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

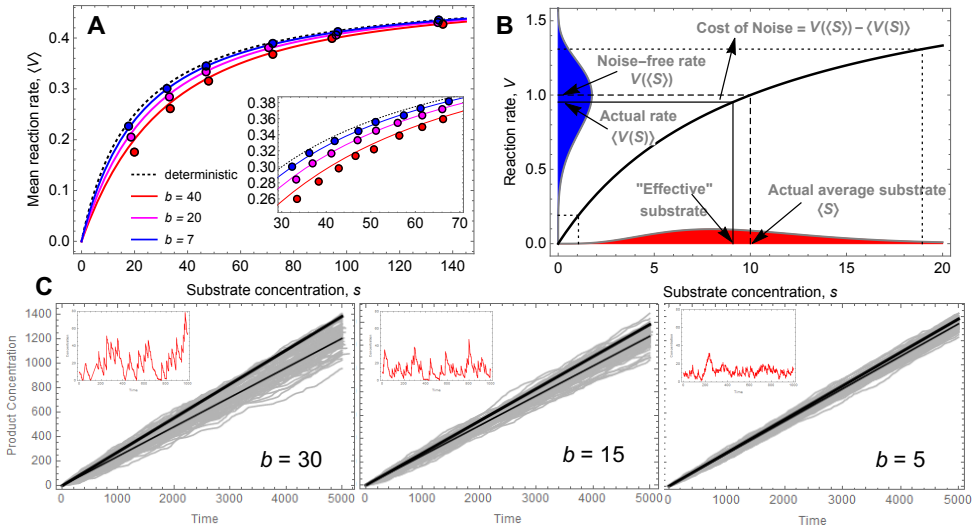
166 s, e and p are reactant, enzyme and product concentration, respectively (following
167 convention lowercase denotes concentration, which unless otherwise noted will take units
168 of molecules per cell), $V_{max} = k_{cat}e$ is the asymptotic reaction rate (k_{cat} is the number of
169 substrate 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).



207

208 ***Figure 1:*** **Noise-induced slowdown of hyperbolic reactions.** Stochastic
209 simulations employing Gillespie's (Gillespie 1977) exact stochastic simulation algorithm
210 (SSA) of the microscopic system of elementary reactions for the Michaelis-Menten
211 reaction (see SI). Points represent the rate of product formation over time steps 100-800
212 (providing a burn-in more than 10 times the half life of P or S) ensemble averaged over
213 1000 realizations of the stochastic process. Results fit well with the QSSA model (Eqn. 3,
214 solid lines in **a**, **b** and thin black line in **c**). The substrate burst input, b, is varied to alter
215 the substrate noise level. Substrate input rate, k_{in} is divided by b so that mean substrate is
216 not affected by bursting, and is varied to generate different substrate concentrations. All
217 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)**
218 The rate law curve demonstrating that, for a given average substrate concentration,
219 substrate noise slows the reaction, but only if the reaction is not in its first or zeroth order
220 regime (low or high substrate, respectively). Note that the accuracy of the stochastic
221 QSSA requires that s, $K_m \ll F$, which fails for the red points in **a** and **b** (where $K_m = 20$
222 and $F = 20.5$). Inset is a zoomed in view. **B)** A geometric illustration of noise-induced
223 slowdown, showing that variance (noise) in a state variable reduces the average of a
224 hyperbolic function (i.e., Jensen's inequality). **C)** 100 realizations of the stochastic
225 process (gray) compared to the deterministic (thick black line) and stochastic (thin black
226 line) predictions. Red traces in inset are example outputs from a single realization of the
227 substrate dynamics for a short time window.
228
229 A more rigorous and general expansion method is used in the following section
230 ("Noisy Non-linear Reaction Networks in Finite Volumes"). For now, there are two main
231 points: 1) reaction kinetics depend not only on average molecule concentrations, but on
232 the mean and variance in molecule numbers, and 2) for a given average substrate
233 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

stochastic simulations very well provided that $F, K_m \ll s$. Equation 2 gives a very good approximation to 3 under this same restriction (Fig. S2), which provides an important benchmark for the accuracy of the more rigorous expansion approximations used below.

