Phase-transition-mediated Switching in a Multisite Phosphorylation Chain

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(Dated: May 25, 2004)

We construct and analyze a stochastic model of a population of N protein molecules, each of which can be phosphorylated and dephosphorylated at J sites by the same kinase and phosphatase respectively. In addition, fully dephosphorylated (phosphorylated) proteins feed back to act as catalysts for all intermediate dephosphorylation (phosphorylation) reactions. The population is treated as a vector of occupancies of J + 1 sites in a one-dimensional lattice representing the phosphorylation states of each protein molecule. This model exhibits a continuous phase transition at any $J \ge 2$, between a symmetric and a symmetry-breaking state. In addition, we find that the universality class of the phase transition at finite J is different than that at $J \to \infty$.

PACS numbers: 02.50.Ey, 05.40.-a, 87.16.Yc, 87.16.Ac, 82.39.Rt

Molecular switches are a key component of many signal transduction and gene expression circuits that control cellular behavior such as the cell cycle [1, 2], differentiation [3, 4] and memory [5]. One sense of switching is termed ultrasensitivity, in which the character of a given steady state changes significantly within a small signal range [6]. A different functionality is bistable (toggle) switching, the capacity of a deterministic circuit to flip between two states in response to a transient signal [7]. Toggle switching is associated with "checkpoints" and is implemented through some form of feedback.

Activation states in molecular signal transduction are often defined in terms of different numbers of phosphorylations of a target protein. Phosphorylation is the covalent attachment of a phosphate group (consumed from an energy-rich ATP molecule), generally at a tyrosine, serine or threonine residue of a protein. Both phosphorylation and dephosphorylation are catalyzed concurrently, by exogenously supplied kinase and phosphatase enzymes. Their rates may also be enhanced by feedback catalysis [3], wherein the fully phosphorylated product increases the rate of phosphorylation of the remaining partially phosphorylated proteins.

Since the total number of protein molecules involved in these reactions is not large, stochasticity places important limits on the stability of a switch. Thermal fluctuations can jeopardize the reliability or even existence of switches constructed from signaling pathways. Understanding their consequences is important, both empirically [8, 9] and theoretically [10–13].

In this Letter, rather than considering one empirically derived signal transduction network in detail, we explore a fictitious (though possible) circuit that unites features present in many more complex networks: multisite phosphorylation Despite its abstraction, such a model illustrates important issues present in more realistic cases [15] and enables us to present the refinement of formal tools with which we can begin to address those cases.

The model assumes a set of N proteins that can undergo J phosphorylation reactions each. Dephosphorylation also occurs concurrently from each state except 0.

In addition, the rates of phosphorylation and dephosphorylation are enhanced by positive feedback from states Jand 0. We analyse the master equation of this problem, using many-body techniques applied previously to stochastic models of gene expression [16]. The Letter demonstrates four points. First, we show that a multisite phosphorylation chain with both positive and negative feedback allows switching behavior to emerge from population-level cooperative effects. The switching behavior of such a system is qualitatively distinct from one originating through single-molecule kinetic effects, such as saturation through complex formation [4, 5]. Second, both the emergence of a bistable phase and its stability are endogenous functions of the parameters of the problem, which we can compute with our method, thus generalising the analysis presented in [17]. Third, in this minimal model at large N, bistability occurs for any $J \geq 2$. Thus multiple phosphorylation is essential for phase-transition-mediated switching, in contrast to an abstraction based on saturation where only J = 1 [5] is required. (Known empirical J values range from 1 to 18 [18]). We find, in addition, that the universality class of this phase transition from unistability to bistability, is *different* for finite J and for infinite J. We do not know of any other non-equilibrium model which shows this behaviour. Fourth, we find that the phase transition may be brought about either by keeping the input signal (numbers of kinase and phosphatase molecules) constant and varying the number of target protein molecules or by keeping the latter fixed and changing the former. Molecular biologists typically think of control within a fixed circuit architecture, as varying the input signal. We see that the number of target molecules also constitutes a degree of freedom for control. In other words a circuit can be switched with input signals clamped constant, by changing the expression levels of target proteins.

A schematic representation of our model is shown in Fig. 1. Phosphorylation states are represented as J + 1 lattice sites indexed by $j \in 0, \ldots, J$, among which the N protein molecules hop independently forward (by individual phosphorylation) or backward (by dephosphory-



FIG. 1: The symmetrized multisite phosphorylation chain. The number of phosphorylated sites of the target protein defines states $0 \dots J$ depicted as a one-dimensional lattice. Dashed arrows represent phosphorylation and dephosphorylation transitions between states. Solid arrows designate catalytic action (thick: phosphorylation, thin: dephosphorylation). *I* is the number of externally-supplied kinase particles, *P* the number of externally-supplied phosphatase particles, and n_J and n_0 the number of target proteins feeding back with kinase and phosphatase activity, respectively.

