Fitness costs of noise in biochemical reaction networks and the evolutionary limits of cellular robustness

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ABSTRACT

Gene expression is inherently noisy, but little is known about whether noise affects cell function or, if so, how and by how much. Here I present a theoretical framework to quantify the fitness costs of gene expression noise and identify the evolutionary and synthetic targets of noise control. I find that gene expression noise reduces fitness by slowing the average rate of nutrient uptake and protein synthesis. This is a direct consequence of the hyperbolic (Michaelis-Menten) kinetics of most biological reactions, which I show cause "hyperbolic filtering", a process that diminishes both the average rate and noise propagation of stochastic reactions. Interestingly, I find that transcriptional noise directly slows growth by slowing the average translation rate. Perhaps surprisingly, this is the largest fitness cost of transcriptional noise since translation strongly filters mRNA noise, making protein noise largely independent of transcriptional noise, consistent with empirical data. Translation, not transcription, then, is the primary target of protein noise control. Paradoxically, selection for protein-noise control favors increased ribosome-mRNA binding affinity, even though this increases translational bursting. However, I find that the efficacy of selection to suppress noise decays faster than linearly with increasing cell size. This predicts a stark, cell-size-mediated taxonomic divide in selection pressures for noise control: small unicellular species, including most prokaryotes, face fairly strong selection to suppress gene expression noise, whereas larger unicells, including most eukaryotes, experience extremely weak selection. I suggest that this taxonomic discrepancy in selection efficacy contributed to the evolution of greater gene-regulatory complexity in eukaryotes.

ARTICLE SUMMARY

Gene expression is a probabilistic process, resulting in random variation in mRNA and protein abundance among cells called "noise". Understanding how noise affects cell function is a major problem in biology. Here I present theory demonstrating that gene expression noise slows the average rate of cell division. Furthermore, by modeling stochastic gene expression with non-linearity, I identify novel mechanisms of cellular robustness. However, I find that the cost of noise, and therefore the strength of selection favoring robustness, decays faster than linearly with increasing cell size. This may help explain the vast differences in gene-regulatory complexity between prokaryotes and eukaryotes.

INTRODUCTION

The probabilistic nature of chemical reactions, along with the small volumes of living cells, create substantial molecular randomness called "noise" (Berg 1978; McAdams and Arkin 1997). Gene expression noise, for example, is measured as variation in mRNA or protein abundance within a single cell over time (Taniguchi et al. 2010), or, equivalently, among cells in an isogenic cell population (Elowitz et al. 2002). Because the abundance of most mRNAs and proteins are on the order of 1 to 1000 copies per cell (Taniguchi et al. 2010), a random birth or death of a single copy can instantaneously change the cellular concentration by 0.1-100%. These random fluctuations are amplified by bursting, the synthesis of multiple mRNAs or proteins in brief pulses (Raser and O'Shea 2004; Raser and O'Shea 2005; Raj et al. 2006; Pedraza and Paulsson 2008; Thattai and van Oudenaarden 2001). Using fluorescent probes and fluorescently tagged proteins,

74 researchers have quantified the noise statistics of mRNA and/or proteins in *E. coli*,
75 budding yeast, and mammalian cell lines, demonstrating the ubiquity of gene expression
76 noise and its considerable variability across the genome and among taxa (Elowitz et al.
77 2002; Blake et al. 2003; Golding et al. 2005; Raj et al. 2006; Zenklusen, Larson, and
78 Singer 2008; Taniguchi et al. 2010; Newman et al. 2006; Balázsi, van Oudenaarden, and
79 Collins 2011).

80 A central question in cell biology and evolution is whether or not cellular noise
81 has a functional consequence. Answering this question is necessary for establishing
82 rational design criteria for synthetic genetic circuits and for providing evolutionary
83 explanations for empirical patterns of noise among genes and taxa. For example, why
84 does *E. coli* have an average mRNA Fano factor (variance/mean) of $F = 1.6$ (Taniguchi et
85 al. 2010), which is just above the theoretical minimum of $F = 1$, whereas mammalian
86 cells have 40-100 times stronger mRNA noise (Raj et al. 2006; Balázsi,
87 van Oudenaarden, and Collins 2011; Dar et al. 2012)? While the mechanistic causes of
88 this discrepancy are becoming clearer (Suter et al. 2011), the evolutionary forces
89 permitting such a discrepancy remain murky.

90 Mounting empirical evidence suggests that natural selection has acted to limit
91 gene expression noise in many cases (Metzger et al. 2015; Newman et al. 2006; Alemu et
92 al. 2014; Batada and Hurst 2007; Fraser et al. 2004; Lehner 2008), implying that noise is
93 often costly to cells. Yet identifying and quantifying this cost remains a major challenge.
94 Perhaps the most widely invoked cost of cellular noise is imprecision, the lack of fine
95 control over cellular processes. Evolutionarily, this cost of noise is typically modeled as
96 random phenotypic deviations from the adaptive optimum, which is opposed by

97 stabilizing selection (Wagner et al. 1997; Draghi and Whitlock 2015). Several molecular
98 mechanisms linking imprecision to suboptimal fitness deviations have been proposed.
99 The dosage-control hypothesis proposes that noise disrupts control over the
100 stoichiometric balance of interacting proteins (Fraser et al. 2004; Lehner 2008).
101 Consistent with this hypothesis, proteins that form complexes have lower average noise
102 than other proteins (Fraser et al. 2004; Lehner 2008; Bar-Even et al. 2006), but models
103 currently do not predict the magnitude of this effect so that quantitative correspondence
104 between predictions and observations remain unknown. More commonly, researchers
105 focus on how noise disrupts the fine control over cell states regulated by bistable
106 (ON/OFF) switches (Balázsi, van Oudenaarden, and Collins 2011; Csete and Doyle
107 2002). Noise in the input parameter can cause the cell to flip stochastically from the
108 desired to undesired state, or to respond slowly to environmental cues, creating a
109 maladaptive mismatch between the cell state and the selecting environment. However,
110 the least noisy genes, those presumably experiencing the strongest selection to suppress
111 noise, are constitutively expressed and involved in core cell functions, not control over
112 bistability (Fraser et al. 2004; Lehner 2008; Bar-Even et al. 2006). This cost is also
113 difficult to quantify in any general way because its effects are highly context dependent,
114 to the extent that in some contexts the cost of imprecision can change signs, becoming
115 evolutionarily beneficial (Acar, Mettetal, and van Oudenaarden 2008; Rao, Wolf, and
116 Arkin 2002; Kussell and Leibler 2005; Eldar and Elowitz 2010; Kaern et al. 2005; Thattai
117 and Van Oudenaarden 2004).
118 As an alternative, here I demonstrate a general, readily quantifiable and consistent
119 cost of cellular noise. I show that noise in substrate abundance slows the average rate of

120 product formation in Michaelis-Menten-type (i.e., non-cooperative bimolecular) chemical
121 reactions, which constitute most reactions in the cell including those most closely tied to
122 fitness: nutrient uptake and protein synthesis. This result is a straightforward consequence
123 of the non-linearity of bimolecular reactions. While it is well known that noise alters the
124 mean of nonlinear stochastic systems, this effect is typically ignored, giving rise to the
125 widespread use of linear approximations (e.g., the "linear noise approximation" (LNA)
126 (van Kampen 2007)), particularly in studies of gene expression noise (Thattai and van
127 Oudenaarden 2001; Shahrezaei and Swain 2008; Paulsson 2004). The LNA is well-
128 justified when applied to investigating steady-state concentrations in noisy chemical
129 networks, since the difference between linear and non-linear predictions at steady-state
130 are on the order of a single molecule. However, fitness in living cells is determined by
131 rates—not concentrations--integrated over an enormous number of reactions over the
132 lifetime of a cell and its genetic lineage. For example, an *E. coli* cell must carry out a
133 minimum of 2.5×10^6 translation reactions to produce a single daughter cell. A minor
134 slowing of reaction rates within cells, if heritable, may pose a non-negligible selective
135 cost. From this perspective, the effects of noise on fitness cannot be ignored, and indeed,
136 I find that they can be substantial in small cells.
137
138 **RESULTS**
139 The goal of the paper is to quantify the strength of selection favoring the attenuation of
140 gene expression noise, and to identify the targets of noise attenuation. The paper proceeds
141 as follows. First, I show that noise slows the average rate of biochemical reactions that
142 have non-linear, hyperbolic kinetics. Then, I extend this result to networks of coupled

143 non-linear reactions in order to determine how noise effects total flux through
144 biochemical networks, as well as how the network architecture itself affects the
145 propagation of noise. This enables us to quantify the cost of noise, measured as the loss in
146 average rate of end-product formation, and to quantify the noise statistics of the system. I
147 then apply the model to gene expression, focusing on the consequences of non-linear,
148 hyperbolic translation kinetics on the propagation of mRNA noise to the protein level,
149 and to the average rate of biomass synthesis. Finally, I investigate how the volume of
150 reaction compartments and whole-cells influences the cost of noise, concluding that the
151 cost of noise, and therefore the selection pressures favoring low gene expression noise,
152 decays rapidly with increasing cell size, leading to a testable prediction about levels of
153 gene expression noise across unicellular species.
154
155 **Noise-induced slowdown of Michaelis-Menten reactions.** The purpose of this section is
156 to begin building the theory from the simplest component of a network: a single isolated
157 reaction. We will see that input noise slows the reaction rate of a single, isolated reaction
158 obeying Michaelis-Menten type kinetics. In the following section, the analytical results
159 from this section are put on a more rigorous theoretical footing and then extended to
160 networks of coupled reactions in open systems.
161 The cornerstone of biochemistry is the Michaelis-Menten (MM) reaction, which
162 describes the enzyme catalyzed conversion of substrate, *S*, into product, *P*. The rate of
163 product formation is described by the hyperbolic MM equation (Michaelis and Menten
164 1913; Briggs and Haldane 1925; Ingalls 2013):
165

$$\frac{dp}{dt} = V = V_{max}\frac{s}{K_m + s} \qquad 1$$

166 s, e and p are reactant, enzyme and product concentration, respectively (following
167 convention lowercase denotes concentration, which unless otherwise noted will take units
168 of molecules per cell), $V_{max} = k_{cat}e$ is the asymptotic reaction rate (k_{cat} is the number of
169 substrate molecules converted to product per enzyme per unit time), and K_m, the
170 Michaelis or half-saturation constant, is the substrate concentration at $V = V_{max}/2$. This
171 equation is derived from a system of elementary reactions by applying the law of mass
172 action and the quasi-steady state approximation (QSSA) (Briggs and Haldane 1925;
173 Ingalls 2013) (Sup. Mat.). The QSSA is a separation of timescales approximation that
174 eliminates fast variables by setting them to their steady-state. The law of mass action is
175 valid in cases where the timescale of molecular diffusion is fast relative to the reaction
176 rate, so that the system behaves as if it were well-mixed. The rate of enzymatic reactions
177 in cells is far below the diffusion limit (Bar-Even et al 2006), so that mass action is a
178 reasonable approximation in most cases. For stochastic reactions, wherein s, e, and p
179 experience random number fluctuations, Eqn. 1 takes on a probabilistic interpretation as
180 the "reaction propensity" (van Kampen 2007) giving the transition probability for $n_P \rightarrow$
181 $n_P + 1$ and $n_S \rightarrow n_S - 1$, where n denotes particle number. In general, the QSSA is valid
182 for stochastic systems under the same conditions for which it is valid in deterministic
183 systems (Rao and Arkin 2003; Sanft, Gillespie, and Petzold 2011), notwithstanding
184 finite-volume corrections (Grima 2009b; Grima 2009a; Grima 2010) (see below).
185 Because Eqn. 1 is hyperbolic in s, Jensen's inequality proves that, for random s,
186 $\langle V(s)\rangle \leq V(\langle s\rangle)$ (angle brackets denote ensemble average). That is, the average reaction
187 rate is less than or equal to a noise-free (i.e., deterministic) reaction with $s = \langle s\rangle$ (Figs. 1,
188 S1). This is seen by expanding Eqn. 1 to second order in Taylor series about $\langle s\rangle$ and

taking the expectation (see SI),

$$\langle V(s)\rangle = \underbrace{V_{max}\frac{\langle s\rangle}{K_m + \langle s\rangle}}_{V(\langle s\rangle)} - \underbrace{\frac{\langle \epsilon_S{}^2\rangle}{\langle s\rangle^2} V_{max} a(1-a)^2}_{noise-induced\ slowdown} + O(\epsilon_S{}^3) \qquad 2$$

$a = K_m/(K_m + \langle s\rangle)$ is the "kinetic order" of the MM reaction evaluated at the average substrate concentration, and ($\eta^2 = \langle \epsilon_S{}^2\rangle/\langle s\rangle^2$) the squared coefficient of variation, which is often simply called "the noise". The leading order correction in Eqn. 2 takes the sign of the second derivative of Eqn. 1, which is everywhere negative for hyperbolic transition rates, thus demonstrating the noise-induced slowdown of MM type reactions.

The kinetic order parameter, *a,* is a key parameter that appears repeatedly below. *a* is identical to the steady-state fraction of unbound enzyme. In metabolic control analysis (MCA), *a* is equivalent to the "elasticity coefficient" (Kacser and Burns 1973). In general, *a* measures the nonlinearity of the reaction rate at a given mean substrate concentration. First and zeroth order reactions (a = 1 and 0, respectively) are both linear, and as a consequence there is no noise-induced slowdown, consistent with Jensen's Inequality. The rate of a first order reaction is linearly dependent on the substrate concentration, whereas the rate of a zeroth order reaction is independent of substrate concentration. In a MM type reaction, the reaction is in its first order regime when substrate is scarce, and in the zeroth order regime when substrate is abundant (the flat part of the rate law curve in Fig. 1).

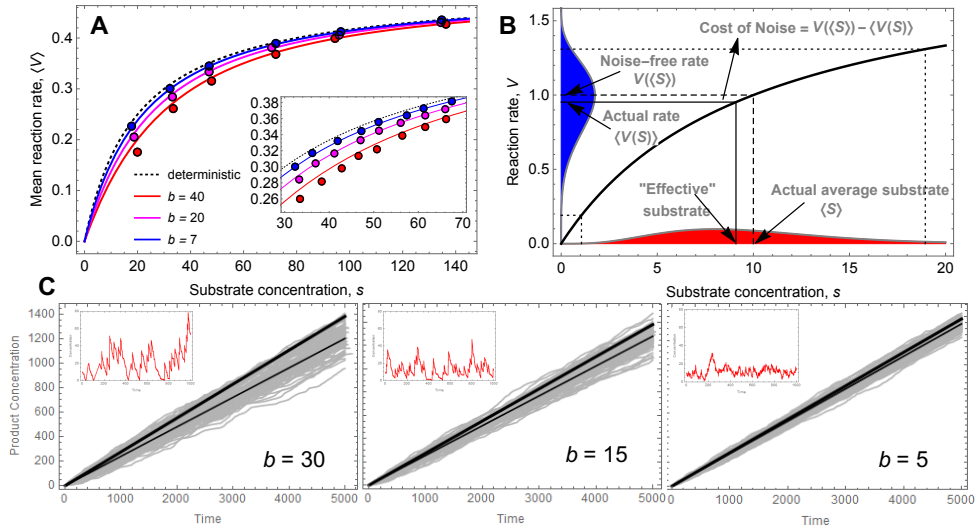


Figure 1: **Noise-induced slowdown of hyperbolic reactions.** Stochastic simulations employing Gillespie's (Gillespie 1977) exact stochastic simulation algorithm (SSA) of the microscopic system of elementary reactions for the Michaelis-Menten reaction (see SI). Points represent the rate of product formation over time steps 100-800 (providing a burn-in more than 10 times the half life of P or S) ensemble averaged over 1000 realizations of the stochastic process. Results fit well with the QSSA model (Eqn. 3, solid lines in **a**, **b** and thin black line in **c**). The substrate burst input, b, is varied to alter the substrate noise level. Substrate input rate, k_{in} is divided by b so that mean substrate is not affected by bursting, and is varied to generate different substrate concentrations. All other parameters are constant ($e = 1$, $k_{ON} = 1$, $k_{OFF} = 19.5$, $k_{cat} = 0.5$, $\delta_S = 0.1$, $\delta_P = 0.1$). **A)** The rate law curve demonstrating that, for a given average substrate concentration, substrate noise slows the reaction, but only if the reaction is not in its first or zeroth order regime (low or high substrate, respectively). Note that the accuracy of the stochastic QSSA requires that s, $K_m \ll F$, which fails for the red points in **a** and **b** (where $K_m = 20$ and $F = 20.5$). Inset is a zoomed in view. **B)** A geometric illustration of noise-induced slowdown, showing that variance (noise) in a state variable reduces the average of a hyperbolic function (i.e., Jensen's inequality). **C)** 100 realizations of the stochastic process (gray) compared to the deterministic (thick black line) and stochastic (thin black line) predictions. Red traces in inset are example outputs from a single realization of the substrate dynamics for a short time window.

