilon = 150$ for all tested noise levels. The resulting signals had standard deviation values of σ = [0, 6, 12, 24, 48, 84, 120].

The global noise term was added to synthesis terms (R_x^+) of each gene, *x*. Each circuit was simulated and reconstructed for each of the seven noise levels. We found that presence of this global variability can affect reconstruction but that for circuit motifs, this occurs only at very large variance (Figure S8).

Data sampling frequency

We investigated how data sampling frequency impacts reconstruction. As a proof of concept we selected two regulatory motifs: A activates B and A and B are co-regulated, simulated each using 50 randomly chosen parameter sets, and reconstructed using data sampled at different time intervals (Figure S9). As expected, we found that reconstruction remains robust as long as the sampling captures the dynamics of data.

List of primers used

pGAL1_fwd: GGAA GGGCCC TTCTCCTTGACGTTAAAGTATAGAGGT

pGAL1_rev: GGAA CTCGAG TTGGATGGACGCAAAGAAGT

pADH1_fwd: GGAA GGGCCC GGCAACCAAACCCATACATC

pADH1_rev: GGAA CTCGAG GTCGACCTGCAGAAAAAGAAACA

pHSP12_fwd: GGAA GGGCCC AGGTGGAGTGCGATTTGTTC

pHSP12_rev: GGAA CTCGAG TTTTTGTTTTGAGTTGTTTGTTTGA

Msn2_fwd: GGAA GTCGAC AATAAA ATGACGGTCGACCATGATTT

Msn2_rev: GGAA GCGGCCGC TGAAGGTACCGGAAAAATGG

Venus_fwd: GGAA ctcgag AATAAA atgtctaaaggtgaagaattat

Venus_rev: GGAA AGATCT TTTGTACAATTCATCCATACC

mKate_fwd: GGAA ctcgag AATAAA atggtgagcgagctgattaagg

mKate_rev: ggaa ggtacc TTAtctgtgccccagtttgcta

TetR_fwd: GGAA ctcgag AATAAA ATGTCTAGATTAGATAAAAG

TetR_rev: ggaa ggtacc ggacccactttcacatttaag

mCherry_fwd: GGAA GGATCC atgacagtcaacactaagacc

mCherry_rev:ggaa GCGGCCGC TTATAATTGGCCAGTCTTTTTCAAA

References:

(1) Engblom, S. (2006) Computing the moments of high dimensional solutions of the master equation. App Math and Comp 180, 498-515.

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(4) Paulsson, J. (2005) Models of stochastic gene expression. Phys of Life Rev 2, 157-175.

(5) Elowitz, M. B., Levine, A. J., Siggis, E. D., Swain, P. S. (2002) Stochastic Gene Expression in a single cell. Science 297(5584), 1183-1186.

(6) Bar-Evan, A., Paulsson, J., Maheshari, N., Carmi, M., O'Shea, E., Pilpel, Y., Barkai, N. (2006) Noise in protein expression scales with natural protein abundance. Nat Genet 38, 636-643.

(7) Stewart-Ornstein, J., Weissman, J. S., El-Samad, H. (2012) Cellular noise regulons underlie fluctuations in Saccharomyces cerevisiae. Mol Cell 45(4), 483-493.

(8) Dunlop, M. J., Cox, III R. S., Levine, J. H., Murray, R. M., Elowitz, M. B. (2008) Regulatory activity revealed by dynamic correlations in gene expression noise. Nat Genet 40, 1493-1498

Table 1. Parameter range

Parameter	Range
α_a	(0, 250000]
α_b	$(0, 10^{13}]$
γ_a, γ_b	(0, 15]
K	$(0, 10^6]$
n	(0, 5]

Figure S1. The dynamic noise equations accurately predict trajectories of noise for activation and inhibition motifs. Prediction accuracy was computed using 60 SSA simulations of activation and inhibition networks using different, randomly chosen parameter sets. **(A)** Change in mean expression of A and B between the pre-stimulus and post-stimulus steady-states for each of the 60 tested networks. **(B)** Prediction error between SSA and dynamic noise equations for total noise of A and B and of their shared noise. Prediction error was quantified as percent of total noise averaged over all time-points (251 or 501 time-points). **(C)** Error for the decomposed noise of B. Prediction error was quantified as percent of total noise averaged over all time-points (251 or 501 time-points)