Figure 1 shows that the effect of noise on MM-type reactions is to increase the observed Michaelis constant. Therefore, a stochastic formulation of Eqn. 1 that accounts for noisy substrate while retaining the classical form is obtained simply by substituting into Eqn. 1 an "effective", stochastic Michaelis constant, $\widetilde{K}_m$, where $\widetilde{K}_m \geq K_m$ with equality occurring only with zero noise, zero curvature of the rate law, and macroscopic reaction volume (S6).

Noisy Non-linear Reaction Networks in Finite Volumes. In the previous section, we showed that substrate noise slows the rate of product formation in a single hyperbolic reaction in a closed system. To apply this result, the statistics of the substrate species are required. We now take a more rigorous approach and generalize the noise-induced slowdown phenomenon to reaction networks in open systems. Further, the model allows one to predict the noise statistics of the system solely with knowledge of the network structure and reaction kinetics. Of particular interest is how network architectures may create feedbacks that buffer the system flux against noise-induced slowdown. The following theory builds on the work of van Kampen (van Kampen 2007) and Elf and Erhenberg (Elf and Ehrenberg 2003) (see also Grima (Grima 2010)).

The time evolution of the joint probability density of all *N* nodes in a chemical reaction network with *R* edges is given by the following "system-size expansion" (van 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 i^{th} 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 j^{th} 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

vanish when $s \rightarrow 0$ (first order kinetics), $s \rightarrow$ Infinity (zeroth order kinetics) or when $\Omega \rightarrow$ Infinity (macroscopic volume). Two of the second moments (Sup. Mat.) for the general MM case can be written compactly:

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

While the present approach builds on that derived by Grima (Grima 2010) the results and conclusions differ: Grima (Grima 2010) found that noise had no effect on product concentration in MM reactions. There are two reasons for this discrepancy. First, Grima assumed no dilution of the substrate ($\delta_S = 0$). As can be seen from equation 6, this causes the finite volume correction term for $\langle p \rangle$ to vanish. Thus, Grima's results are in agreement with the present results for this special case, and all of the results in his papers are correct. However, following (Grima 2010) exactly for $\delta_S > 0$ also gives zero stochastic deviation of the product concentration (see SI), which is incorrect. Here is why: when the system-size expansion of the chemical master equation is applied to a system of elementary reactions, as in (Grima 2010) and most other methods, the rate of product formation, V, is proportional to the intermediate complex concentration, c. Because c is unimolecular, the method reads this rate as linear (its second derivative is zero), and so the mean cannot be affected by noise (as proven by Jensen's inequality). However, in MM reactions V is decidedly non-linear. Indeed, it is hyperbolic. Thus, the elementary reaction conceals an essential non-linearity of the system, and will give incorrect results in the general case. To rectify this, one must apply Eqn. 4 to a QSSA reduced system of biochemical equations as shown in the SI. Note that the use of 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 $a = 0.1\text{-}0.2$ 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