A more rigorous and general expansion method is used in the following section ("Noisy Non-linear Reaction Networks in Finite Volumes"). For now, there are two main points: 1) reaction kinetics depend not only on average molecule concentrations, but on the mean and variance in molecule numbers, and 2) for a given average substrate concentration, substrate noise reduces the rate of biochemical reactions. The difference

234 between the average stochastic rate and deterministic rate grows monotonically with
235 increasing noise, and equality occurs only with zero noise or zero curvature of $V(s)$.
236 Mechanistically, this can be understood as follows: positive fluctuations in substrate
237 oversaturate available catalysts leaving many unbound S molecules that are not actively
238 creating product, causing inefficiency. As a consequence, the same amount of substrate
239 would produce a faster net reaction rate if spread out evenly over the same time interval
240 (i.e., the same mean with zero noise).

241 If the substrate statistics are known, then the probability density of V with random
242 s can be solved explicitly for a number of common probability distributions. mRNA and
243 protein counts are typically gamma distributed within a cell over time or among cells in a
244 clonal population (Taniguchi et al. 2010). The gamma distribution has shape parameter α
245 $= CV^{-2}$ and rate parameter $\beta = F$, where $CV^2 = \sigma_x^2/\langle x\rangle^2$ and F = $\sigma_x^2/\langle x\rangle$ are the squared
246 coefficient of variation (the noise) and the Fano factor (often called "the noise strength"),
247 respectively (Taniguchi et al. 2010). F and CV^2 are common measures of noise intensity,
248 with convenient mechanistic interpretations in gene expression: F is closely related to the
249 burst size (e.g., number of proteins synthesized per mRNA lifetime) and CV^{-2} to the burst
250 frequency (e.g., the number of mRNA's synthesized per protein lifetime). F is
251 independent of cell volume, whereas CV^2 decreases with cell volume (they are intensive
252 and extensive variables, respectively). For gamma distributed s, the probability density
253 function of the Michaelis-Menten reaction rate (for $V < V_{max}$) is exactly,

254 $$Pr(V = v) = \frac{V_{max}}{k_m}\frac{v^{\alpha-1}e^{-v/z\beta}}{z(z\beta)^{\alpha}\Gamma(\alpha)}, \qquad z = \frac{V_{max} - v}{k_m} \qquad 3$$

255 Eqn. 3 is verified by Monte Carlo simulations implemented with Gillespie's exact
256 stochastic simulation algorithm (SSA) (Gillespie 1977) (Fig. 1). Eqn. 3 fits data from

257 stochastic simulations very well provided that $F, K_m \ll s$. Equation 2 gives a very good
258 approximation to 3 under this same restriction (Fig. S2), which provides an important
259 benchmark for the accuracy of the more rigorous expansion approximations used below.
260 Figure 1 shows that the effect of noise on MM-type reactions is to increase the
261 observed Michaelis constant. Therefore, a stochastic formulation of Eqn. 1 that accounts
262 for noisy substrate while retaining the classical form is obtained simply by substituting
263 into Eqn. 1 an "effective", stochastic Michaelis constant, $\widetilde{K}_m$, where $\widetilde{K}_m \geq K_m$ with
264 equality occurring only with zero noise, zero curvature of the rate law, and macroscopic
265 reaction volume (S6).
266
267 **Noisy Non-linear Reaction Networks in Finite Volumes.** In the previous section, we
268 showed that substrate noise slows the rate of product formation in a single hyperbolic
269 reaction in a closed system. To apply this result, the statistics of the substrate species are
270 required. We now take a more rigorous approach and generalize the noise-induced
271 slowdown phenomenon to reaction networks in open systems. Further, the model allows
272 one to predict the noise statistics of the system solely with knowledge of the network
273 structure and reaction kinetics. Of particular interest is how network architectures may
274 create feedbacks that buffer the system flux against noise-induced slowdown. The
275 following theory builds on the work of van Kampen (van Kampen 2007) and Elf and
276 Erhenberg (Elf and Ehrenberg 2003) (see also Grima (Grima 2010)).
277 The time evolution of the joint probability density of all *N* nodes in a chemical
278 reaction network with *R* edges is given by the following "system-size expansion" (van
279 Kampen 2007) of the multidimensional chemical master equation (derived in the SI):

$$\frac{\partial \Pi(\vec{\epsilon},t)}{\partial t} = \overbrace{-\sum_{i,k}^{N} J_{ik}\frac{\partial(\epsilon_k \Pi)}{\partial \epsilon_i} + \frac{1}{2}\sum_{i,k=1}^{N} D_{ik}\frac{\partial^2 \Pi}{\partial \epsilon_i \partial \epsilon_k}}^{linear\ Fokker-Planck\ equation}$$

$$+\underbrace{\frac{\Omega^{-1/2}}{2}\left(-\sum_{i,k,l=1}^{N}\frac{\partial J_{ik}}{\partial \overline{x_l}}\frac{\partial(\epsilon_l \epsilon_k \Pi)}{\partial \epsilon_i} + \sum_{i,k,l=1}^{N}\frac{\partial D_{ik}}{\partial \overline{x_l}}\frac{\partial^2(\epsilon_l \Pi)}{\partial \epsilon_i \partial \epsilon_k}\right)}_{correction\ for\ non-linearity\ and\ finite\ volume} + O(\Omega^{-1}) \qquad 4$$

$\Pi = \Pi(\vec{\epsilon},t)$ is the joint probability density of all reacting species, Ω is the reaction volume, ϵ_i is the random perturbation of the ith species from $\overline{x_i}$, its macroscopic steady-state (overbars will denote the macroscopic steady-state value of a variable throughout), S_{ij} is the stoichiometric coefficient of species i in the jth reaction, and

$$J_{ik} = \sum_{j=1}^{R} S_{ij}\frac{\partial f_j(\vec{\bar{x}})}{\partial \overline{x_k}}, \quad D_{ik} = \sum_{j=1}^{R} S_{ij}S_{kj}\, f_j(\vec{\bar{x}})$$

are entries of the Jacobian and diffusion matrices, respectively, of the continuous deterministic system of equations. Equation 4 is a linearization of the chemical master equation obtained by applying the ansatz that concentrations fluctuate about their macroscopic steady-state value, with fluctuation size scaling inversely with the square root of the reaction volume, Ω (van Kampen 2007). This inverse square-root dependence of noise on volume is a formal statement of the Law of Large Numbers, and is formally equivalent to the relationship between sample size and the standard deviation of a sampling distribution. One thus substitutes, $x_i = \overline{x_i} + \Omega^{-1/2}\epsilon_i$, for each species i in the deterministic system of equations, and then expands each transition rate, $V_j(x)$, in powers of $\Omega^{-1/2}$ about $\overline{x_i}$, truncating the series at the desired order (van Kampen 2007). The terms of order $O(\Omega^0)$ in (4) give the "linear noise approximation" (LNA) (van Kampen 2007), which is equivalent to the standard linear Fokker-Planck equation, while the terms in Ω^-

$^{1/2}$ account for noise-induced deviations caused by non-linear rate laws in finite volumes, which we show cannot be ignored in living cells.

To apply 4, one begins by writing a macroscopic description of a reaction network in QSSA reduced form. Here we analyze a generalized MM scheme (S17) in an open system obeying the following macroscopic system of ODEs (Sup. Mat.):

$$\frac{ds}{dt} = b_S k_{in,S} - hV - \delta_S s, \qquad \frac{de}{dt} = b_E k_{in,E} - \delta_E e, \qquad \frac{dp}{dt} = V - \delta_P p \qquad 5$$

δ_i and $k_{in,i}$ are the decay and input rates, respectively, of i, and $V = k_{cat}es/(K_m+s)$. Substrate (S) and enzyme (E) are fed into the system via a burst process with burst size b_S and b_E, respectively ($n_S \rightarrow n_S + b_S$; $n_E \rightarrow n_E + b_E$). $h = \{0,1\}$ is an indicator variable that toggles between reactions where S is consumed ($h = 1$), as in most metabolic reactions, and reactions where S is not consumed ($h = 0$), as with template-mediated reactions such as translation where E denotes ribosomes and S mRNA, which is not consumed in translation.

From System 5 and Eqn 4, we obtain the following steady-state average concentrations of each species in a generalized, open MM reaction network (Sup. Mat.):

$$\langle s \rangle = \bar{s} \underbrace{+h\left[a\frac{\langle \epsilon_S^2 \rangle}{\bar{s}} - K_m \frac{\langle \epsilon_S \epsilon_E \rangle}{\bar{e}\bar{s}}\right] A\Omega^{-1}}_{stochastic\ amplification}, \qquad \langle e \rangle = \bar{e}\ ,$$

$$\langle p \rangle = \bar{p} - \frac{\delta_S}{\delta_P}\left[a\frac{\langle \epsilon_S^2 \rangle}{\bar{s}} - K_m \frac{\langle \epsilon_S \epsilon_E \rangle}{\bar{e}\bar{s}}\right] A\Omega^{-1}, \qquad 6$$

$$\langle V \rangle = \bar{V} \underbrace{-\delta_S\left[a\frac{\langle \epsilon_S^2 \rangle}{\bar{s}} - K_m \frac{\langle \epsilon_S \epsilon_E \rangle}{\bar{e}\bar{s}}\right] A\Omega^{-1}}_{noise-induced\ slowdown}$$

Where $A = (k_{cat}\bar{e}\bar{s}/(hk_{cat}\bar{e}K_m + \delta_S(K_m + \bar{s})^2))$ and $a = K_m/(K_m + \bar{s})$ is the MM reaction order. Note that in the general case, $\langle s \rangle$ is increased and $\langle p \rangle$ is decreased by noise compared to the macroscopic system. The non-linear mesoscopic correction terms

320 vanish when $s \rightarrow 0$ (first order kinetics), $s \rightarrow$ Infinity (zeroth order kinetics) or when

321 $\Omega \rightarrow$ Infinity (macroscopic volume). Two of the second moments (Sup. Mat.) for the

322 general MM case can be written compactly:

323 $$\langle \epsilon_E^2 \rangle = \frac{1}{2}\bar{e}(b_E + 1), \quad \langle \epsilon_S \epsilon_E \rangle = -h\langle \epsilon_E^2 \rangle \frac{k_{cat}\bar{s}}{hak_{cat}\bar{e} + (\delta_E + \delta_P)(K_m + \bar{s})} \qquad 7$$

324 While the present approach builds on that derived by Grima (Grima 2010) the

325 results and conclusions differ: Grima (Grima 2010) found that noise had no effect on

326 product concentration in MM reactions. There are two reasons for this discrepancy. First,

327 Grima assumed no dilution of the substrate ($\delta_S = 0$). As can be seen from equation 6, this

328 causes the finite volume correction term for $\langle p \rangle$ to vanish. Thus, Grima's results are in

329 agreement with the present results for this special case, and all of the results in his papers

330 are correct. However, following (Grima 2010) exactly for $\delta_S > 0$ also gives zero

331 stochastic deviation of the product concentration (see SI), which is incorrect. Here is

332 why: when the system-size expansion of the chemical master equation is applied to a

333 system of elementary reactions, as in (Grima 2010) and most other methods, the rate of

334 product formation, V, is proportional to the intermediate complex concentration, c.

335 Because c is unimolecular, the method reads this rate as linear (its second derivative is

336 zero), and so the mean cannot be affected by noise (as proven by Jensen's inequality).

337 However, in MM reactions V is decidedly non-linear. Indeed, it is hyperbolic. Thus, the

338 elementary reaction conceals an essential non-linearity of the system, and will give

339 incorrect results in the general case. To rectify this, one must apply Eqn. 4 to a QSSA

340 reduced system of biochemical equations as shown in the SI. Note that the use of

341 elementary or QSSA reactions is not up to choice: the elementary reaction scheme will

342 give the wrong answer. Note also that applying the method of (Grima 2010) to a QSSA
343 reduced system also gives incorrect results, for reasons discussed in the SI.
344
345 **Noise propagation in gene expression with hyperbolic filtering.** Because gene
346 expression noise is costly, there will ostensibly be selection pressure for noise control.
347 But how can a cell suppress gene expression noise? That is, what are the evolutionary
348 targets of noise-control? To answer these questions, we must model the process of gene
349 expression, form transcription through translation, to identify how noise is generated and
350 filtered.
351 We deviate from previous models of gene expression noise by assuming that
352 translation obeys non-linear reaction kinetics. Previous models of gene expression noise
353 have considered translation as a first-order process (Thattai and van Oudenaarden 2001;
354 Shahrezaei and Swain 2008; Paulsson 2004; Bar-Even et al. 2006; Pedraza and Paulsson
355 2008; McAdams and Arkin 1997). However, translation in living cells may actually be
356 closer to zeroth-order than first-order, with a reaction order of a = 0.1-0.2 in log-phase
357 growth in *E. coli* and budding yeast, based on the fraction of unoccupied ribosomes
358 (Arava et al. 2003; Zenklusen, Larson, and Singer 2008; Ingolia 2014). We now
359 investigate the effects of hyperbolic translation kinetics on gene expression noise.
360 Following previous models of gene expression noise (Thattai and van
361 Oudenaarden 2001; Shahrezaei and Swain 2008; Paulsson 2004; Bar-Even et al. 2006;
362 Pedraza and Paulsson 2008; McAdams and Arkin 1997), we assume for now that
363 translation is initiation limited (see "Cell fitness" section below for generalization), such
364 that it relies only on the concentrations of ribosomes, r, and mRNA, m. If mRNA is

transcribed in bursts of size b_t, then the steady-state variance and Fano factor of mRNA are (Sup. Mat.),

$$\sigma_{mRNA}{}^2 = \frac{1}{2}\overline{m}(b_t + 1), \qquad F_{mRNA} = \frac{1}{2}(b_t + 1) \tag{8}$$

which is a special case of (Elf and Ehrenberg 2003) and which is in excellent agreement with simulations (Figs 2, 3).