We have effectively assumed a specific sequence for phosphate attachment, in not assigning combinatorial factors for attachment rates which grow at intermediate j. Our abstraction does not include the formation of enzyme-substrate complexes, but can be considered as approximating a situation in which the enzyme-substrate complex has a high dissociation (Michaelis) constant. Finally, as in Ref. [16], we omit spatial structure and dimensionality, as could be induced in real systems if reactions are scaffolded at membranes or other cellular structures.

Our symmetrizing assumption that the unphosphorylated state j = 0 is a catalyst for dephosphorylation, permits closed-form analysis of many interesting quantities. This assumption can be relaxed in numerical investigations [15]. There are two respects in which nonsymmetric systems, either topologically (where only the j = J state acts as a catalyst) or through $I \neq P$, can differ from the system idealized here. If the topology is symmetric but $I \neq P$ we expect that one of the two steady states becomes metastable and the transition first-order. If the topology is asymmetric however, sufficiently large N always ensures that the feedback wins and that there is a unique stable state.

Before presenting details of the analysis, we summarize our results below. The system of Fig. 1 is describable in terms of a single parameter $g \equiv N/q$. At small g values the N particles are homogenously distributed over the



FIG. 2: Order parameter $|\langle n_J - n_0 \rangle| / N$ for the phosphorylation chain: mean-field theory (lines) and simulations (symbols). J + 1 = [5, 10, 100] corresponds to [dot, dash, solid] for lines and $[+, 0, \times]$ for symbols. Particle numbers used in the simulations are respectively N = [4000, 2000, 400].

J + 1 sites of the lattice. In particular, average occupancy $\langle n_0 \rangle$ of site 0 is equal to the average occupancy $\langle n_J \rangle$ of site J, where the averages are over the steady state distribution. As g increases, at a value $g = g_c$, the system undergoes spontaneous symmetry breaking and the occupancy at either site 0 or J becomes greater than at the other, until at very large g, almost all the particles are found at either the fully dephosphorylated state j = 0 or the fully phosphorylated state j = J. The relevant order parameter describing the system is hence $|\langle n_J - n_0 \rangle| / N \in [0, 1]$. Fig. 2 shows our numerical and analytical estimates of this quantity.

The phase transition occurs at any J > 1 at a critical point whose mean-field value is $g_c = (J+1)/(J-1)$. In a neighborhood of order $g_c \leq g \leq 1+2(g_c-1)$, the order parameter scales with g as in Curie-Weiss mean-field ferromagnetism [19], with a J-dependent normalization:

$$\frac{|\langle n_J - n_0 \rangle|}{N} \approx \frac{\sqrt{6J}}{J+1} \left(\frac{g}{g_c} - 1\right)^{1/2}.$$
 (1)

For $g \gtrsim 1+2(g_c-1)$ the order parameter saturates to a *J*-independent envelope value

$$\frac{|\langle n_J - n_0 \rangle|}{N} \to 1 - \frac{1}{g}.$$
 (2)

Since $g_c - 1 \rightarrow 2/J$ for large J, Eq. (2) also gives the behavior in the formal $J \rightarrow \infty$ limit. The derivative of the order parameter converges to one in arbitrarily small neighborhoods of the critical point, rather than to ∞ as in the Curie-Weiss regime; thus $J \rightarrow \infty$ defines a different universality class than any finite J, as shown in Fig. 2. Qualitatively, the distinction between small and large-J is determined by whether one or both reflecting boundaries are sensed by the near-critical symmetrybroken state.

The fluctuations of the order parameter, normalized by

the Poisson value 2N/(J+1), are dominated by a peak

,

$$\frac{\left\langle \left(n_J - n_0\right)^2 \right\rangle - \left\langle n_J - n_0 \right\rangle^2}{2N/(J+1)} \approx \frac{\text{const} \sim 1}{(J+1)\left|g - g_c\right|} \qquad (3)$$

The factor 1/(J+1) arises because a single mode out of J+1 in the diffusive spectrum goes unstable at the critical point. This mode describes collective fluctuation of particles between the j = 0 and j = J limits, and corresponds to the fluctuations in aggregate magnetization in the Curie-Weiss solution. The results for the fluctuation spectrum are shown in Fig. 3.