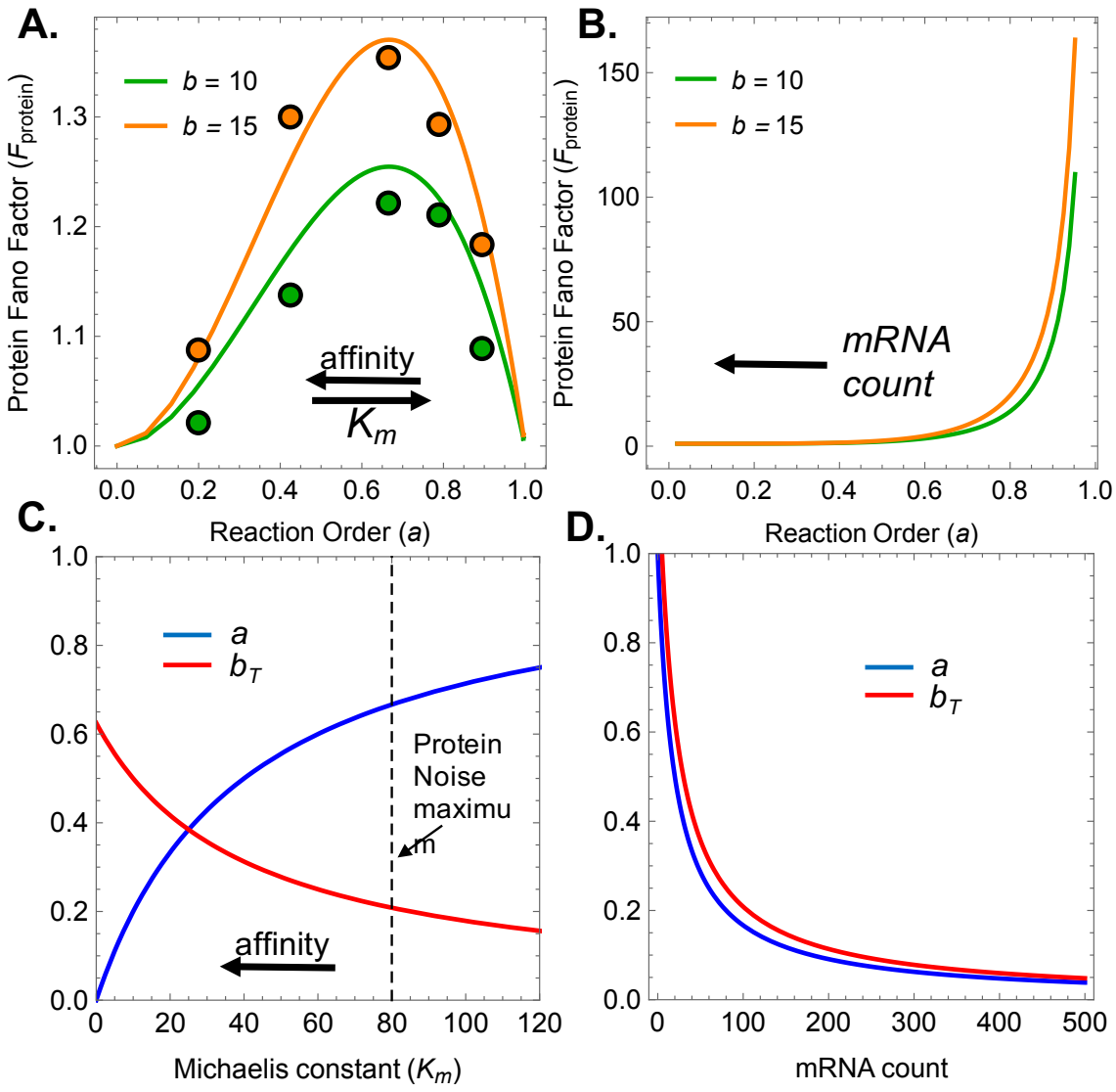
365 transcribed in bursts of size b_t, then the steady-state variance and Fano factor of mRNA

366 are (Sup. Mat.),

367 $$\sigma_{mRNA}{}^2 = \frac{1}{2}\overline{m}(b_t + 1), \qquad F_{mRNA} = \frac{1}{2}(b_t + 1) \qquad 8$$

368 which is a special case of (Elf and Ehrenberg 2003) and which is in excellent agreement

369 with simulations (Figs 2, 3).



370

371 ***Figure 2:*** **Protein noise with hyperbolic filtering.** Translation propagates mRNA noise
372 to the protein level, but hyperbolic reaction kinetics ($0<a<1$) can substantially ameliorate
373 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.