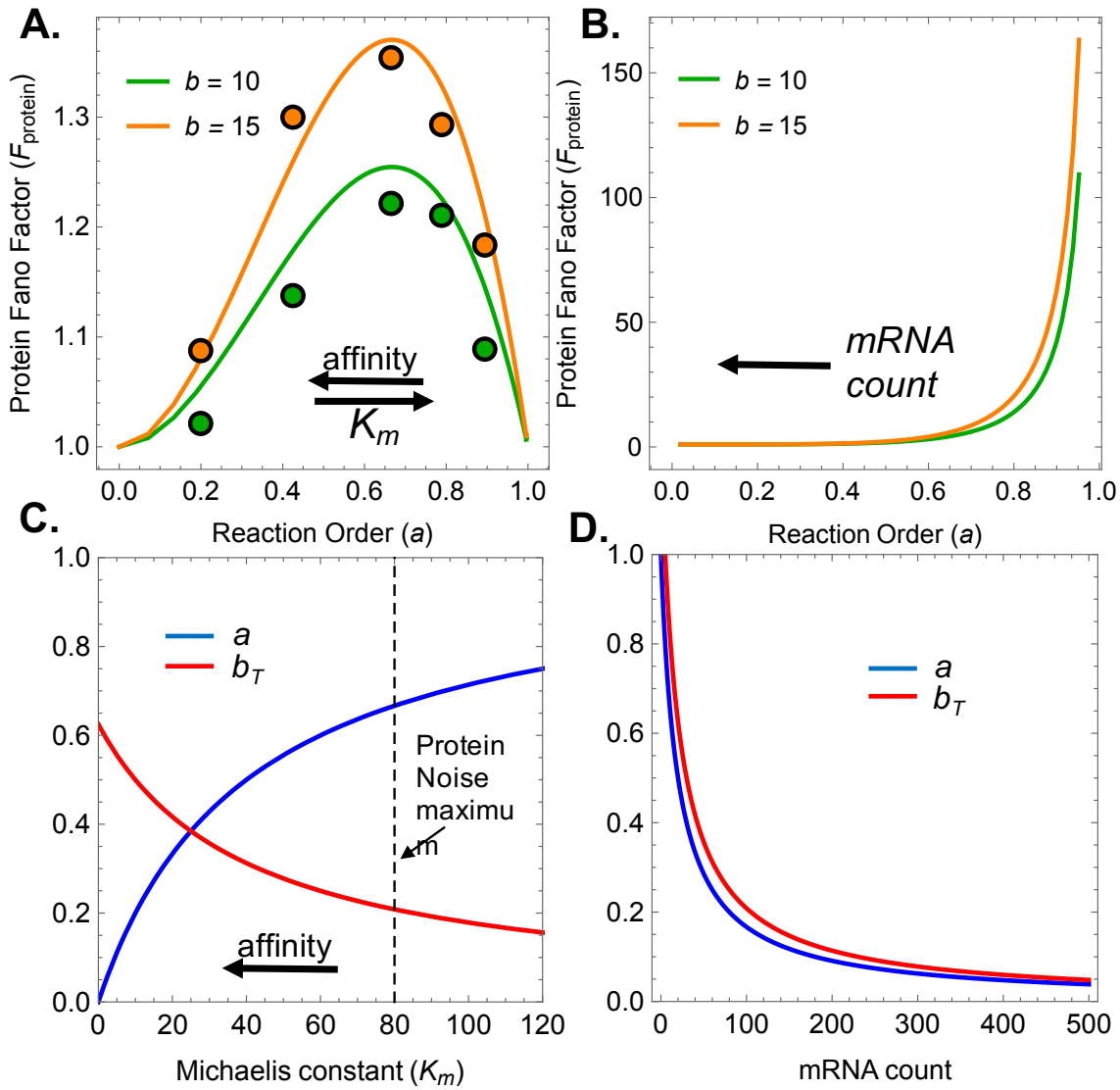


Figure 2: **Protein noise with hyperbolic filtering.** Translation propagates mRNA noise to the protein level, but hyperbolic reaction kinetics ($0<a<1$) can substantially ameliorate this noise and cause highly non-linear relationships between noise and underlying

parameters. Points represent ensemble variance of protein number from the last of 110 time steps over 10,000 realizations of the SSA (see SI), lines represent theoretical prediction (from Eqns 9 and S21). **A, C)** Increasing ribosomal binding affinity (i.e., reducing K_m) has two opposing effects on protein noise: it reduces reaction order but increases translational bursting. Which effect dominates depends on the population's starting point relative to the protein noise peak at a* = 2/3. Most species have translational $a < 2/3$, suggesting that increasing ribosomal binding affinity will actually reduce protein noise, even though it increases bursting. **B, D)** Increasing mRNA abundance, holding mRNA noise constant, monotonically decreases protein noise for all parameter values, as both the reaction order and translational burst parameters decrease with increasing mRNA (Sup. Mat. for theoretical derivation of this result). Parameters: e = 5, k_{ON} =1, k_{OFF} = 19.5 (varied in **A)**, k_{cat} = 0.5, δ_S = 0.1, δ_P = 0.1, k_{in} = 4/b (varied in **B**). K_m was tuned by varying k_{OFF}, s was tuned by varying k_{in}, while mRNA noise was held constant with a burst input of either b =10 or 15.

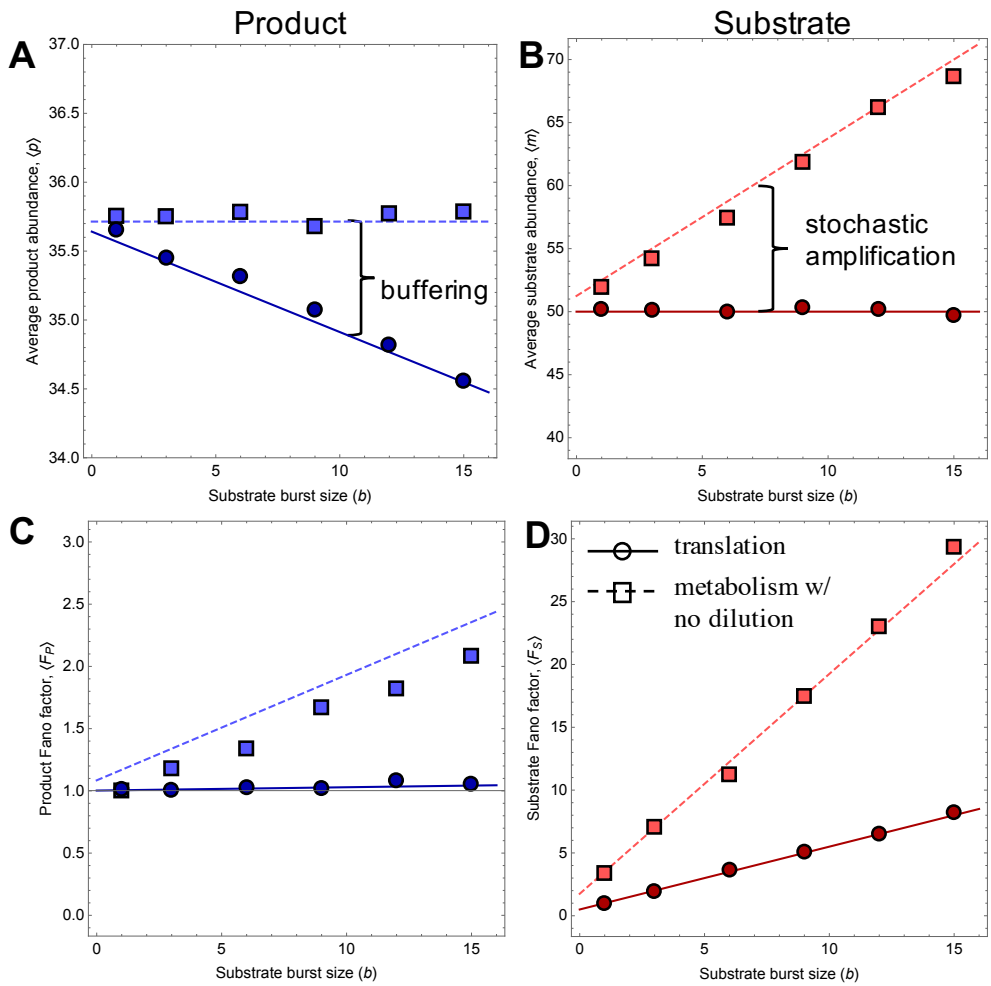


Figure 3: **Effect of network architecture on the cost of noise.** Theoretical predictions (Eqns. 6-8, S20, S21) for metabolic reactions (dashed lines; h = 1, δ_S = 0) and translation (solid lines; h =0, δ_S = 0.1) compared to ensemble average (blue) and variance (red) of

1,000-5,000 SSA realizations (points). **A)** Noise-induced slowdown causes an accumulation of substrate ("stochastic amplification") which completely buffers reaction flux for metabolic reactions when substrate dilution/degradation is zero ($\delta_S = 0$) **(B)**. Because mRNA is not consumed in translation ($h = 0$), its concentration is independent of reaction rate so does not experience stochastic amplification. Thus, translation is not buffered by network feedback. **C,D)** The effect of network architecture on substrate (C) and product (D) noise. e = 1, k_{ON} =1, k_{OFF} = 19.5, k_{cat} = 0.5, , δ_P = 0.01, and k_{in} = 5/b (solid lines) or 0.357/b (dashed lines).

The variance in protein numbers at steady-state is, $\langle \epsilon^2_{protein} \rangle \cong \bar{p}(1 + a^2 b_T F_{mRNA} + CV_R^2 \bar{p}/2)$, where the parameter $a^2 = [K_m/(K_m + m)]^2$ is the squared reaction order and , $CV^2_{ribo} = \sigma^2_{ribo}/\bar{r}^2$ is the ribosomal noise (Sup. Mat.). The translational burst parameter, b_T, is the number of proteins synthesized by a single mRNA molecule over its lifetime, which with hyperbolic translation kinetics is, $b_T = k_T r/((K_m + m)\delta_S)$. Previous work assumed first-order translation, such that $b_T = k_T r/(K_m \delta_S)$ (Thattai and van Oudenaarden 2001; Shahrezaei and Swain 2008). Introducing non-linearity causes the translational burst size to become a non-linear, decreasing function of mRNA abundance: all else equal, an mRNA molecule competing with fewer other mRNA's will be translated more often in its lifetime, increasing its burst size. Alternatively, in the zeroth order regime ($m >> K_m$), translational bursting goes to zero because ribosomes are so oversaturated with mRNA's that any given mRNA is more likely to be degraded than translated. The protein noise strength and noise, then, are, respectively,

$$F_{protein} \cong 1 + a^2 b_T F_{mRNA} + CV^2_{ribo} \bar{p}/2$$

416 $$CV^2_{protein} \cong \underbrace{\frac{1}{\bar{p}} + \frac{a^2 b_T F_{mRNA}}{\bar{p}}}_{\substack{intrinsic \\ noise}} + \underbrace{\frac{CV^2_{ribo}}{2}}_{\substack{extrinsic \\ noise}} \quad 9$$

417 Eqn. 9 agrees well with simulation results (Figs. 2, 3) and retrieves the result of
418 Thattai and van Oudenaarden (Thattai and van Oudenaarden 2001) ($F_{protein} \cong 1 + b_T$)
419 as a special case when there is no transcriptional bursting ($b_t = 1 \Rightarrow F_{mRNA} = 1$), no
420 ribosome fluctuations (CV^2_{ribo} = 0), and translation is first order (i.e., linear) in mRNA
421 abundance (a = 1). The first terms in $F_{protein}$ and $CV^2_{protein}$ (1 and $1/\bar{p}$, respectively) are
422 simply Poisson terms reflecting low copy number fluctuations (Paulsson 2004; Pedraza
423 and Paulsson 2008), while the second and third terms introduce factors that increase noise
424 above Poisson levels. The second terms ($a^2 b_T F_{mRNA}$ and $a^2 b_T F_{mRNA}/\bar{p}$, respectively)
425 incorporate the consequences of translational and transcriptional bursting. These terms
426 propagate mRNA fluctuations, F_{mRNA}, to the protein level. Importantly, hyperbolic
427 translation kinetics (0< a < 1) dampen the effect of mRNA fluctuations on protein noise,
428 thus attenuating the propagation of mRNA noise via a process that might be called
429 "hyperbolic filtering". Together the first and second terms are considered "intrinsic
430 noise" (Swain, Elowitz, and Siggia 2002; Raser and O'Shea 2005; Paulsson 2004; Bar-
431 Even et al. 2006; Sanchez and Golding 2013). The last term ($CV^2_{ribo}\bar{p}/2$ and $CV^2_{ribo}/2$)
432 accounts for fluctuations in ribosome numbers, which create positively correlated
433 fluctuations among protein species called "extrinsic noise" (Swain, Elowitz, and Siggia
434 2002; Raser and O'Shea 2005; Paulsson 2004; Bar-Even et al. 2006; Sanchez and
435 Golding 2013). This extrinsic noise term dominates for abundant proteins (large p),
436 consistent with the observation that protein noise scales inversely with protein abundance

437 for low copy number proteins, but is independent of abundance for high copy number
438 proteins (Blake et al. 2003; Raser and O'Shea 2004; Bar-Even et al. 2006).
439 Translation initiation in *E. coli* and yeast has a reaction order of $a \sim 0.1\text{-}0.2$ (3,
440 49), which is comfortably to the left of the protein noise peak at $a^* = 2/3$ (see Sup. Mat.;
441 Fig 2a). This implies that, contrary to predictions based on first-order translation
442 initiation models (Thattai and van Oudenaarden 2001; Raser and O'Shea 2005), evolving
443 or engineering increased binding affinity of mRNA to ribosomes (i.e., reduced
444 translational K_m) will actually lower protein noise, not increase it. This occurs because
445 reducing K_m has two complementary effects on protein noise: it increases translational
446 bursting, b_T, but reduces reaction order, a, which attenuates noise via a phenomenon we
447 term "zeroth-order insensitivity". The latter effect is not accounted for in previous gene
448 expression models, but has implications for the evolutionary and synthetic targets of
449 noise-amelioration (see Discussion). Hyperbolic filtering also has practical implications
450 when making inferences from data. For example, translational burst size, b_T, can be
451 estimated from Eqn 9 with knowledge of the mRNA and protein statistics; however,
452 assuming first order reaction kinetics ($a = 1$) can lead to large underestimates of this
453 parameter from data. For example, for $a \sim 1/5$, assuming first order translation kinetics
454 can lead to a 25-fold underestimate of translational burst size.
455
456 **Correlation between mRNA and protein noise with hyperbolic translational**
457 **filtering:** We now quantify the effect of hyperbolic translation kinetics on the correlation
458 between mRNA and protein noise. Emprical work in yeast, E. coli and human cell lines
459 have found that there is no correlation between mRNA and protein levels in the cell, or

460 between mRNA and protein noise (Chen et al. 2002; Ghaemmaghami et al. 2003;
461 Taniguchi et al. 2010; Gygi et al. 1999). Here I show that this lack of correlation can be
462 explained, in part, by modeling translation as a hyperbolic reaction.

463 Assuming as before that mRNA lifetimes are much shorter than protein lifetimes
464 ($\delta_{mRNA} >> \delta_{protein}$), the covariance between mRNA and protein noise, , is found to be,
465 $\sigma_{mp} \cong ab_T\sigma_{mRNA}^2$. The linear regression coefficient, $\beta_{mp} \equiv \sigma_{mp}/\sigma_{mRNA}^2$ is then,
466 $\beta_{mp} \cong ab_T$. Both of these show that hyperbolic translation kinetics ($a < 1$) diminish the
467 statistical association between mRNA and proteins. This is most usefully illustrated by
468 the Pearson correlation coefficient, $\rho \equiv \sigma_{mp}/\sigma_{mRNA}\sigma_{protein}$, which is approximately
469 equal to,

470 $$\rho \cong a\sqrt{\left(\frac{\delta_{protein}}{\delta_{mRNA}}\right)\left(\frac{b_T F_{mRNA}}{1 + a^2 b_T F_{mRNA} + CV_{ribo}^2\bar{p}/2}\right)} \qquad 10$$

471 Consistent with the theoretical predictions of Tanaguchi et al (3), Eqn. 10 shows that the
472 mRNA-protein correlation is increased with increasing transcriptional noise strength,
473 F_{mRNA}, is diminished with larger differences in lifetimes of mRNA and protein
474 ($\delta_{protein}/\delta_{mRNA} < 1$), and is diminished by extrinsic noise ($CV_{ribo}^2\bar{p}/2$). In addition, the
475 covariance, linear regression coefficient, and correlation between mRNA and protein
476 fluctuations all increase with increasing translational bursting, b_T. The most important
477 novel result here is that the correlation between mRNA noise and protein noise is
478 diminished by the hyperbolic filtering of translation (that is, when $a < 1$).

479

480 **Network architecture, feedback, and buffering*:*** While noise slows a reaction for a
481 given substrate concentration, network feedbacks cause adjustment to substrate levels

482 that may in turn alter reaction rates. Specifically, if a reaction proceeds more slowly, then
483 substrate levels accumulate, which then cause the reaction to speed up again. Accounting
484 for this feedback is crucial in quantifying the costs of noise. We now ask: How exactly
485 does this feedback influence the total flux through noisey reaction networks? To address
486 this problem, we now analyze how network architecture creates feedbacks that alter
487 chemical flux, potentially compensating for noise-induced slowdown. We treat three
488 limiting cases: 1) $h = 1$, $\delta_S = 0$; 2) $h = 1$, $\delta_S > 0$; and 3) $h = 0$.