FIG. 3: Fluctuations in the order parameter scaled for g above and below critical. var(n') stands for the variance $\langle (n_0 - n_J)^2 \rangle - \langle n_0 - n_J \rangle^2$. Lines are leading-order expansion in fluctuations about the symmetric mean-field solution, continued through g_c ; symbols from simulation, J + 1 values and markers as in Fig. 2. Large panel shows convergence of var(n') to N/g at all J, and a fit to $(N/g) (1 - g^{-2.5})$ for J + 1 = 100. Inset shows convergence of var(n') to Poisson result 2N/(J+1) as $g \to 0$.

At large J, the prefactor 1/(J+1) in Eq. (3) reduces the single-mode Curie-Weiss fluctuation peak to zero weight. The $J \to \infty$ fluctuation spectrum is hence therefore characterized entirely by renormalized diffusion like in an effectively coupled Bose gas. If fluctuations were enirely independent, the mean-field spectrum would be $\left[\left\langle n_0^2 \right\rangle - \left\langle n_0 \right\rangle^2\right]_{\rm MFT} = N\left(1 - 1/g\right)\left(1/g\right)$. As shown in Fig. 3, however, the large-J background converges to $\left\langle n_0^2 \right\rangle - \left\langle n_0 \right\rangle^2 \to N\left(1 - 1/g^{2.5}\right)(1/g)$, indicating that correlated fluctuations dominate.

We now present some details of the analysis of the model. The phosphorylation chain is described instantaneously by a state vector of occupation numbers $n \equiv (n_0, n_1, \ldots, n_J)$, and the ensemble by a joint probability density P(n). $N \equiv \sum_{j=0}^{J} \langle n_j \rangle$ is a constant.

The master equation corresponding to the stochastic dynamics of Fig. 1 is

$$\frac{d}{dt}P(n) = \sum_{j=0}^{J-1} \left[(I+n_J - \delta_{J,j+1}) (n_j+1) P(n+1_j - 1_{j+1}) \right]$$

$$- (I + n_J) n_j P(n) + (P + n_0 - \delta_{0,j}) (n_{j+1} + 1) P(n - 1_j + 1_{j+1}) - (P + n_0) n_j P(n)],$$
(4)

where 1_j represents the vector of zeros with a 1 in the j^{th} position, and $\delta_{j,j'}$ is the Kronecker delta. Eq. (4) can be solved perturbatively by introducing an operator algebra on a basis of number states, and so converting P(n) into an equivalent representation as a state vector. Creation and annihilation operators are introduced [20, 21] with standard commutation relations $\left[a_j, a_{j'}^{\dagger}\right] \equiv \delta_{j,j'}$, and a zero-particle state vector $|0\rangle$ and its conjugate (0) are defined by the conditions $a_j |0\rangle \equiv 0, \forall j, (0|a_j^{\dagger} \equiv 0, \forall j.$ Number states are defined as

$$|n) \equiv \prod_{j=0}^{J} \left(a_{j}^{\dagger}\right)^{n_{j}} |0\rangle, \qquad (5)$$

and normalized as [21] $(0|\exp\left(\sum_{j}a_{j}\right)|n) = 1, \forall n$. The j^{th} number operator has the usual representation $\hat{n}_{j} \equiv a_{j}^{\dagger}a_{j}$, and $(0|\exp\left(\sum_{j'}a_{j'}\right)\hat{n}_{j}|n) = n_{j}$.

The master equation (4) has the corresponding representation

$$\frac{d}{dt}\left|\psi\right) = -\Omega\left|\psi\right),\tag{6}$$

with $|\psi\rangle \equiv \sum_{n} P(n) |n\rangle$, and the evolution operator

$$\Omega = q \sum_{j=0}^{J-1} \left(a_{j+1}^{\dagger} - a_{j}^{\dagger} \right) \left[\left(1 + \frac{\hat{n}_{0}}{q} \right) a_{j+1} - \left(1 + \frac{\hat{n}_{J}}{q} \right) a_{j} \right]$$
(7)