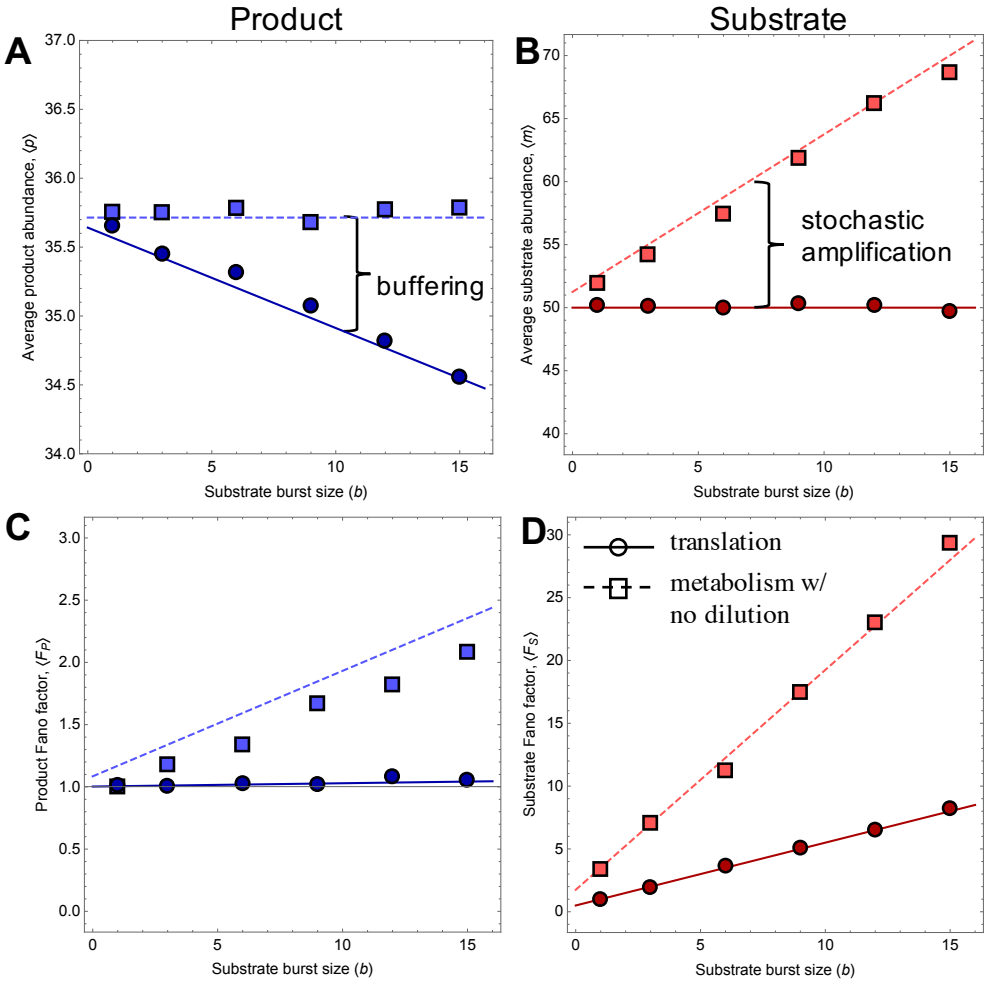


Figure 3: **Effect of network architecture on the cost of noise.** Theoretical predictions (Eqns. 6-8, S20, S21) for metabolic reactions (dashed lines; h = 1, δ_S = 0) and translation (solid lines; h =0, δ_S = 0.1) compared to ensemble average (blue) and variance (red) of

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,

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

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

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

for low copy number proteins, but is independent of abundance for high copy number proteins (Blake et al. 2003; Raser and O'Shea 2004; Bar-Even et al. 2006).