489 *Cases 1 ($h = 1$, $\delta_S = 0$): Stochastic substrate amplification and buffering:* One
490 important feedback in noisy networks is the amplification of substrate concentration by
491 noise (Eqn. 6), which has been studied in a process called "stochastic focusing"
492 (Paulsson, Berg, and Ehrenberg 2000). Eqn. 6 shows that stochastic amplification will
493 occur whenever substrate is consumed by the reaction ($h = 1$), as in metabolic reactions.
494 Consequently, this steady-state adjustment of substrate creates intrinsic buffering of
495 reaction flux that ameliorates noise-induced slowdown: the reaction is slowed by noise,
496 allowing substrate to accumulate, which then speeds up the reaction. The extent of
497 buffering depends on the substrate dilution parameter, δ_S. In the MM network studied by
498 Grima (Grima 2010; Grima 2009b), for example, the substrate entered the reaction
499 volume at rate k_{in} and exited only once consumed by the focal reaction at rate V. Flux
500 balance requires that $V = k_{in}$ in such a system, so that the reaction rate in steady-state is
501 unaffected by noise, as found by Grima (Grima 2010; Grima 2009b). Thus, under the flux
502 constraint $V = k_{in}$, stochastic amplification completely buffers the system against noise-
503 induced slowdown (Fig 3).

504 *Case 2 (h = 1, δ_S > 0): Incomplete amplification and buffering.* However, the
505 precise balancing of V with k_{in} does not apply in living cells, where substrate exits the
506 system via multiple alternative channels ($\delta_S > 0$), primarily dilution by cell division, but
507 also through excretion, titration by other reactions and enzymatic degradation.
508 Extracellular substrate also experiences non-zero δ_S, as it is diluted by bulk flow (such as
509 in a chemostat) or by uptake from neighboring cells. Accounting for these effects
510 modifies the flux constraint to: $k_{in} = V + \delta_S s$. With this network architecture, stochastic
511 amplification compensates for some of the diminution of V by noise, but not all.
512 Consequently, stochastic amplification fails to completely buffer metabolic reactions in
513 living cells against noise-induced slowdown.
514 *Case 3 (h = 0, δ_S > 0): Gene expression is especially sensitive to noise-induced*
515 *slow-down; no stochastic amplification, no buffering.* The $V = k_{in}$ flux constraint is
516 entirely abolished in template-mediated reactions (h = 0), such as protein synthesis via
517 translation of mRNA. Because mRNA is not consumed by translation, steady-state
518 mRNA concentration is set by the flux constraint $k_{in} = \delta_S s$, which is independent of
519 translation rate, V. Thus, stochastic amplification does not occur in translation and so
520 cannot buffer translation from mRNA noise (Fig. 3). As a consequence, gene expression
521 is unusually sensitive to noise.
522
523 **Cell fitness.** Noise slows biochemical reactions, but how does it affect fitness? We now
524 consider a reaction network representing a coarse-grained model of cell growth. We then
525 analyze this reaction network in the stochastic case using the method developed above in
526 order to quantify the effects of gene expression noise on the average rate of cell division.

527 During balanced exponential growth, it has been shown empirically that fitness,
528 here defined as the division rate of a cell, is equivalent to the total rate of protein
529 synthesis by translation in a cell (Shahrezaei and Marguerat 2015; Scott et al. 2010; Scott
530 et al. 2014). We denote the rate of cell division by the parameter λ . In general, biomass
531 synthesis involves the import of extracellular nutrients followed, ultimately, by their
532 conversion into new biomass via translation. Translation initiation involves the binding of
533 mRNA, in abundance m, to a ribosome, in abundance r, followed by elongation via the
534 polymerization addition of amino acids (aa) to a growing protein chain. Elongation rate is
535 limited by the intracellular nutrient, in abundance s_I, in scarcest supply (i.e., von Leibig's
536 Law of the Minimum (Droop 1974; De Baar 1994; Tilman 1982)), which is typically
537 either ATP or aa-charged tRNA's. In eutrophic conditions, where nutrients are saturating,
538 translation is initiation-limited (Shah et al. 2013), whereas in oligotrophic conditions,
539 where nutrients are scarce, translation is elongation-limited (Tuller et al. 2010).
540 This coarse-grained cell model follows a QSSA system of ODE's (S22) with
541 growth rate,

542 $$\frac{dp}{dt} = k_T r \frac{s_I m}{K_1 + K_2 s_I + K_3 m + s_I m} - \lambda p \qquad 11$$

543 which is equivalent to a compulsory-order two-substrate enzymatic reaction with
544 independent (i.e., non-cooperative) binding events (Ingalls 2013). k_T is the translation
545 elongation rate per mRNA per ribosome (which absorbs the number of S_I per protein),
546 K_1/ K_2/ K_3 are affinity constants, and λ is the protein dilution rate, which during steady-
547 state growth is equivalent to the rate of cell division. Eutrophic and oligotrophic regimes,
548 corresponding to initiation- and elongation-limited translation, respectively, are found
549 from this equation in the limit as s_I or m, respectively, goes to infinity. Eqn. 11 can be

550 rearranged to form a Monod equation, which maps growth rate to extracellular nutrient

551 supply with a number of practical and conceptual benefits (S24).

552 The expected steady-state rate of cell division (cells/time) is found by setting Eqn.

553 11 to zero and solving for λ,

554 $$\langle \lambda \rangle = \frac{\langle V \rangle}{p_{ss}} \qquad 12$$

555 p_{ss} is defined as the species-specific total protein content of a cell (protein/cell), which is

556 assumed constant for a given species, V is the synthesis rate of proteins (protein/time).

557

558 **Fitness with noise.** Following the method above, we write down a QSSA reduced system

559 of equations for growth rate according to our coarse-grained cell model (S22), giving a

560 system in five equations describing the time evolution of mRNA, uptake proteins,

561 extracellular substrate, intracellular substrate and protein biomass. We assume that

562 mRNA and extracellular nutrients undergo a linear birth-death process, supplied with

563 burst inputs of size b_t and b_{SE} respectively, and ignore ribosome fluctuations.

564 Intracellular nutrients are consumed by protein synthesis, but mRNA is not. Expected

565 fitness, then, is (Sup. Mat.):

566 $$\langle \lambda \rangle \cong \frac{\bar{V}}{p_{ss}} \left[1 - \underbrace{\overbrace{\frac{\langle \epsilon_m^2 \rangle}{\bar{m}} \frac{A_1}{\Omega}}^{mRNA\ noise} - \overbrace{\frac{\langle \epsilon_{PU}^2 \rangle}{\bar{p}_U} \frac{A_2}{\Omega}}^{protein\ noise}}_{cost\ of\ gene\ expression\ noise} - \overbrace{\frac{\langle \epsilon_{SE}^2 \rangle}{\overline{s_E}} \frac{A_3}{\Omega}}^{extracellular\ noise} \right] \qquad 13$$

567 Where the A_i's are positive constants given in the SI. In reality, the protein noise term

568 will contain contributions from all proteins involved in the pathway importing and

569 processing nutrients to their final form utilized in translation. The MCA model of Wang

570 and Zhang (Wang and Zhang 2011) then fits within this term. The extracellular noise

571 term quantifies the strength of selection favoring phenotypic "bet hedging" (Starrfelt and
572 Kokko 2012) strategies in response to environmental unpredictability.

573 Surprisingly, transcriptional noise directly reduces fitness. This occurs because
574 fluctuations in mRNA levels reduce the average translation rate. This is counter to the
575 typical view, which holds that transcriptional noise impinges on cell function only to the
576 extent that it propagates to generate protein noise. Unlike the cost of protein or
577 extracellular nutrient noise, which require a number of parameter estimates that are
578 highly context-dependent, the cost of mRNA noise is easily estimated from available data
579 if we assume that cells are growing in their eutrophic regime ($s_I \rightarrow$ infinity), where
580 growth rate is limited by translation initiation. Taking the eutrophic limit of Eqn 11
581 gives, $dp/dt \cong k_T rm/(K_m + m) - \lambda p$, which is simply a MM reaction with mRNA
582 serving as the substrate and ribosomes as the catalyst. Noting that fluctuations in total
583 ribosome number and mRNA number are uncorrelated, we find from equations 4, 7 and
584 9,

585 $$\langle\lambda\rangle \cong \frac{\bar{V}}{p_{ss}}\left[1 - \underbrace{\frac{(b_t+1)}{2\bar{m}} a(1-a)\Omega^{-1}}_{cost\ of\ trancriptional\ bursting}\right] \qquad 14$$

586 Where $\bar{V} = k_T rm/(K_m + m)$. The cost of transcriptional bursting vanishes with zeroth-
587 or first-order translation (a = 0 and 1, respectively) and/or as cell volume and/or mRNA
588 abundance become large.

589 During balanced exponential growth of *E. coli* in rich medium: $m \sim 1400$
590 molecules, $r \sim 21{,}000$ molecules, and $k_T \sim 21aa/s \sim 4.6$ proteins/min (given a mean
591 protein length of 275*aa*). The total protein count of a cell is $p_{ss} = 2.4\text{x}10^6$ molecules.
592 During growth in nutrient rich medium, $(1 - a) \sim 0.85$ (Arava et al. 2003; Ingolia 2014;

593 Zenklusen, Larson, and Singer 2008). Applying these parameter estimates to $\bar{V}/p_{ss}$ gives
594 a doubling time for *E. coli* of ~30 minutes. Similar calculations with parameters for the
595 budding yeast *Saccharomyces cerevisiae* (m ~ 15,000-60,000, r ~ 243,000, k_T ~ 15aa/s ~
596 2 proteins/min (average protein length 450aa), $p_{ss} = 5x10^7$, a = .85) gives a doubling
597 time of ~120 minutes. Both values are in close agreement with experiments in nutrient
598 rich media, providing an important benchmark.

599 In an *E. coli* cell, according to Eqn. 14, a mutation causing one unit increase in the
600 total mRNA Fano factor results in an average loss of 7.5 proteins/min, 223 proteins/cell
601 division, and $1.5\text{x}10^{-4}$ cells/generation, such that selection against mRNA noise is more
602 than 10,000 times stronger than random drift in this species (the effective population size
603 determines the strength of random allele frequency fluctuations and is $N_e = 2x10^8$ in *E.*
604 *coli*). In contrast, the same mutation in yeast, which has 40-fold higher m, has a cost of
605 $2.1\text{x}10^{-6}$ cells/generation, which is only about 20 times stronger than random drift ($N_e =$
606 $1x10^7$ in *S. cerevisiae*). This illustrates how fitness magnifies small differences in
607 reaction rates by integrating over the whole cell and cell lifetime, and highlights the
608 importance of cell volume (or more directly, molecule number) in influencing the
609 efficacy of selection against noise.

610

611 **Rapid decay of selection efficacy with cell volume.** As we just showed, the cost of
612 noise depends on reaction volume. Because unicellular species differ over several orders
613 of magnitude in their cell volumes, it may be possible to use these two facts in order to
614 make predictions about taxonomic patterns of gene expression noise.

615 The fate of a mutation that modifies cellular noise depends on the relative
616 magnitude of selection and random genetic drift. Selection efficiently overcomes random
617 drift provided that $2N_e|s| > 1$ (Kimura 1957; Kimura 1962). The boundary at $2N_e|s| = 1$
618 defines the" drift barrier" separating efficient and inefficient selection regimes (Sung et
619 al. 2012; Lynch 2007b). The drift barrier acts as a kind of evolutionary attractor
620 preventing organismal perfection: recurrent deleterious mutations with effects smaller
621 than $2N_e|s|$ push species away from their adaptive optima, and selection is too weak to
622 push them back. Thus, the balance between recurrent mutation, drift and selection
623 establishes a suboptimal phenotypic steady-state. We now derive a scaling relationship
624 between cell volume and selection efficacy demonstrating that the drift boundary for
625 noise attenuation scales superlinearly with cell volume.
626
627 **Selection strength scales inversely with cell size.** Selection strength, s, is defined as the
628 difference in absolute fitness of mutant (m) and resident (r) alleles scaled by the mean
629 fitness of the population. For a rare mutant we have: $s = (\lambda_m - \lambda_r)/\lambda_r$. This is simply
630 the difference in the absolute number of daughter cells produced by the mutant per
631 resident doubling. For simplicity, we assume a haploid population and that the mutant
632 and resident genotypes differ only in transcriptional noise. When the only source of noise
633 is intrinsic mRNA fluctuations, the selection coefficient is approximately (see SI for
634 derivation),

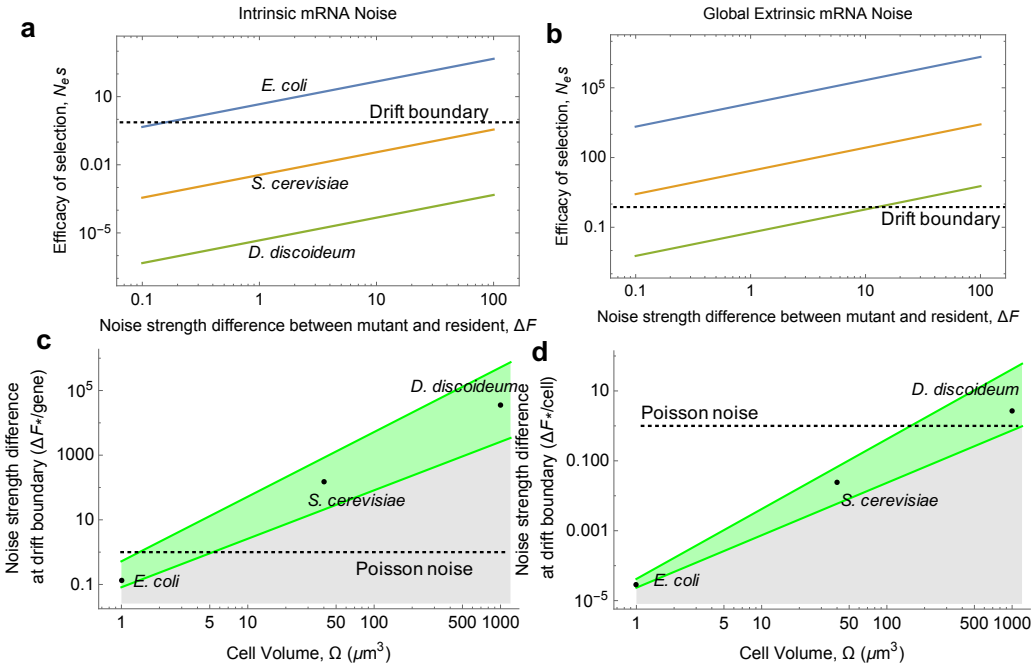
635 $$\mathrm{s} \cong \Delta F_{mRNA} \frac{K_m}{(K_m + m)^2} {\Omega_c}^{-1} = \Delta CV^2_{mRNA} a(1-a){\Omega_c}^{-1} \quad 15$$

636 Ω_c denotes the volume of the reaction compartment. The sign of selection is determined
637 by $\Delta F = (F_r - F_m)$ or $\Delta CV^2 = (CV_r^2 - CV_m^2)$, the difference in Fano factors (noise