Figure S2. Evaluation of the method *in silico* using data obtained from SSA. Reconstruction of regulatory motifs: A activates B, A inhibits B, co-regulation of A and B, a feedback loop in which A and B regulate each other, and an indirect regulation of B by A (hidden, unmeasured node in between). Data was obtained for 153 different, randomly chosen parameter sets. Choice of parameters for the feedback loop was restricted to those that assured that both A and B were susceptible to one another ($H_{ab} > 1.5$ and $H_{ba} > 1.5$). Each regulatory motif was tested for all regulatory permutations between A and B (rows). The results are reported as histograms of the correlation between measured and estimated noise for that particular topology. A low or negative correlation value for the correct topology indicates error in reconstruction. The zero correlation indicates that the numerical integrator failed to solve the noise equation.

Figure S3. Dissection of cases that could not be reconstructed with noise information. (A) In this case, B (α_b = 1062, γ_b = 1.0, K_b = 41860, n_b = 2) and its noise were insensitive to changes in concentration of the upstream protein A (α_a = 452/2712, γ_a = 0.9) (left panel) and hence noise did not propagate (right panel). **(B)** In this case, the approximations inherent in the model were inadequate for both mean and noise, a problem that might be alleviated by inclusion of higher order terms to the mean and noise equations. (α_a = 4/56, γ_a = 0.7, α_b = 696, γ_b = 1.0, K_b = 9202, n_b = 4) **(C)** In this case synthesis of B was fully inhibited by A which caused the noise of B to be dominated by degradation alone and no noise from A was propagated (α_a = 24/384, γ_a = 2.0, α_b = 221968, γ_b = 2.0, K_b = 50, n_b = 1). Insets in (A), (B) and (C): propagated noise and intrinsic noise in B expressed as a fraction of total noise.

Figure S4: The dynamic propagation of noise depends on parameter values. Each plot is the propagated (green) and shared (blue) noise of a downstream node of an inhibitory network versus time. Each trace shows noise trajectory for different parameter value for **(A)** Michaelis-Menten parameter *K,* which is a key determinant of susceptibility, **(B)** degradation rate of the downstream protein, γ_b, which is a key determinant of time averaging **(C)** hill coefficient *n*, **(D)** synthesis rate of the downstream protein, α_b, and **(E)** upstream rate of activation, α_a. Red arrow indicates ascending parameter values.

Figure S5. Using *in vivo* data collected for synthetic circuits, mean alone was insufficient for distinguishing between the possible regulatory relationships. (**A)** Activation circuit. Plot of means for the topology A→B, B→A and A and B are co-regulated by an input function (gray line) versus time. (**B)** Inhibition circuit. Plot of means for the topology A--|B, B--|A and A and B are co-regulated versus time. **(C)** Co-regulation circuit. Plot of means for the topology A→B, B→A and A and B are co-regulated versus time.

Figure S6. Inclusion of higher moments in the dynamic noise equation does not substantially improve accuracy of predictions. Error incurred by not including higher moments is quantified as percent of total noise and plotted for each topology and parameter set tested. The values reported are averaged over the entire trajectory of the downstream protein.

Figure S7. *In silico* intrinsic noise for our models is well represented by the dynamic noise equation as well as the inverse of the mean (1/b) even at non-steady state. (**A)** An example trajectory of intrinsic noise of protein B calculated by an *in silico* noise decomposition of SSA results (blue), the dynamic noise equation (green) or the relationship 1/b(t) (red) for a circuit in which A inhibits B, (α_a = 574/2098, γ_a = 4.8, α_b = 5.3259E7, γ_b = 4.0, K_b = 18606, n_b = 2). Inset: mean behavior B. **(B)** Comparison of intrinsic noise to its measured value computed using all three methods for 40 different parameter sets.

Figure S8. Exploration of Global noise. Reconstruction of two regulatory motifs: A activates B and A and B are co-regulated. Each motif was simulated using 60 randomly chosen parameter sets and for seven different levels of global noise (left column). Each histogram reports on the obtained correlations between measured and estimated noise for a specific regulatory relationship (columns) and level of global noise (rows).

Figure S9. Dependence on sampling frequency. Reconstruction of two *in silico* regulatory motifs: A activates B and A and B are co-regulated, using data sampled at different time interval (left column) – from top: using all 501 time points, using every 5th, 10th, 54th, and 100th time point. Each histogram shows reconstruction of these motifs for 50 randomly chosen parameter sets.