Translation initiation in *E. coli* and yeast has a reaction order of $a \sim 0.1\text{-}0.2$ (3, 49), which is comfortably to the left of the protein noise peak at $a^* = 2/3$ (see Sup. Mat.; Fig 2a). This implies that, contrary to predictions based on first-order translation initiation models (Thattai and van Oudenaarden 2001; Raser and O'Shea 2005), evolving or engineering increased binding affinity of mRNA to ribosomes (i.e., reduced translational K_m) will actually lower protein noise, not increase it. This occurs because reducing K_m has two complementary effects on protein noise: it increases translational bursting, b_T, but reduces reaction order, *a,* which attenuates noise via a phenomenon we term "zeroth-order insensitivity". The latter effect is not accounted for in previous gene expression models, but has implications for the evolutionary and synthetic targets of noise-amelioration (see Discussion). Hyperbolic filtering also has practical implications when making inferences from data. For example, translational burst size, b_T, can be estimated from Eqn 9 with knowledge of the mRNA and protein statistics; however, assuming first order reaction kinetics ($a = 1$) can lead to large underestimates of this parameter from data. For example, for $a \sim 1/5$, assuming first order translation kinetics can lead to a 25-fold underestimate of translational burst size.

Correlation between mRNA and protein noise with hyperbolic translational filtering: We now quantify the effect of hyperbolic translation kinetics on the correlation between mRNA and protein noise. Emprical work in yeast, E. coli and human cell lines have found that there is no correlation between mRNA and protein levels in the cell, or

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

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

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

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

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

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

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

Case 2 ($h = 1$, $\delta_S > 0$): Incomplete amplification and buffering. However, the precise balancing of V with k_{in} does not apply in living cells, where substrate exits the system via multiple alternative channels ($\delta_S > 0$), primarily dilution by cell division, but also through excretion, titration by other reactions and enzymatic degradation. Extracellular substrate also experiences non-zero δ_S, as it is diluted by bulk flow (such as in a chemostat) or by uptake from neighboring cells. Accounting for these effects modifies the flux constraint to: $k_{in} = V + \delta_S s$. With this network architecture, stochastic amplification compensates for some of the diminution of V by noise, but not all. Consequently, stochastic amplification fails to completely buffer metabolic reactions in living cells against noise-induced slowdown.

Case 3 ($h = 0$, $\delta_S > 0$): Gene expression is especially sensitive to noise-induced slow-down; no stochastic amplification, no buffering. The $V = k_{in}$ flux constraint is entirely abolished in template-mediated reactions ($h = 0$), such as protein synthesis via translation of mRNA. Because mRNA is not consumed by translation, steady-state mRNA concentration is set by the flux constraint $k_{in} = \delta_S s$, which is independent of translation rate, V. Thus, stochastic amplification does not occur in translation and so cannot buffer translation from mRNA noise (Fig. 3). As a consequence, gene expression is unusually sensitive to noise.

Cell fitness. Noise slows biochemical reactions, but how does it affect fitness? We now consider a reaction network representing a coarse-grained model of cell growth. We then analyze this reaction network in the stochastic case using the method developed above in order to quantify the effects of gene expression noise on the average rate of cell division.

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

This coarse-grained cell model follows a QSSA system of ODE's (S22) with growth rate,

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

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

rearranged to form a Monod equation, which maps growth rate to extracellular nutrient supply with a number of practical and conceptual benefits (S24).

The expected steady-state rate of cell division (cells/time) is found by setting Eqn. 11 to zero and solving for λ,

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

p_{ss} is defined as the species-specific total protein content of a cell (protein/cell), which is assumed constant for a given species, V is the synthesis rate of proteins (protein/time).

Fitness with noise. Following the method above, we write down a QSSA reduced system of equations for growth rate according to our coarse-grained cell model (S22), giving a system in five equations describing the time evolution of mRNA, uptake proteins, extracellular substrate, intracellular substrate and protein biomass. We assume that mRNA and extracellular nutrients undergo a linear birth-death process, supplied with burst inputs of size b_t and b_{SE} respectively, and ignore ribosome fluctuations. Intracellular nutrients are consumed by protein synthesis, but mRNA is not. Expected fitness, then, is (Sup. Mat.):