The integral of Eq. (6), $|\psi_t\rangle \equiv e^{-\Omega t} |\psi_0\rangle$, is converted to a functional integral by the insertion of the coherentstate representation of the identity operator

$$\int \frac{d\phi_{t'}^{\dagger} \, d\phi_{t'}}{\pi} e^{-\phi_{t'}^{\dagger} \cdot \phi_{t'}} e^{a^{\dagger} \cdot \phi_{t'}} \left| 0 \right) \left(0 \right| e^{\phi_{t'}^{\dagger} \cdot a} = \sum_{n} \left| n \right) \left(n \right| = I$$
(8)

at a set of times $t' = k\Delta t \in (0,t)$. ϕ_t is a column vector of J + 1 complex coefficients, and ϕ_t^{\dagger} its adjoint. If we take as a convenient choice of initial state $|\psi_0\rangle = \exp\left(\sum_j \bar{n}_j \left(a_j^{\dagger} - 1\right)\right)|0\rangle$, we obtain by standard methods [21] the relation for the normalized partition function

$$(0|\exp\left(\sum_{j}a_{j}\right)|\psi_{t}) = \int \mathcal{D}\tilde{\phi}\,\mathcal{D}\phi e^{-\int dtL}e^{\tilde{\phi}_{0}\cdot(\bar{n}-\phi_{0})},$$
(9)

in which the diffusion-"Lagrangian" is

$$L\left(\tilde{\phi},\phi\right) = \tilde{\phi} \cdot \frac{\partial\phi}{\partial t} + \Omega\left(\tilde{\phi}+1,\phi\right).$$
(10)

The field ϕ_t^{\dagger} is shifted in notation as $\phi_t^{\dagger} \equiv \tilde{\phi}_t + 1$, to cancel the surface term from the normalization, and the function $\Omega\left(\tilde{\phi}+1,\phi\right)$ in Eq. (10) has the form of Eq. (7), with the substitutions $a_j^{\dagger} \rightarrow \tilde{\phi}_j + 1$, $a_j \rightarrow \phi_j$. The mean number density is related to the integra-

The mean number density is related to the integration variables as $\langle \phi_{j_t} \rangle = \langle n_{j_t} \rangle$, where the first $\langle \rangle$ denotes average in the functional integral (9), and the second the equivalent average under P(n). The mean-field solution for the order parameter is obtained from the stationary-point approximation of $\langle \phi_{j_t} \rangle$ by the solution $\bar{\phi}$ to $\partial L / \partial \tilde{\phi} \Big|_{\bar{\phi} \equiv 0} = 0$, and recovers Eq. (1) and Eq. (2) in appropriate limits. Fluctuations are computed by shifting the integration variables $\tilde{\phi} \equiv 0 + \tilde{\phi}', \phi \equiv \bar{\phi} + \phi',$ and expanding L to second order in primes, to obtain a matrix equation of the form

$$L = \tilde{\phi}' D_0 \phi' + \tilde{\phi}' D_2 \tilde{\phi}'^T.$$
(11)

 D_0 is the diffusion operator in the stationary background, and D_2 is a kernel whose eigenvalues govern the Gaussian noise spectrum of the stochastic process.

Manipulation of the operators in the functional integral gives the corresponding fluctuation relation in the symmetric phase, used in Fig. 3:

$$\frac{\left\langle \left(n_{J} - n_{0}\right)^{2} \right\rangle - \left\langle n_{J} - n_{0} \right\rangle^{2}}{2N/\left(J+1\right)} = 1 + \frac{J+1}{N} \left\langle \left(\frac{\phi_{J}^{'} - \phi_{0}^{'}}{\sqrt{2}}\right)_{t}^{2} \right\rangle$$
(12)

The important property of the matrix D_2 in Eq. (11) is that it has a single negative eigenvalue at all g, whose fluctuations may be removed in exchange for a Langevin

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field, which then drives the noise spectrum of the ensemble, just as in classical reaction-diffusion [21]. All other eigenvalues of D_2 are zero in the symmetric phase, and a single positive eigenvalue appears in the symmetrybroken phase.

Also from Eq. (11), the Green's function which propagates the Langevin fluctuations may be expanded in eigenvectors of D_0 . Elementary algebra shows that only the lowest antisymmetric eigenvector becomes degenerate at the critical point, leading to the Curie-Weiss divergence with weight 1/(J+1) of Eq. (3). The remainder of the eigenvectors remain close to ordinary diffusive solutions in the symmetric phase, leading to the regular component of the fluctuation spectrum in Fig. 3. These details, as well as an analysis of the symmetry-broken phase, will be provided in a longer paper [22].

To conclude: in attempting to understand as a manybody effect the stability of a signal-transduction element based on multiple phosphorylation with feedback, we have found that the onset of bistability is a second-order phase transition at large N, with distinct small-J and large-J behaviours.

An important feature of the model is the residence time within states in the symmetry-broken phase as a function of N and g. This feature is of biological interest, since it pertains to the memory of switches at finite temperature. For a system described by a single occupation variable, assuming the form of the switching potential, this has been bounded above [17] by an exponential in N. For a multi-variate system, this bound should only receive polynomial corrections. An analysis of our model leads to a first-principles computation of these corrections [22].

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