638 strength) or squared coefficients of variation (noise), respectively, between resident and
639 mutant alleles. A mutation that increases transcriptional noise will be deleterious (s < 0),
640 while a decrease in noise is advantageous (s>0). More generally, $s \cong \sum_i^n (\Delta F_i A_i)\, \Omega_{ci}{}^{-1}$,
641 where the sum is over all *n* sources of noise affected by the mutation, the A_i are constants,
642 and Ω_{ci} is the reaction volume of the ith noise source. Note that selection cannot act on
643 noise levels in first or zeroth order reactions (*a* = 1 or 0, respectively), since noise does
644 not alter the mean in these cases. Noise will, however, alter the heritability (e.g.,
645 penetrance) of a trait (Wang and Zhang 2011), which will reduce the response to
646 selection, but we do not consider this effect here. Importantly, the strength of selection
647 against noise explicitly depends on reaction volume.
648
649 **Drift strength scales positively with cell size.** A species population size tends to scale
650 inversely with its body size: a species must divide a limited resource pool amongst its
651 members, resulting either in a large number of small individuals or a small number of
652 large ones (Damuth 1981; Damuth 1987). For multicellular species the empirical scaling
653 relationship between body mass and population size, known as "Damuth's Law", follows
654 a power law with a scaling exponent of -¾, likely reflecting the ¾ power scaling of body
655 size with energetic demands (Damuth 1981; Damuth 1987). However, in unicellular
656 species' metabolic rate scales either linearly (eukaryotes) or superlinearly (prokaryotes)
657 with cell mass (DeLong et al. 2010). Assuming that N_e scales linearly with *N*, that mass
658 scales linearly with cell volume (i.e., cells have constant density), then $N_e = B\Omega_w{}^{-g_{drift}}$,
659 where the subscript on Ω_w denotes whole cell volume and g_{drift} = [3/4, 5/4] is the scaling
660 exponent. The constant, *B,* is proportional to the population size of a species with unit

661 volume, and absorbs cell density as the proportionality constant between mass and
662 volume.
663
664 **Combining forces: Selection efficacy scales superlinearly with cell size.** Combining
665 these results implies that the efficacy of selection against noise is proportional to
666 $({\Omega_w}^{-g_{drift}}{\Omega_c}^{-1})$. For translation, it is possible to establish a relationship between Ω_c and
667 Ω_w, allowing us to reduce the number of independent variables.
668 Protein synthesis occurs in the cytoplasm/cytosol, suggesting that reaction-
669 compartment volume may simply equal whole-cell volume ($\Omega_c \sim \Omega_w$) for translation. This
670 relationship appears to hold between *E. coli* and yeast (*m* = 1500 and 60,000,
671 respectively, with a 40 fold difference in cell volume) and within mammalian cells as
672 they change volume throughout the cell cycle (Kempe et al. 2015; Padovan-Merhar et al.
673 2015). However, Gillooly et al (Gillooly et al. 2005) found that RNA concentration scales
674 as body mass to the -1/4 power over a broad range of eukaryotes, corresponding to RNA
675 number scaling with mass to the ¾ power. Although unicells were underrepresented in
676 their sample, this agrees well with the scaling of mRNA between *E. coli* and the amoeba
677 *Dictyostelium discoideum* where *m* = 155,000 and cell size is approximately 500-1000
678 fold larger than *E. coli*, such that $\Omega_c \sim \Omega_w^{2/3}\ to\ \Omega_w^{3/4}$.
679 Thus, for translation, we assume that reaction volume scales with whole cell
680 volume according to, $\Omega_c = \Omega_w^{-g_{sel.}}$, where g_{sel} = [3/4, 1]. Therefore, the efficacy of
681 selection against mRNA noise can be written as an allometric scaling law:
682 $$N_e|s| \cong \Delta C V_{mRNA}^2 a(1-a) B {\Omega_w}^{-g} \quad 16$$

683 The superlinear scaling exponent, $g = g_{sel} + g_{drift} = [3/2, 2¼]$, makes selection against
684 noise extremely weak in large cells. For example, all else equal, selection against a
685 mutation that increases expression noise is thirty thousand to one million times stronger
686 in *E. coli* than in a species, such as the amoeba *Dictyostelium discoideum*, which is about
687 1000 times larger (Fig. 4).



688

689 ***Figure 4:*** **Power-law scaling of selection efficacy with cell volume. A,B)** The predicted
690 efficacy of selection, $N_e|s|$, for *E. coli*, *S. cerevisae* and *Dictyostelium disoicdeum*, as a
691 function of the jump size in noise caused by a mutation, $|\Delta F|$, for mutations acting at a
692 single locus (left-hand column) and mutations acting on the noise of the global
693 transcriptome (right-hand column). The mRNA concentrations and effective population
694 size (N_e) estimates used for the figure are found in Table S1 (m = 1500, 60,000 and
695 155,000, respectively, and ploidy-adjusted $N_e = 2x10^8$, $1x10^7$ and $1.1x10^5$, respectively).
696 Reaction order was assumed to be a = 0.8 for all three species. The "drift boundary" is
697 $2N_e|s| = 1$. **C,D)** The predicted noise jump size at the drift boundary as a function of cell
698 volume. The green region represents a range of values for the scaling exponent (-2 to -
699 3/2), reaction order (0.75-0.9), and in **C)** the gene number (3250-12,500). Above the
700 green region (or individual points) selection is strong, below mutations are effectively
701 neutral.
702

703 Solving for $|\Delta F|$ at the drift boundary ($2N_e|s| = 1$), gives the jump size in noise
704 strength "visible" to natural selection. If the only cost of transcriptional noise is reduced
705 translation, then the selectively impermissible jump sizes in transcriptional noise follows
706 the scaling relationship,

707 $$|\Delta F_{mRNA}{}^{*}| > b\Omega_w{}^{g} \quad 17$$

708 where $b = (K_m + m)^2/BK_m$. Mutations causing jumps smaller than this critical size are
709 "effectively neutral", and thus invisible to selection acting on translation rate. Adaptive
710 mutations that decrease noise by an amount less than this jump size cannot efficiently
711 spread, while deleterious mutations with effect size below this threshold cannot be
712 efficiently purged by selection.

713 Parameterizing our model with literature estimates of cell volume, effective
714 population size, genome size, ribosomal occupancy, and translation kinetics, we predict
715 that *E. coli* should be strongly selected to minimize intrinsic transcriptional noise simply
716 because this noise slows translation. Whereas the cost of transcriptional noise is too weak
717 per gene (on average) in *S. cerevisiae* or *Dictyostelium* for selection to maintain intrinsic
718 noise at the Poisson minimum if noise-induced slowdown of translation is the only cost
719 of mRNA noise. Fig 4 shows that in cells below about 5fL (most prokaryotes), selection
720 favors suppression of intrinsic transcriptional noise to its theoretical minimum defined by
721 Poisson statistics, even if the only cost of transcription noise is reduced translation rate.
722 But because of the superlinear scaling of selection efficacy, cells only 100 times larger
723 (most eukaryotes) cannot efficiently oppose intrinsic noise jumps even as large as $\Delta F =$
724 1000 (again, if this is the only selection pressure for noise-minimization acting).
725 Alternatively, jumps in global transcriptional noise (i.e., extrinsic noise) have a much

726 greater selective effect, meaning that traits affecting extrinsic noise, such as ribosome
727 fluctuations (Eqn 9), should be under strong selection for noise-amelioration even in
728 moderately sized species.
729
730 **DISCUSSION**
731 Biochemical reactions are non-linear, but are typically modeled as linear in stochastic
732 models of gene expression due to technical limitations (van Kampen 2007). Here we have
733 introduced a novel procedure for easily and more accurately incorporating non-linearity
734 into models of coupled stochastic chemical reactions. We have found that hyperbolic
735 reaction kinetics filter input noise in such a way that the average reaction rate is slowed
736 by noise, while attenuating noise propagation. These results have a number of
737 implications.
738
739 **A drift barrier hypothesis for gene expression noise and robustness.** Cell size places
740 severe constraints on the evolution of noise suppression. The magnitude of noise-induced
741 slowdown of Michaelis-Menten reactions is inversely proportional to the reaction
742 volume, which follows intuitively from the fact that, for a given molecular density,
743 greater volumes contain more molecules and thus generate less noise. Lynch (Lynch
744 2007b) has previously noted the "genomic perils of evolving large body size". Large
745 bodied species tend to be less efficient at purging deleterious mutations due to the
746 positive scaling of genetic drift strength with body size, leading to a number of broad
747 taxonomic patterns including greater genomic mutation rate, transposon abundance and
748 intron number in unicellular eukaryotes and metazoans relative to prokaryotes and

749 viruses (Lynch 2007b; Lynch 2007a; Sung et al. 2012). Unique to gene expression noise,
750 cell size affects not only drift but also fitness, resulting in a negative superlinear scaling
751 of selection efficacy with cell size (Eqns. 15-17). Unicellular species span more than four
752 orders of magnitude in cell volume (~0.1 μm^3 Mycoplasma to more than 1000 μm^3 in
753 amoebazoans and other protists), with a sharp taxonomic divide separating prokaryotes
754 and eukaryotes. "Typical" cell diameters are 0.5-5um for prokaryotes and 10-100um for
755 unicellular eukaryotes. Taking a conservative value of a 100-fold average volume
756 difference between prokaryotes and eukaryotes, this translates to a 1000-10,000-fold
757 difference in the average efficacy of selection against noise for non-compartmentalized
758 processes (Eqn. 16).

759 Of course, numerous other fitness effects of gene expression noise occur (Eqn. 13
760 and (Fraser et al. 2004; Wang and Zhang 2011; Lehner 2008; Raj and van Oudenaarden
761 2008; Balázsi, van Oudenaarden, and Collins 2011; Raser and O'Shea 2005)), but these
762 must all scale with cell (or compartment) volume, such that selection for noise-
763 amelioration will often be weaker in eukaryotes than prokaryotes. It is possible that the
764 greater transcriptional complexity in eukaryotic cells, which employ multisubunit
765 regulatory complexes, enhancer regions, heterochromatic gene silencing, nuclear
766 localization into "transcription factories" and other features that greatly increase the
767 burstiness of transcription, evolved only once greater cell size freed populations from the
768 selective constraint of noise minimization. The cause of the size disparity between
769 prokaryotes and eukaryotes is thought to be due to differences in cellular respiration:
770 prokaryotes synthesize ATP via the electron transport chain embedded in the cell's
771 plasma membrane, which requires a high surface area to volume ratio for the whole cell

772 in order to balance energy production and demand. Respiration via mitochondria free
773 eukaryotes from this constraint, allowing them to achieve large cell sizes without
774 bankrupting the cell's energy budget. Thus, the evolution of mitochondria may have
775 paved the way for the greater transcriptional sophistication of eukaryotes.
776
777 **Translation attenuates the propagation of transcriptional noise:** Gene expression
778 noise is generated at two levels: transcription and translation. While the molecular
779 mechanisms controlling noise at each of these levels are becoming increasingly well
780 understood, much remains unclear about the propagation of noise between levels. I have
781 shown here that hyperbolic translation kinetics greatly attenuate the propagation of
782 mRNA noise to the protein level. Given parameter estimates of translation kinetics in E.
783 coli and yeast, the results here imply that hyberbolic translation will reduce mRNA noise
784 by between 25 and 100 fold, substantially attenuating the propagation of mRNA noise to
785 the protein level. The hyperbolic filtering of mRNA noise by translation also manifests as
786 a substantial reduction in the correlation between mRNA and protein abundance (Eqn.
787 10). This result is consistent with data from *E. coli* showing that there is zero correlation
788 between a gene's mRNA noise and its protein noise (Tanaguchi et al 2010). We have
789 shown that hyperbolic translation kinetics can help explain this lack of correlation, since
790 the correlation coefficient between mRNA and protein noise is proportional to the
791 reaction order parameter, a, which is between 0.1 and 0.2 in E. coli, implying a large
792 reduction in the correlation. Geometrically, one can visualize this diminution of
793 correlation by observing the Michaelis-Menten rate curve: on the flat part of the curve, all

794 mRNA concentrations lead to the same rate of protein synthesis, thus creating a zero
795 correlation between mRNA and protein.
796 A major implication of these results is that the vast majority of all intrinsic protein
797 noise comes from translation, not transcription. Consequently, if protein noise, rather
798 than mRNA noise, determines the functional consequences of gene expression noise, then
799 natural selection on transcriptional noise will be extremely weak, and synthetic systems
800 desiring control of protein noise should target translation, not transcription. We have thus
801 come to two conclusions about transcriptional noise: 1) mRNA noise directly inhibits
802 growth by slowing translation and 2) very little mRNA noise propagates to the protein
803 level. These two predictions cast new light on the functional consequences of
804 transcriptional noise in living cells.
805
806 **Survival of the flattest.** We have shown that hyperbolic reactions, by filtering out high-
807 amplitude input fluctuations, cause a reduction in both signal (average output) and noise
808 (relative output variance) compared to first-order reactions. The strength of selection
809 favoring organismal robustness is determined by the former, while the target of selection
810 for increased robustness is determined by the latter. Both the strength of noise and its cost
811 are mediated by the reaction order, $a = K_m/(K_m + s)$ (Eqns. 6, 9), making this the obvious
812 target for noise-attenuation. Noise-induced slowdown is minimal for first and zeroth
813 order reactions (a =1, 0, respectively), while noise itself is minimal only in zeroth order
814 (a = 0). Indeed, zeroth order kinetics completely filter out super-Poisson intrinsic input
815 noise (Eqn. 9; Fig. 2), making the output entirely insensitive to input noise. We might call
816 this phenomenon "zeroth order insensitivity". Selection for robustness, then, should push

817 reactions towards their zeroth order regime. Geometrically, the zeroth order regime
818 corresponds to the flat part of the rate curve, such that adaptive robustness by this
819 mechanism promotes "survival of flattest", a term previously invoked to describe
820 selection for robustness via very different mechanisms (Wilke et al. 2001; Codoñer et al.
821 2006).

822 There are two targets of modification for reaction order minimization: increasing
823 substrate abundance, s, which moves the substrate distribution to the flatter part of the
824 rate curve, or reducing K_m, which shifts the rate curve itself. Importantly, these strategies
825 are not equivalent (Fig. 2). Decreasing K_m (while holding s constant) causes a unimodal
826 change in product noise because K_m has two complementary effects: it reduces reaction
827 order, a, which filters noise, but it also increases product burst size, b_T (Fig. 2).
828 Alternatively, increasing mRNA concentration (with K_m held constant) monotonically
829 decreases protein noise because both reaction order, a, and translational bursting, b_T,
830 decline. This strategy increases fitness monotonically, thus avoiding the mRNA/protein
831 noise trade-off. Importantly, increasing robustness by increasing substrate concentration
832 is different from increasing robustness by decreasing substrate noise. To highlight this,
833 one could force noise strength to stay constant while increasing mean substrate. The
834 result would be increased robustness without a decrease in noise strength. This strategy
835 undoubtedly generates other costs not accounted for in the current model, such as the
836 energetic cost of increased mRNA synthesis. Future work should explicitly examine the
837 evolutionary feasibility of cellular robustness via this mechanism in light of its potential
838 trade-offs.
839

840 **Robustness via compartmentalization:** The present theory provides new insight into the
841 recent argument that compartmentalization of reactions in eukaryotic cells is a strategy
842 for noise control (Stoeger, Battich, and Pelkmans 2016). If cell compartments are filled
843 with reactants via active transport by membrane transporters, then the resulting
844 hyperbolic filtering (described above) will attenuate copy number fluctuations of
845 reactants potentially down to the Poisson minimum, though not lower (e.g., Eqns 9 and
846 S21). But hyperbolic filtering also slows the average rate of transport, creating
847 inefficiency. In addition, reducing the reaction volume increases the relative size of
848 fluctuations (the noise), which slows the absolute average rate of reactions within the
849 compartment. Future work is necessary to determine exactly when the conflicting costs
850 and benefits of hyperbolic filtering favor reaction compartmentalization as a strategy for
851 cellular robustness. In eukaryotes, this takes on another dimension, because mRNA is
852 actively transported out of the nucleus and into the cytoplasm before translation. The
853 hyperbolic filtering of transcriptional noise at this level will greatly ameliorate
854 transcriptional noise even before mRNA is filtered by translation. Future work should
855 model the consequences of this serial transcriptional noise filtering in eukaryotes.
856
857 **Metabolic flux.** Wang and Zhang (Wang and Zhang 2011) used metabolic control
858 analysis (MCA) to study the effect of enzyme fluctuations on metabolic flux, concluding
859 that enzyme noise diminishes fitness in pathways with more than one enzyme. This result
860 arises from the hyperbolic relationship between enzyme concentration and steady state
861 flux in multi-enzyme metabolic pathways (Kacser and Burns 1973), and so, like the
862 present paper, is a straightforward consequence of nonlinear averaging. However, MCA

863 models assume first-order enzyme kinetics (Kacser and Burns 1973). In light of the
864 present results, it is not immediately clear if this assumption makes the results of (Wang
865 and Zhang 2011) an underestimate of the true cost of gene expression noise, because
866 hyperbolic rate laws cause noise-induced slowdown at each pathway step, or an
867 overestimate of cost, because hyperbolic rate laws attenuate noise at each step, thus
868 damping noise propagation through the pathway. Future work should distinguish between
869 these two possibilities.
870
871 **The crucial assumption:** All of the major results of this paper rely on the validity of
872 hyperbolic reaction kinetics as an accurate description of *in vivo* biochemical reactions.
873 This assumption is not guaranteed, especially for translation. Translation is a unique
874 biochemical reaction because a single substrate molecule (mRNA) is simultaneously
875 bound to multiple enzymes (ribosomes), and it is therefore easy to imagine that
876 translation kinetics may not be well-described by Michaelis-Menten reaction kinetics.
877 Therefore, the present theoretical results must remain in question until future empirical
878 work carefully measures the *in vivo* rate law of protein translation.
879
880 **METHODS**
881 All Monte Carlo simulations were run on the elementary (microscopic) system of
882 reactions (S17) using Gillespie's Exact Stochastic Simulation Algorithm (SSA) (Gillespie
883 1977) implemented in *Mathematica* v10.3.0.0 with the open source xSSAlite package,
884 freely available from the xCellerator project (http://www.xlr8r.info/SSA/). Each
885 realization of the simulation is a Markov Chain with irregular (exponentially distributed)
886 time intervals. For statistical analysis, each realization was regularized using the

TimeSeriesAggregate[] command. Mean[] and Variance[] commands of the regularized time series were used to compute the moments of the Markov Chain. Data points in figures 1,2 and 3 are ensemble averages or variances over 1000-10,000 simulated realizations of the stochastic process; each realization was run for at least 10 times the half life of S or P (half-life = ln(2)/ δ), whichever was longer, and the value for that realization was *s* or *p* during the final second of the simulation run.