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

Where the A_i's are positive constants given in the SI. In reality, the protein noise term will contain contributions from all proteins involved in the pathway importing and processing nutrients to their final form utilized in translation. The MCA model of Wang and Zhang (Wang and Zhang 2011) then fits within this term. The extracellular noise

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

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

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

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

During balanced exponential growth of *E. coli* in rich medium: $m \sim 1400$ molecules, $r \sim 21{,}000$ molecules, and $k_T \sim 21aa/s \sim 4.6$ proteins/min (given a mean protein length of 275aa). The total protein count of a cell is $p_{ss} = 2.4\text{x}10^6$ molecules. 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, $k_T \sim 15aa/s \sim$
596 2 proteins/min (average protein length 450*aa*), $p_{ss} = 5x10^7$, 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 $$\mathrm{s} \cong \Delta F_{mRNA} \frac{K_m}{(K_m + m)^2} {\Omega_c}^{-1} = \Delta CV^2_{mRNA} a(1-a){\Omega_c}^{-1} \qquad 15$$

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 i^{th} 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 $$N_e|\mathrm{s}| \cong \Delta C V_{mRNA}^2 a(1-a) B \Omega_w{}^{-g} \qquad 16$$

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

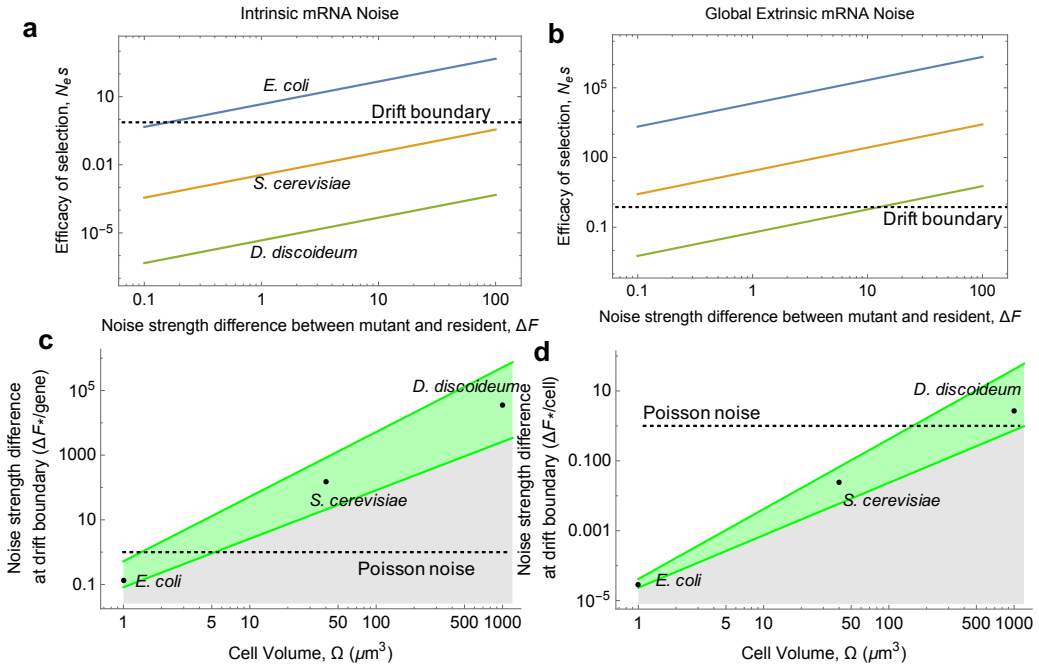


Figure 4: **Power-law scaling of selection efficacy with cell volume. A,B)** The predicted efficacy of selection, $N_e|s|$, for *E. coli*, *S. cerevisae* and *Dictyostelium disoicdeum*, as a function of the jump size in noise caused by a mutation, $|\Delta F|$, for mutations acting at a single locus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

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

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

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

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

greater selective effect, meaning that traits affecting extrinsic noise, such as ribosome fluctuations (Eqn 9), should be under strong selection for noise-amelioration even in moderately sized species.

DISCUSSION

Biochemical reactions are non-linear, but are typically modeled as linear in stochastic models of gene expression due to technical limitations (van Kampen 2007). Here we have introduced a novel procedure for easily and more accurately incorporating non-linearity into models of coupled stochastic chemical reactions. We have found that hyperbolic reaction kinetics filter input noise in such a way that the average reaction rate is slowed by noise, while attenuating noise propagation. These results have a number of implications.

A drift barrier hypothesis for gene expression noise and robustness. Cell size places severe constraints on the evolution of noise suppression. The magnitude of noise-induced slowdown of Michaelis-Menten reactions is inversely proportional to the reaction volume, which follows intuitively from the fact that, for a given molecular density, greater volumes contain more molecules and thus generate less noise. Lynch (Lynch 2007b) has previously noted the "genomic perils of evolving large body size". Large bodied species tend to be less efficient at purging deleterious mutations due to the positive scaling of genetic drift strength with body size, leading to a number of broad taxonomic patterns including greater genomic mutation rate, transposon abundance and 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

mRNA concentrations lead to the same rate of protein synthesis, thus creating a zero correlation between mRNA and protein.

A major implication of these results is that the vast majority of all intrinsic protein noise comes from translation, not transcription. Consequently, if protein noise, rather than mRNA noise, determines the functional consequences of gene expression noise, then natural selection on transcriptional noise will be extremely weak, and synthetic systems desiring control of protein noise should target translation, not transcription. We have thus come to two conclusions about transcriptional noise: 1) mRNA noise directly inhibits growth by slowing translation and 2) very little mRNA noise propagates to the protein level. These two predictions cast new light on the functional consequences of transcriptional noise in living cells.

Survival of the flattest. We have shown that hyperbolic reactions, by filtering out high-amplitude input fluctuations, cause a reduction in both signal (average output) and noise (relative output variance) compared to first-order reactions. The strength of selection favoring organismal robustness is determined by the former, while the target of selection for increased robustness is determined by the latter. Both the strength of noise and its cost are mediated by the reaction order, $a = K_m/(K_m + s)$ (Eqns. 6, 9), making this the obvious target for noise-attenuation. Noise-induced slowdown is minimal for first and zeroth order reactions (a =1, 0, respectively), while noise itself is minimal only in zeroth order ($a = 0$). Indeed, zeroth order kinetics completely filter out super-Poisson intrinsic input noise (Eqn. 9; Fig. 2), making the output entirely insensitive to input noise. We might call 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

models assume first-order enzyme kinetics (Kacser and Burns 1973). In light of the present results, it is not immediately clear if this assumption makes the results of (Wang and Zhang 2011) an underestimate of the true cost of gene expression noise, because hyperbolic rate laws cause noise-induced slowdown at each pathway step, or an overestimate of cost, because hyperbolic rate laws attenuate noise at each step, thus damping noise propagation through the pathway. Future work should distinguish between these two possibilities.

The crucial assumption: All of the major results of this paper rely on the validity of hyperbolic reaction kinetics as an accurate description of *in vivo* biochemical reactions. This assumption is not guaranteed, especially for translation. Translation is a unique biochemical reaction because a single substrate molecule (mRNA) is simultaneously bound to multiple enzymes (ribosomes), and it is therefore easy to imagine that translation kinetics may not be well-described by Michaelis-Menten reaction kinetics. Therefore, the present theoretical results must remain in question until future empirical work carefully measures the *in vivo* rate law of protein translation.

METHODS

All Monte Carlo simulations were run on the elementary (microscopic) system of reactions (S17) using Gillespie's Exact Stochastic Simulation Algorithm (SSA) (Gillespie 1977) implemented in *Mathematica* v10.3.0.0 with the open source xSSAlite package, freely available from the xCellerator project (http://www.xlr8r.info/SSA/). Each realization of the simulation is a Markov Chain with irregular (exponentially distributed) 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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