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Supplemental Material: "Fitness costs of noise in biochemical reaction networks and the evolutionary limits of cellular robustness"

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Derivations of Theoretical Results

I.a. *Stochastic Michaelis-Menten quasi steady-state approximation (MM QSSA)*

Consider the canonical bimolecular reaction scheme representing the catalyzed conversion of substrate into product (3, 4):

$$S + E \xleftrightarrow{k_{on}, k_{off}} C \xrightarrow{k_{cat}} P + E$$

Substrate *S* reversibly binds catalyst *E* to form a reaction intermediate complex, *C*, that then decays irreversibly into *E* and product *P*. Irreversibility is allowed because living systems exist far from thermodynamic equilibrium. Because three or more body collisions are rare, all catalytic reactions can be decomposed into a series of bimolecular reactions of this type, which is why this reaction scheme provides the cornerstone of biochemistry.

This microscopic description is converted into a system of first-order non-linear differential equations (ODE's) by applying the law of mass action. This system cannot be solved explicitly, so we apply the quasi-steady state approximation (QSSA), which assumes that the timescale of intermediate (*C*) formation is fast relative to product (*P*) formation, such that *C* equilibrates to its steady-state concentration effectively instantaneously to changes in *S* (4, 5).This separation of timescales allows us to set the concentration of *C* to it's steady-state value, giving the steady-state rate of product formation,

$$\frac{dp}{dt} = V = V_{max}\frac{s}{K_m + s} \qquad \text{S1}$$

Here, *s* is reactant concentration (following convention, lowercase denotes concentration), $V_{max} = k_{cat}e$ is the maximum achievable reaction rate, and $k_m = (k_{off} + k_{cat})/k_{on}$, the Michaelis or half-saturation constant, is the substrate

concentration at $V = V_{max}/2$. This rate law function is a rectangular hyperbola, with V approaching V_{max} asymptotically as s goes to infinite (Figure 1).

Because S1 is hyperbolic, Jensen's inequality proves that, for random s, $\langle V(s)\rangle \leq V(\langle s\rangle)$. This says that the average reaction rate is less than or equal to a noise-free (i.e., deterministic) reaction with $s = \langle s\rangle$. This is shown by Taylor expanding the Michaelis Menten reaction rate about the average value of s. First, multiply the numerator and denominator of S1 by the reaction volume, Ω, to obtain a description in terms of particle numbers, $n_S = \langle n_S\rangle + \epsilon_S$, where $\langle n_S\rangle$ is the ensemble average number of species S, and ϵ_S is a random variable with finite variance and zero mean. Expanding S1 in powers of ϵ_S and taking the expected value gives,

$$\langle V(s)\rangle = \underbrace{V_{max}\frac{\langle n_S\rangle}{k_m + \langle n_S\rangle}}_{V(\langle s\rangle)} - \underbrace{\langle {\epsilon_S}^2\rangle V_{max}\frac{k_m}{(k_m + \langle n_S\rangle)^3}}_{cost\ of\ noise} + O(\epsilon^3) \qquad \text{S2}$$

Where $k_m = \Omega K_m$. The variance in V is,

$$\sigma_V^2 = \langle V^2\rangle - \langle V\rangle^2 = \langle {\epsilon_S}^2\rangle {V_{max}}^2 \frac{{k_m}^2}{(k_m + \langle n_S\rangle)^4} + O(\epsilon^3) \qquad \text{S3}$$

Substrate noise reduces the rate of biochemical reactions. The difference between the average stochastic rate and deterministic rate grows monotonically with increasing noise, and equality occurs only with zero noise or zero curvature of $V(s)$.

For gamma distributed n_S, the probability density function of the Michaelis-Menten reaction rate (for $V < V_{max}$) is exactly,

$$P(V = v) = \frac{V_{max}}{k_m}\frac{v^{\alpha-1}e^{-v/z\beta}}{z(z\beta)^\alpha\Gamma(\alpha)}, \qquad z = \frac{V_{max} - v}{k_m} \qquad \text{S4}$$

which has mean,

$$\langle V\rangle = V_{max}\frac{e^{\frac{k_m}{F}}}{\eta^2}\left(\frac{F}{k_m}\right)^{-\eta^{-2}}\Gamma\left(-\eta^{-2}, \frac{k_m}{F}\right) \qquad \text{S5}$$

where Γ(.,.) is the incomplete gamma function. Equation S4 is verified by Monte Carlo simulations implemented with Gillespie's Exact Stochastic Simulation Algorithm (SSA) (1) (Fig. 1).

1205 Figure 1 shows that the effect of noise on MM-type reactions is to increase the
1206 observed Michaelis constant. Therefore, a stochastic formulation of S1 that accounts for
1207 all stochastic effects of fluctuating *s* at steady-state is obtained simply by substituting an
1208 "effective", stochastic Michaelis constant using equations or S5, respectively:

$$\langle V \rangle = V_{max} \frac{\langle s \rangle}{\widetilde{K}_m + \langle s \rangle} \qquad \text{S6}$$

1209

1210 where,

1211 $$\widetilde{K}_m = \frac{e^{-\frac{K_m}{F}} F \left(\frac{F}{K_m}\right)^{-\eta^{-2}}}{\Gamma\left(-\eta^{-2}, \frac{K_m}{F}\right)} - F\eta^{-2}, \quad \widetilde{K}_m \cong K_m \left[\frac{(K_m + \langle s \rangle)^2 + \langle s \rangle F}{(K_m + \langle s \rangle)^2 - K_m F}\right]$$

1212

1213 Note that $\widetilde{K}_m \geq K_m$ and $\widetilde{K}_m$ increases with increasing noise strength (measured by the
1214 Fano factor, *F*. This simple substitution retains the form of the classical MM equation
1215 (Equation S1) and provides a simple, phenomenological short-hand for the effect of noise
1216 on MM reaction kinetics.

1217

1218 **I.b. Reaction networks**

1219 **I.b.i. The multivariate system-size expansion of the chemical master equation**

1220 We now solve for the time evolution of the moments (mean and variance) for each
1221 species in a chemical reaction network. We employ the chemical master equation and its
1222 expansion in orders of the inverse square root of system size, Ω. The chemical master
1223 equation describes the time evolution of the probability distribution of chemical species.
1224 Because these equations include bimolecular reactions they are non-linear, and are
1225 typically not explicitly solvable. The system size expansion is an approximation method,
1226 where the parameter Ω provides an objective criterion for truncation of the series
1227 expansion allowing for moment closure. For thorough reviews and extensions of the
1228 method see van Kampen (6), Elf and Erhenberg (7), and Grima (8). van Kampen
1229 introduced the system size expansion, Elf and Erhenberg generalized the system size
1230 expansion to systems of chemical reactions, and Grima extended this further by deriving
1231 a multivariate system size expansion that retained terms beyond the linear noise

approximation (LNA). Here we build on this work to provide a novel method that accurately models the statistics of gene expression and metabolic networks in living cells.

We define $P(\vec{n},t)$ as the joint probability density function of particle numbers for all N species in a system of R reactions. Its time evolution follows the chemical master equation (7),

$$\frac{\partial P(\vec{n},t)}{\partial t} = \Omega \sum_{j=1}^{R} \left(\prod_{i=1}^{N} E_i^{-S_{ij}} - 1 \right) f_j(\vec{n},\Omega) P(\vec{n},t) \qquad \text{S7}$$

where $\vec{n}$ is the vector of particle numbers of all species, Ω is the reaction volume, $f_j(\vec{n},\Omega)$ is the transition rate between states for the *j*th reaction, S_{ij} is the stoichiometric coefficient for the net reaction, and *E* is the step operator which acts as follows: $E_i^{-S_{ij}} f(n_1,\dots,n_i,\dots,n_N) = f\left(n_1,\dots,n_i - S_{ij},\dots,n_N\right)$. The particle number is then written as a random perturbation about the macroscopic concentration (thus translating from the extensive variable *n* to the intensive variable *x*) according to the ansatz that the relative perturbation size scales as the inverse square root of the reaction volume, in accordance with the Law of Large Numbers, leading to the substitution:

$$\frac{n_i}{\Omega} = \bar{x}_i + \Omega^{-1/2}\epsilon_i \qquad \text{S8}$$

where $\bar{x}_i$ is the macroscopic steady-state concentration of species *i*, and ϵ_i is a continuous random variable. Substituting into Eqn. S7 and simplifying (6, 7),

$$\frac{\partial P(\vec{n},t)}{\partial t} = \frac{\partial \Pi(\vec{\epsilon},t)}{\partial t} - \Omega^{1/2} \sum_{i=1}^{N} \frac{\partial \mathrm{x}_i}{\partial t} \frac{\partial \Pi(\vec{\epsilon},t)}{\partial \epsilon_i} \qquad \text{S9}$$

Taylor expansion of the transition rate for the *j*th reaction, $f_j(\vec{x})$, about the macroscopic rate gives,

$$f_j(\vec{x}) = f_j(\bar{\vec{x}}) + \Omega^{-1/2} \sum_{i}^{N} \frac{\partial f_j(\bar{\vec{x}})}{\partial x_i} \epsilon_i + \frac{\Omega^{-1}}{2} \sum_{i,k=1}^{N} \frac{\partial^2 f_j(\bar{\vec{x}})}{\partial x_i \partial x_k} \epsilon_i \epsilon_j + O(\Omega^{-3/2}) \qquad \text{S10}$$

$$= f_j(\bar{\vec{x}}) + \Omega^{-1/2} \nabla f_j(\bar{\vec{x}}) \vec{\epsilon} + \frac{\Omega^{-1}}{2} \vec{\epsilon}^T \boldsymbol{H}(\bar{\vec{x}}) \vec{\epsilon} + O(\Omega^{-3/2})$$

where ∇ is the gradient operator and $\boldsymbol{H}$ is the Hessian matrix.

Note that S10 differs from that of Grima (equation 13 in (8)), who included an additional term accounting for the special case of homodimerization. While Grima's expansion works for all elementary reactions, it gives erroneous results when applied to bimolecular reactions written in QSSA reduced from. We therefore use Eqn. S10 with the caveat that it will be inaccurate for homodimerization reactions at low copy numbers.

Taylor expansion of $\prod_{i=1}^{N} E_i^{-S_{ij}}$ to order $O(\Omega^{-3/2})$ gives the differential operator (6–8),

$$\prod_{i=1}^{N} E_i^{-S_{ij}} = 1 - \Omega^{-1/2} \sum_{i}^{N} S_{ij} \frac{\partial}{\partial \epsilon_i} + \frac{\Omega^{-1}}{2!} \sum_{i,k=1}^{N} S_{ij} S_{kj} \frac{\partial^2}{\partial \epsilon_i \partial \epsilon_k} - \frac{\Omega^{-1}}{3!} \sum_{i,k,l=1}^{N} S_{ij} S_{kj} S_{lj} \frac{\partial^3}{\partial \epsilon_i \partial \epsilon_k \partial \epsilon_l} + O(\Omega^{-3/2}) \quad \text{S11}$$

Combining S8-S11, and ignoring third derivatives (which vanish for first and second moments) we obtain,

$$\frac{\partial \Pi(\vec{\epsilon}, t)}{\partial t} - \Omega^{\frac{1}{2}} \sum_{i=1}^{N} \frac{\partial \mathrm{x}_i}{\partial t} \frac{\partial \Pi(\vec{\epsilon}, t)}{\partial \epsilon_i} = \Omega \sum_{j=1}^{R} \left(-\Omega^{-1/2} \sum_{i}^{N} S_{ij} \frac{\partial}{\partial \epsilon_i} + \frac{\Omega^{-1}}{2} \sum_{i,k=1}^{N} S_{ij} S_{kj} \frac{\partial^2}{\partial \epsilon_i \partial \epsilon_k} \right) \left(f_j(\vec{\bar{x}}) + \Omega^{-1/2} \sum_{i}^{N} \frac{\partial f_j(\vec{\bar{x}})}{\partial x_i} \epsilon_i + \frac{\Omega^{-1}}{2} \sum_{i,j=1}^{N} \frac{\partial^2 f_j(\vec{\bar{x}})}{\partial x_i \partial x_j} \epsilon_i \epsilon_j \right) \Pi(\vec{\epsilon}, t)$$

Noting that terms of order $O(\Omega^{1/2})$ cancel, after some algebra we arrive at the following multivariate master equation,

$$\frac{\partial \Pi(\vec{\epsilon}, t)}{\partial t} \qquad \text{S12}$$

$$= -\sum_{i,k}^{N} J_{ik} \frac{\partial(\epsilon_k \Pi)}{\partial \epsilon_i} + \frac{1}{2} \sum_{i,k=1}^{N} D_{ik} \frac{\partial^2 \Pi}{\partial \epsilon_i \partial \epsilon_k}$$

$$+ \frac{\Omega^{-1/2}}{2} \left(- \sum_{i,k,l=1}^{N} \frac{\partial J_{ik}}{\partial x_l} \frac{\partial(\epsilon_l \epsilon_k \Pi)}{\partial \epsilon_i} + \sum_{i,k,l=1}^{N} \frac{\partial D_{ik}}{\partial x_l} \frac{\partial^2(\epsilon_l \Pi)}{\partial \epsilon_i \partial \epsilon_k} \right) + O(\Omega^{-1})$$

where $\Pi = \Pi(\vec{\epsilon}, t)$, and

$$J_{ik} = \sum_{j=1}^{R} S_{ij} \frac{\partial f_j(\vec{\bar{x}})}{\partial x_k}$$

$$D_{ik} = \sum_{j=1}^{R} S_{ij} S_{kj} f_j(\vec{\bar{x}})$$

The first two terms of S12 (of order Ω^0) together give the standard linear Fokker-Planck equation which is the sum of directional motion (e.g., convection, transport, drift) and diffusion, respectively. The terms of order $\Omega^{-1/2}$ are the nonlinear, finite volume corrections to these terms, which vanish for linear reactions and/or reactions in large volumes (i.e., in the macroscopic limit).

The stationary solution of the linear Fokker-Planck equation is a Gaussian distribution. We expect that if the relative fluctuation size is small compared to the curvature of the transition rate, then the stationary solution of S12 remains approximately Gaussian even in finite volumes with non-linear transition rates. Of course, the actual product distribution of a reaction with a hyperbolic rate law is positively skewed (and a sigmoidal rate law is leptokurtic). Assuming relatively small fluctuations, only the first two moments of $\Pi(\vec{\epsilon}, t)$ are needed to approximately characterize the full distribution in small volumes.

The time derivative of the first moment of the *i*th species is found by multiplying both sides of S12 by ϵ_i and integrating over all ϵ:

$$\int \epsilon_i \frac{\partial \Pi(\vec{\epsilon}, t)}{\partial t} = \frac{\partial \langle \epsilon_i \rangle}{\partial t}$$

$$= -\int \epsilon_i \sum_{k}^{N} J_{ik} \frac{\partial(\epsilon_k \Pi)}{\partial \epsilon_i} d\epsilon_i + \frac{1}{2} \int \epsilon_i \sum_{k=1}^{N} D_{ik} \frac{\partial^2 \Pi}{\partial \epsilon_i \partial \epsilon_k} d\epsilon_i$$

$$-\frac{\Omega^{-1/2}}{2} \left(\int \epsilon_i \sum_{k,l=1}^{N} \frac{\partial J_{ik}}{\partial x_l} \frac{\partial(\epsilon_l \epsilon_k \Pi)}{\partial \epsilon_i} d\epsilon_i - \int \epsilon_i \sum_{k,l=1}^{N} \frac{\partial D_{ik}}{\partial x_l} \frac{\partial^2(\epsilon_l \Pi)}{\partial \epsilon_i \partial \epsilon_k} d\epsilon_i \right)$$

This is straightforwardly computed using integration by parts (iteratively for the second derivative terms) and applying the definitions of the mean and variance, leading to:

$$\frac{\partial \langle \epsilon_i \rangle}{\partial t} = -\sum_{k}^{N} J_{ik} \langle \epsilon_k \rangle + \frac{\Omega^{-1/2}}{2} \sum_{k,l=1}^{N} \frac{\partial J_{ik}}{\partial x_l} \langle \epsilon_l \epsilon_k \rangle + O(\Omega^{-1}) \qquad \text{S13}$$

which has matrix form:

$$\frac{\partial \langle \vec{\epsilon} \rangle}{\partial t} = -\boldsymbol{J} \cdot \langle \vec{\epsilon} \rangle + \Omega^{-\frac{1}{2}} \vec{\theta} + O(\Omega^{-1}) \qquad \text{S14}$$

where $\vec{\theta}$ is a vector with kth element:

$$\theta_k = \frac{1}{2} \sum_{i,j}^{N} \frac{\partial J_{ki}}{\partial x_j} \langle \epsilon_i \epsilon_j \rangle$$

Substituting $\vec{\epsilon} = \Omega^{1/2}(\vec{x} - \vec{\bar{x}})$, setting S14 equal to zero and solving for the average concentration vector, $\langle \vec{x} \rangle$**,** finally gives the matrix equation for first moments of the steady-state system:

$$\langle \vec{x} \rangle = \vec{\bar{x}} + \Omega^{-1} \boldsymbol{J}^{-1} \cdot \vec{\theta} \qquad \text{S15}$$

Thus, one can solve for the average of all species in a discrete, stochastic system with finite volume directly from the continuous, deterministic system of equations for the system.

One solves the second moments of the system using the same approach. One multiplies S12 by ${\epsilon_i}^2$ integrates (again, using integration by parts), then solves for $\langle \epsilon^2 \rangle$ when the distribution is stationary ($\frac{d\langle \epsilon^2 \rangle}{dt} = 0$). The full solution is a function of

the first three moments of the joint distribution, such that the moment hierarchy does not close. Further, solving the second moments of the linear multivariate Fokker-Planck equation is already computationally intensive, without consider higher order corrections. Thus, we will ignore the non-linear finite volume correction to the second moments for the purposes of the present paper. Simulation results suggest that this leads to small error (Fig. 2, 3 main text). Therefore, we can solve for the second moments by solving the Lyapunov Equation (7):

$$0 = \mathbf{J} \cdot \mathbf{C} + \mathbf{C} \cdot \mathbf{J}^{\boldsymbol{T}} + \mathbf{D} + O(\Omega^{-\frac{1}{2}}) \qquad \text{S16}$$

for the covariance matrix **C** which has elements,

$C_{ij} = \langle \epsilon_i \epsilon_j \rangle$

This can be solved symbolically using the *Mathematica* (Wolfram Research) command: LyapunovSolve[**J**,-**D**]. **D** is the noise-generating diffusion matrix given by,

$$\mathbf{D} = \mathbf{S} \cdot \mathbf{R} \cdot \mathbf{S}^{\boldsymbol{T}}$$

S is the stoichiometric matrix of the network and **R** is the matrix of the macroscopic rate function.

Comparison to Grima (2010):

Grima (8) previously derived results for a multivariate system size expansion to order $O(\Omega^{-1})$, as we have just done. However, our final equations differ in two ways, with both quantitative and qualitative disagreement. Grima's master equation does not include finite volume non-linear corrections to the diffusion term, which we include here only for the sake of generality and completeness, although it disappears for first moments and we neglect it for our computation of second moments. However, the following difference prevents Grima's method from being applied accurately to a QSSA reduced system: Grima's (8) first moments are solved using:

$$\langle \vec{x} \rangle = \vec{\bar{x}} + \Omega^{-1} \boldsymbol{J}^{-\mathbf{1}} \cdot \vec{\Delta}$$

where,

$$\Delta_k = \frac{1}{2}\left(\sum_{i,j}^{N} \frac{\partial J_{ki}}{\partial \phi_j}\langle \epsilon_i \epsilon_j \rangle - \sum_{i}^{N} \phi_i \frac{\partial J_{ki}}{\partial \phi_i}\right)$$

Compare this Δ to our θ above. Grima's expression is valid when applied to all systems of elementary reactions, where the second term in Δ_k accounts for homodimerization reactions (since homodimerization rates are proportional to $n(n\text{-}1)$, not n^2). However, Grima's expression does not work when applied to reduced (e.g., "elementary complex", pseudo-first order, biochemical) reactions. As a trade-off, the present approach is inaccurate when applied to homodimerization reactions in small volumes.

I.b.ii. Application to a general enzyme-catalyzed reaction in an open system

We consider the following generalized MM reaction scheme:

$$\begin{gathered} \emptyset \xrightarrow{k_{in}} b_S S, \qquad \emptyset \xrightarrow{k_{in,E}} b_E E \\ S + E \xleftrightarrow{k_1, k_{-1}} C \xrightarrow{k_{cat}} P + E + (1-h)S \\ S \xrightarrow{\delta_S} \emptyset, \qquad P \xrightarrow{\delta_P} \emptyset, \qquad E \xrightarrow{\delta_E} \emptyset, \qquad C \xrightarrow{\delta_C} \emptyset \end{gathered} \tag{S17}$$

This scheme generalizes the three cases of interest. The parameter h is an indicator variable: $h = 1$ in reactions where S is consumed (as with most metabolic reactions) and $h = 0$ for template-mediated reactions (such as when S is mRNA (i.e., translation) or a gene (i.e., transcription)). When $\delta_S = 0$ the only output of S is via the reaction catalyzed by E; this corresponds to the case modeled by Grima (8) who found that noise does not alter pathway flux. This scheme corresponds to the following system of ordinary differential equations (ODE's):

$$\begin{aligned} \frac{ds}{dt} &= b_S k_{in} - k_1 s e_0 + ((1-h)k_{cat} + k_{-1})c - \delta_S s \\ \frac{de}{dt} &= b_E k_{in,E} - k_1 s e_0 + (k_{cat} + k_{-1})c - \delta_E e \\ \frac{dc}{dt} &= k_1 s e_0 - (k_{cat} + k_{-1})c \\ \frac{dp}{dt} &= k_{cat} c - \delta_P p \end{aligned} \tag{S18}$$

Following convention, lower case *s, e,* and *p* denote the concentration of species *S*, *E* and *P*, respectively. The subscript on e_0 is used to make clear that this refers to "free" (unbound) enzyme concentration. Assuming that *c* equilibrates fast relative to the rest of the reaction, we can replace *c* with its steady-state solution which leads to the following QSSA reduction of the system,

$$\frac{ds}{dt} = b_S k_{in,S} - hV - \delta_S s \qquad \text{S19}$$

$$\frac{de}{dt} = b_E k_{in,E} - \delta_E e$$

$$\frac{dp}{dt} = V - \delta_P p$$

where,

$$V = k_{cat} e \frac{s}{K_m + s}$$

and where $e = e_0 + c$ is the total concentration of enzyme.

This system has corresponding Jacobian matrix,

$$\mathbf{J} = \begin{pmatrix} -h\left(\frac{k_{cat}e}{K_m + s}\right)\left(1 - \frac{s}{K_m + s}\right) - \delta_S & -h\left(\frac{k_{cat}s}{K_m + s}\right) & 0 \\ 0 & -\delta_E & 0 \\ \left(\frac{k_{cat}e}{K_m + s}\right)\left(1 - \frac{s}{K_m + s}\right) & \frac{k_{cat}s}{K_m + s} & -\delta_P \end{pmatrix}$$

rate matrix **R**,

$$\mathbf{R} = \begin{pmatrix} k_{in,S} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & k_{in,E} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & V & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & V & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \delta_S s & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \delta_E e & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \delta_P p \end{pmatrix}$$

and stoichiometric matrix,

$$\mathbf{S} = \begin{pmatrix} b_S & 0 & -h & 0 & -1 & 0 & 0 \\ 0 & b_E & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & -1 \end{pmatrix}$$

The noise-generating diffusion matrix is then,

$$\mathbf{D} = \mathbf{S} \cdot \mathbf{R} \cdot \mathbf{S}^{\boldsymbol{T}} = \begin{pmatrix} {b_S}^2 k_{in,S} + hV + \delta_S s & 0 & 0 \\ 0 & {b_E}^2 k_{in,E} + \delta_E e & 0 \\ 0 & 0 & V + \delta_P \end{pmatrix}$$

Finally, the θ vector equals,

$$\vec{\theta} = \left(h\left(\frac{k_{cat}K_m}{(K_m+\bar{s})^2}\right)\left(\langle e\rangle \frac{\langle {\epsilon_S}^2\rangle}{K_m+\bar{s}} - \langle \epsilon_S \epsilon_E\rangle\right) \quad 0 \quad -\left(\frac{k_{cat}K_m}{(K_m+\bar{s})^2}\right)\left(\langle e\rangle \frac{\langle {\epsilon_S}^2\rangle}{K_m+\bar{s}} - \langle \epsilon_S \epsilon_E\rangle\right)\right)^T$$

Substituting **J** and $\vec{\theta}$ into Eqn S15 gives the noise-induced deviation of the steady-state concentrations from the macroscopic prediction:

$$\langle \vec{\mathrm{x}} \rangle - \vec{\boldsymbol{x}} = \left(h\left(\frac{k_{cat}K_m}{\Omega(h\bar{e}k_{cat}K_m + \delta_S(K_m+\bar{s})^2)}\right)\left(\bar{e}\frac{\langle {\epsilon_S}^2\rangle}{K_m+\bar{s}} - \langle \epsilon_S \epsilon_E\rangle\right), \quad 0, \quad -\delta_S\left(\frac{k_{cat}K_m}{\Omega\delta_P(h\bar{e}k_{cat}K_m + \delta_S(K_m+\bar{s})^2)}\right.\right.$$

For translation (h = 0) the vector of steady-state concentrations simplifies to:

$$\langle \vec{\mathrm{x}} \rangle = \begin{pmatrix} \bar{s} & \bar{e} & \bar{p} - \underbrace{\langle {\epsilon_S}^2\rangle \frac{k_{cat}\bar{e}K_m}{\delta_P(K_m+\bar{s})^3}\Omega^{-1}}_{cost\ of\ noise} \end{pmatrix}^T$$

$$= \left(\bar{s} \quad \bar{e} \quad \bar{p}\left(1 - \frac{F_s K_m}{(K_m+\bar{s})^2}\Omega^{-1}\right)\right)^T$$

$$= (\bar{s} \quad \bar{e} \quad \bar{p}(1 - \eta_S^2 a(1-a)\Omega^{-1}))^T$$

where $F_s = \langle {\epsilon_S}^2\rangle/\bar{s}$ is the Fano factor of the substrate (i.e., mRNA) concentration, which equals ½(1+b), where b is the burst size of mRNA.

Four things to note from these results. First, the steady-state enzyme concentration (the second term in each vector) is not affected by noise (it has a deviation equal to 0). This is expected since de/dt has no non-linear terms. Second, if the substrate is not consumed by the reaction (h = 0), then the steady-state substrate concentration is unaffected by noise (the first term in the deviation vector equals 0 when h = 0). Again, this is as expected since the substrate concentration when not consumed follows a simple linear birth-death process, just like the enzyme. Third, noise alters the steady-state

concentration of product (i.e. the reaction flux) only if the substrate is diluted (since the third entry in the deviation vector is proportional to δ_S) or if the reaction is template-mediated ($h = 0$). This is also expected: as described in the Main Text, when $\delta_S = 0$, stochastic focusing, the increase in substrate concentration due to noise, completely buffers the system from noise-induced loss of reaction flux. Finally, the cost of noise is proportional to either the Fano factor (a.k.a. the noise strength) or the squared coefficient of variation (a.k.a. the noise) of the substrate.

I.c. Gene expression noise

The steady-state second moments are given by the covariance matrix, **C**. Many of these moments are too unwieldy to reproduce here. A *Mathematica* (Wolfram Research) notebook with the solution is provided as Supplementary Material. Three of the more compact moments are given here:

$$\langle \epsilon_E^2 \rangle = \frac{1}{2}\bar{e}(1 + b_E) \tag{S20}$$

$$\langle \epsilon_S^2 \rangle = \frac{\left({b_S}^2 k_{in} + \delta_S \bar{s}\right)(\delta_E + \delta_S)(K_m + \bar{s})^4}{2(hK_m k_{cat}\bar{e} + (\delta_E + \delta_S)(K_m + \bar{s})^2)(hK_m k_{cat}\bar{e} + \delta_S(K_m + \bar{s})^2)} + h\frac{k_{cat}(K_m+\bar{s})G}{2(hK_m k_{cat}\bar{e}+(\delta_E+\delta_S)(K_m+\bar{s})^2)(hK_m k_{cat}\bar{e}+\delta_S(K_m+\bar{s})^2)},$$

$$\langle \epsilon_S \epsilon_E \rangle = -h\langle \epsilon_E^2 \rangle \frac{k_{cat}\bar{s}}{hak_{cat}\bar{e}+(\delta_E+\delta_P)(K_m+\bar{s})},$$

$$G = \left[2\langle \epsilon_E^2 \rangle k_{cat}(K_m + \bar{s}) + \left({b_S}^2 k_{in} + \delta_S \bar{s}\right)K_m \bar{e}(K_m + \bar{s}) + \bar{e}\bar{s}(K_m k_{cat}\bar{e} + (\delta_E + \delta_S)(K_m + \bar{s})^2)\right]$$

For the special case of template-mediated reactions ($h = 0$), such as translation, we find:

$$\langle \epsilon_E^2 \rangle = \frac{1}{2}\bar{e}(1 + b_E)$$

$$\langle \epsilon_S^2 \rangle = \frac{1}{2}\bar{s}(1 + b_S)$$

$$\langle \epsilon_S \epsilon_E \rangle = 0$$

For the case when h =0, such as translational, the variance of product (e.g., protein) number is found to be:

$$\langle {\epsilon_P}^2 \rangle = \frac{\bar{p}}{2} + \frac{k_{cat}\bar{e}\bar{s}}{2\delta_P(K_m+\bar{s})} + \frac{{b_S}^2 k_{cat}^2 \bar{e}^2 k_{in,S} K_m^2}{2\delta_S\delta_P(\delta_S+\delta_P)(K_m+\bar{s})^4} + \frac{k_{cat}^2\bar{e}^2\bar{s}K_m^2}{2\delta_P(\delta_S+\delta_P)(K_m+\bar{s})^4} + \frac{k_{cat}^2\bar{s}^2(\delta_E\bar{e}+k_{in,E}{b_E}^2)}{2\delta_E\delta_P(\delta_E+\delta_P)(K_m+\bar{s})^2} \quad \text{S21}$$

This is further simplified by noting the following:

$$\bar{p} = \frac{k_{cat}\bar{e}\bar{s}}{\delta_P(K_m+\bar{s})}$$

$$\bar{s} = \frac{b_S k_{in,S}}{\delta_S}$$

$$\bar{r} = \frac{b_E k_{in,E}}{\delta_E}$$

$$\langle {\epsilon_E}^2 \rangle = \frac{(\delta_R\bar{e}+k_{in,E}{b_E}^2)}{2\delta_E}$$

Making these substitutions and simplifying gives,

$$\langle {\epsilon_P}^2 \rangle = \bar{p} + \bar{p}\frac{k_{cat}\bar{e}K_m^2}{2(\delta_S+\delta_P)(K_m+\bar{s})^3}(b+1) + \langle {\epsilon_E}^2 \rangle \frac{k_{cat}^2\bar{s}^2}{\delta_P(\delta_E+\delta_P)(K_m+\bar{s})^2}$$

Assuming that $\delta_S >> \delta_P$ and that $\delta_E = \delta_P$,

$$\langle {\epsilon_P}^2 \rangle \cong \bar{p} + \bar{p}\frac{k_{cat}\bar{e}K_m^2}{2\delta_S(K_m+\bar{s})^3}(b+1) + \langle {\epsilon_E}^2 \rangle \frac{k_{cat}^2\bar{s}^2}{2\delta_P^2(K_m+\bar{s})^2}$$

Noting that,

$$\frac{k_{cat}^2\bar{s}^2}{\delta_P^2(K_m+\bar{s})^2} = \left(\frac{\bar{p}}{\bar{e}}\right)^2$$

and defining the burst parameter as the number of product molecules produced in the lifetime of a single substrate molecule,

1428 $$b_T \equiv \frac{k_{cat}\bar{e}}{\delta_S(K_m + \bar{s})}$$

1429 and using the definition of a and η_R^2 from the main text and $\langle {\epsilon_S}^2 \rangle$ above, finally leads to,

1430 $$\langle {\epsilon_P}^2 \rangle \cong \bar{p}(1 + a^2 b_T F_S + \eta_E^2 \bar{p}/2)$$

1431 where F_S is the substrate Fano factor, $\langle {\epsilon_S}^2 \rangle / \bar{s}$.

1432 Note that for translation with non-limiting amino acid abundance, fluctuations in
1433 ribosome concentration, $\langle {\epsilon_E}^2 \rangle$, do not influence the the statistics mRNA and do not
1434 influence the mean protein abundance. Ribosome flucutations do, however, alter the
1435 variance of protein abundance, $\langle {\epsilon_P}^2 \rangle$. This latter effect constitutes one source of
1436 "extrinsic noise" (9, 10) in gene expression.

1437 **I.d. The cost of transcriptional bursting**

1438 Substituting these statistics into the steady-state product concentration gives an
1439 expression for the effect of transcriptional bursting, b_t, on protein abundance,

1440 $$\langle p \rangle = \bar{p} - (b_t + 1)\frac{k_{cat}\bar{e}\bar{s}K_m}{2\Omega\delta_P(K_m + \bar{s})^3}$$

1441 $$= \bar{p}\left(1 - \frac{(b_t + 1)K_m}{2\Omega(K_m + \bar{s})^2}\right)$$

1442 and the steady-state rate of protein synthesis ($\langle V \rangle = \langle p \rangle / \delta_P$)

1443 $$\langle V \rangle = \bar{V} - \underbrace{(b_t + 1)\frac{k_{cat}\bar{e}\bar{s}K_m}{2\Omega(K_m + \bar{s})^3}}_{cost\ of\ transcriptional\ bursting}$$

1444 $$= \bar{V}\left(1 - \frac{(b_t + 1)K_m}{2\Omega(K_m + \bar{s})^2}\right)$$

1445

1446 **II.a. Response of protein noise to evolutionary changes in K_m and s.**

1447 Protein noise strength ($F_{protein}$) can be adjusted in two ways: modifying K_m or s. We
1448 now show that these strategies are not equivalent. Specifically, modifying K_m results in a
1449 unimodal change in $F_{protein}$, whereas modifying s has a monotonic effect. Recall from
1450 above,

1451 $$F_{protein} \cong 1 + a^2 b_T F_{mRNA}$$

1452 where the kinetic order parameter, a, is defined as (e.g., (11, 12)),

1453 $$a_{ij} \equiv \left(\frac{\partial f_i}{\partial x_j}\right)\left(\frac{x_j}{f_i}\right) = \frac{\partial \ln (f_i)}{\partial \ln (x_j)}$$

1454 for the reaction rate *f* and concentration *x*. For Michaelis-Menten kinetics,

1455 $$a = \frac{K_m}{K_m + \bar{s}}$$

1456 The reaction order, *a*, is equivalent to the fraction of unbound catalyst.

1457 Evolutionary or synthetic modification of K_m will alter protein noise according to,

1458 $$\frac{\partial F_{protein}}{\partial K_m} = (2a - 3a^2)\frac{k_{cat}\bar{e}F_{mRNA}}{\delta_S(K_m + \bar{s})^2}$$

1459 which has an interior root at,

1460 $$a^* = 2/3$$

1461 for all parameter values, which is also the maximum. Alternatively,

1462 $$\frac{\partial F_{protein}}{\partial \bar{s}} = -3a^2\frac{k_{cat}\bar{e}F_{mRNA}}{\delta_S(K_m + \bar{s})^2}$$

1463 which is a monotonically decreasing function of *a.* Thus, ameliorating noise by
1464 increasing *s* is always a good strategy, all else equal, whereas the direction of evolution
1465 of binding affinity depends on initial conditions. Note that a =2/3 corresponds with weak
1466 binding affinity of an enzyme. Indeed, ribosome binding affinity ranges from *a* = 0.1-0.3,
1467 such that enhancing binding affinity will reduce protein noise.

1468

1469 **II.b. Absolute fitness cost of noise**

1470 We analyze a coarse-grained cell model where cell growth relies on import of
1471 extracellular nutrient, s_E, by nutrient import proteins, p_U, for use in translation of mRNA,
1472 *m*, by ribosomes, *r*. Translation initiation involves the reversible binding of mRNA to
1473 ribosomes. Then elongation proceeds with the sequential addition of *n* intracellular
1474 nutrients, s_I, which we define as the intracellular nutrient most limiting to translational
1475 elongation (either ATP or charged amino acids). posit that fitness, the rate of new
1476 biomass synthesis, *V*, obeys the following QSSA reduced system of biochemical
1477 equations:

$$\frac{ds_E}{dt} = b_{sE}k_{in,SE} - \delta_{SE}s_E \qquad \text{S22}$$

$$\frac{ds_I}{dt} = V_U - \delta_{SI} s_I$$

$$\frac{dm}{dt} = b_t k_t - \delta_M m$$

$$\frac{dp_U}{dt} = \phi V_T - \delta_{PU} p_U$$

$$\frac{dp}{dt} = V_T - \delta_P p$$

where,

$$V_T = k_T r \frac{m s_I}{K_1 + K_2 s_I + K_3 m + s_I m} \qquad \text{S23}$$

V_T is the rate law for a compulsory-order (mRNA first, then s_I second) , two substrate (mRNA and s_I) enzymatic reaction, where ribosomes are the enzyme. The parameter ϕ is the fraction of total mRNA, m, consisting of nutrient importer protein p_U transcripts. In practice, the extracellular nutrient supply, s_E, is much easier to measure and experimentally manipulate than the intracellular nutrient supply, s_I. It is therefore desirable to rewrite Eqn. S23 as a Monod Growth Equation mapping extracellular nutrient supply onto growth rate (13–17). Assuming that nutrient uptake obeys MM kinetics, such that in steady-state, $s_I = k_U p_U s_E /(K_S + s_E)\delta_S$, where k_U is the catalytic constant for uptake, K_S is the uptake half-saturation constant, p_U is the concentration of uptake proteins, and δ_{SI} is the dilution rate of S_I. We further assume the simplest case where extracellular nutrient is used directly for biosynthesis without modification. Substituting into Eqn. S23 and rearranging gives the following Monod Growth equation:

$$\frac{dp}{dt} = \frac{V_{max} s_E}{K_M + s_E} - \lambda p \qquad \text{S24}$$

where,

$$V_{max} = \frac{k_T r k_U p_U}{k_U p_U + \delta_{s_I} K_3} \left(\frac{m}{K_A + m} \right)$$

and where $K_A = (\delta_{s_I} K_1 + k_U p_U K_2)/(k_U p_U + \delta_{s_I} K_3)$ and $K_M = K_s \delta_{s_I}(K_1 + K_3 m)/$ $[\delta_{s_I}(K_1 + K_3 m) + k_U p_U (K_2 + m)]$. The practical and conceptual utility of the Monod equation is that it neatly disentangles cellular and environmental determinants of growth: V_{max} and K_M are intrinsic to the cell, under genetic control, and evolvable, whereas s_E is extrinsic to the cell, not under genetic control and not evolvable. The version derived here unpacks the two fitting parameters, V_{max} and K_M, into their mechanistic components, revealing them both to be jointly determined by translation and uptake kinetics. Faster uptake (higher $k_U p_U$) increases the maximum growth rate (V_{max}) and lowers the half-saturation constants, K_M *and KA*, making growth faster at both high and low nutrient densities. Interestingly, this predicts an uptake-mediated negative correlation between V_{max} and K_M, which is contrary to the common view that V_{max} and K_M are positively correlated and thus in a trade-off conflict.

II.c. Selection strength

Absolute fitness has the following generic form if we ignore extrinsic noise:

$$\langle \lambda \rangle \cong \frac{\bar{V}}{p_{ss}} \left(1 - \sum_i^n A_i F_i \Omega^{-1} \right)$$

where F_i is the Fano factor for substrate *i*, A_i is a constant, and *n* is the number of substrates affecting fitness. The selection gradient on a mutation that alters fitness is,

$$\mathrm{s} = \frac{\langle \lambda \rangle_m - \langle \lambda \rangle_r}{\overline{\langle \lambda \rangle}} \cong \frac{\sum_i^n \left(A_{r,i} F_{r,i} - F_{m,i} A_{m,i} \right)}{1 - \sum_i^n \left(f A_{r,i} F_{r,i} + (1-f) F_{m,i} A_{m,i} \right) \Omega^{-1}} \Omega^{-1}$$

where *f* is the population relative frequency of resident alleles. Because *A* is typically small (in the standard MM reaction $A = K_m /(K_m + \bar{s})^2$), the denominator approximately equals 1 provided that *F* is not too large. For example, for K_m = 350 and *s* = 1400 (values corresponding to translation of mRNA's in *E. coli*), this assumption leads to only a 1% error for *F* as large as 100 and a 10% error for *F* = 1000. These *F* values are very high, and beyond any value considered in the paper. In addition, as Ω increases this error becomes even smaller. We therefore take this assumption as quite reasonable for the situations considered in our paper. If we further assume that genotypes differ only in

noise (i.e., that there is no pleiotropic effect of the mutation), and define ΔF_i as the difference in Fano factors between resident (r) and mutant (m) genotypes, then we have,

$$s \cong \sum_{i}^{n} (\Delta F_i A_i)\, \Omega^{-1}$$

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Supplementary Figures

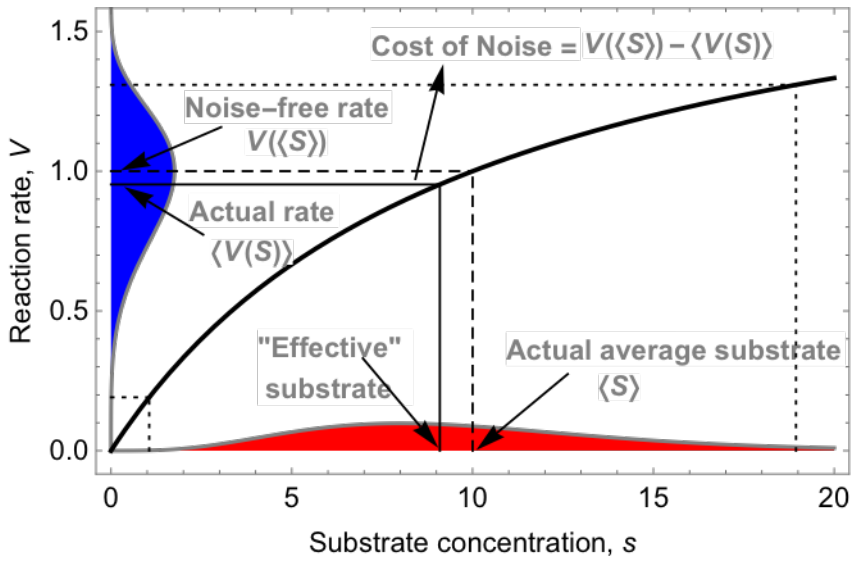

Figure S1. Schematic demonstrating the geometry of noise-induced slowdown for hyperbolic reaction.

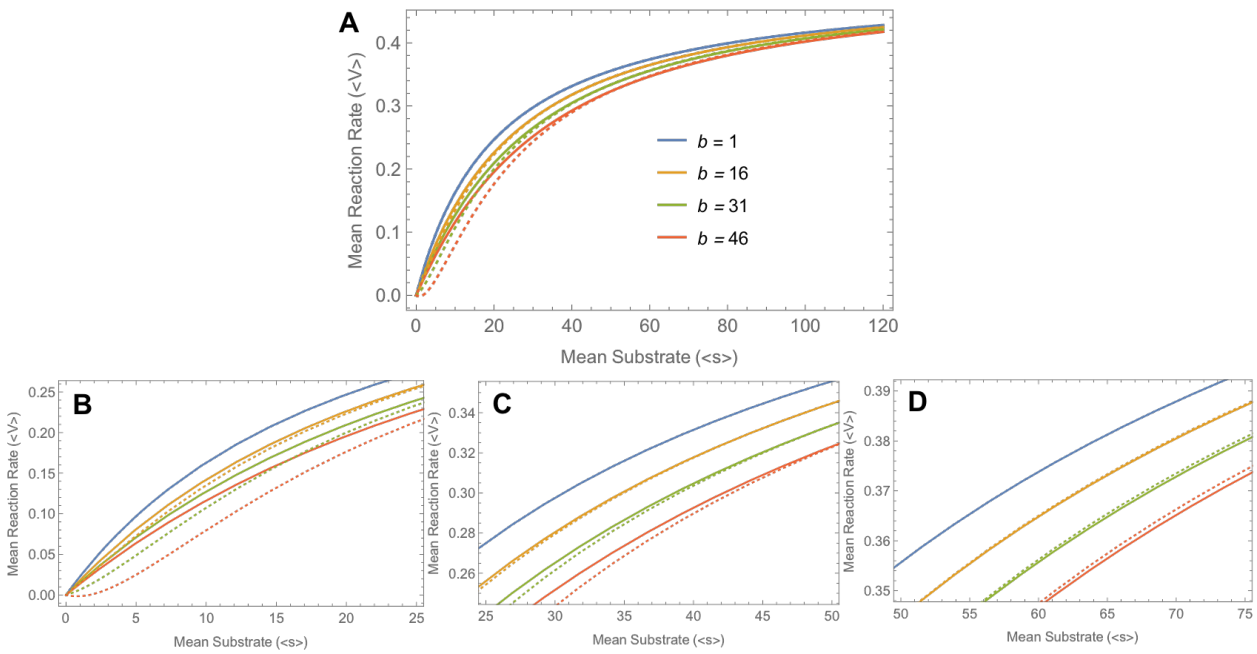

Figure S2. Comparison of exact and approximate predictions for noise-induced slowdown.