Corepressive interaction and clustering of degrade-and-fire oscillators

Bastien Fernandez and Lev S. Tsimring

Phys. Rev. E **84**, 051916 — Published 22 November 2011

DOI: 10.1103/PhysRevE.84.051916

Co-Repressive Interaction and Clustering of Degrade-and-Fire Oscillators

Bastien Fernandez[1] and Lev S. Tsimring[2]
[1] *Centre de Physique Théorique, UMR 6207 CNRS - Aix-Marseille Université, Campus de Luminy Case 907, 13288 Marseille CEDEX 9, FRANCE*
[2] *BioCircuits Institute, University of California, San Diego, La Jolla, CA, 92093-0328*

Strongly nonlinear degrade-and-fire (DF) oscillations may emerge in genetic circuits having a delayed negative feedback loop as their core element. Here we study the synchronization of DF oscillators coupled through a common repressor field. For weak coupling, initially distinct oscillators remain de-synchronized. For stronger coupling, oscillators can be forced to wait in the repressed state until the global repressor field is sufficiently degraded, and then they fire simultaneously forming a synchronized cluster. Our analytical theory provides necessary and sufficient conditions for clustering and specifies the maximum number of clusters which can be formed in the asymptotic regime. We find that in the thermodynamic limit a phase transition occurs at a certain coupling strength from the weakly-clustered regime with only microscopic clusters to a strongly clustered regime where at least one giant cluster has to be present.

PACS numbers: 05.45Xt, 87.16Yc, 87.18Cf

I. INTRODUCTION

Many gene regulatory networks contain negative feedback loops as their core elements [1]. The negative feedback provides robustness and noise-resistance to signaling pathways [2]. They also lie at the center of many natural rhythmic circuits (such as circadian clocks [3]) and synthetic gene oscillators [4–6]. In our recent work [7] the delayed negative feedback was identified as the mechanism of oscillations in the synthetic two-gene oscillator [5]. It is well known that delayed auto-repression can lead to oscillatory gene expression even with only a single regulatory element [8–12]. Typically, the period of delay-induced oscillations is comparable with the delay time and thus this mechanism seemingly could not explain rather slow oscillations observed in gene circuits. However, it turns out that in a strongly nonlinear regime, the period of oscillations is determined by the rate of enzymatic degradation of the repressor protein and therefore can be arbitrarily longer than the transcriptional and translational delay. The essential mechanism of oscillations in this circuit is based on the alternation of two regimes: slow degradation of repressor protein, and the fast production of the repressor (firing) that occurs only when repressor is degraded below a certain critical concentration, hence we termed these sawtooth-like oscillations "degrade-and-fire" in analogy with integrate-and-fire oscillations in neuronal circuits. Bacterial cells containing the DF circuit demonstrated robust oscillations, but due to cell-to-cell variability and stochastic effects, in the absence of cell-cell communication the bacterial colony quickly becomes desynchronized. In our subsequent work [13] we succeeded in synchronizing gene oscillators across bacterial colony, however the synchronized regime was achieved with a different circuit design that incorporated a quorum-sensing machinery producing small molecule AHL which served as signaling element necessary for cell-cell synchronization.

In this paper we study synchronization of DF oscillators through a purely co-repressive interaction. To enable analytical calculations, we replace the original nonlinear delay-differential model of the DF oscillator [7] by a discontinious piecewise linear model which assumes that the concentration x degrades from the maximum value (which can be scaled to 1) linearly with unit rate, $\dot{x} = -1$ (mimicking enzymatic decay with high enzyme affinity), until it reaches the (small) threshold value $\eta > 0$, after which repressor concentration x is immediately reset to 1 ("fire"), and the process repeats. It is easy to see that the protein concentration oscillates with period $1 - \eta$. This model closely resembles the classical "integrate-and-fire" (IF) neuron model which has been extensively studied in the literature [14–17]. We introduce global co-repressive coupling of DF oscillators by assuming that each DF oscillator increases the repressor field of all other oscillators in proportion of its own repressor field. Equivalently, it can be interpreted as if all DF oscillators contribute their respective repressor concentrations to a global "repressor field" that in turn is added to the repressor field of individual oscillators and therefore delays their firing. This mechanism is qualitatively similar to the inhibitory coupling of IF neurons that has been studied by Ernst et al. [17]. However, the important difference is that the oscillators are coupled all the time, and not just during the firing events, and as we see below, the dynamics of co-repressive synchronization of DF oscillators are fundamentally different from those of inhibitory coupled IF neurons. In particular, a critical value of coupling strength exists at which a phase transition from the no-clustering regime to the strong clustering regime occurs.

Consider a population of N degrade-and-fire (DF) oscillators coupled through a common repressor field. We postulate that the dynamics of the concentration of repressor protein x_i is given by the following rules

- if positive, x_i degrades linearly with rate 1, *i.e.* $\dot{x}_i = -1$, or remains constant if it has reached 0,
- when the locally averaged concentration $\chi_i(t) =$

$(1-\epsilon)x_i(t) + \epsilon X(t)$ (where $X(t) = \frac{1}{N}\sum_{i=1}^{N} x_i(t)$ and $0 \leqslant \epsilon \leqslant 1$ is the coupling strength parameter) reaches threshold η, the ith oscillator fires, and its concentration is reset to 1, *i.e.* $x_i(t+) = 1$.

With these simple evolution rules, one can readily see that all oscillators must fire indefinitely (*i.e.* there cannot be "oscillator death" regime for any of the oscillators). Indeed, starting from an arbitrary configuration $\{x_i\}_{i=1}^{N}$ for which all $\chi_i > \eta$, all concentrations decay towards 0 with time (those that have reached $x_i = 0$, remain at zero) and so do all χ_i. Thus the oscillator with the lowest x_i (possibly, more than one if several oscillators have identical concentrations x_i) eventually fires when the corresponding χ_i reaches η. After that, the oscillator j with new lowest x_j has to fire when its χ_j reaches η, and so on. It is also clear that if any two oscillators in a population are in sync at certain time t_*, i.e. $x_i = x_j$, they will remain in sync for all $t > t_*$. What is not obvious however, is under which conditions oscillators that are initially out of sync will synchronize in the course of the dynamics, and what the properties of the resulting clusters are. To answer these questions, we begin with the simple case of two oscillators.

II. INTERACTION OF TWO DF OSCILLATORS

In this case oscillator i ($i = 1, 2$) fires when $\chi_i = (1-\epsilon/2)x_i + \epsilon x_{3-i}/2 = \eta$. Without loss of generality, we can assume that initially, one oscillator has just fired (*i.e.* $(x_1, x_2) = (x, 1)$ with $\eta < x \leqslant 1$) and denote $t_f(x) > 0$ the firing time associated with this configuration. The dynamics can be described through the return map R between ordered concentration pairs just after firings, $(x, 1) \mapsto R(x, 1) := (1 - t_f(x), 1)$.

The 2-dimensional dynamics of two DF oscillators is contained in a parameter dependent subset of the unit square $[0, 1]^2$ (see Fig. 1). For $\epsilon = 0$, firing occurs when either of the individual concentrations $x_i = \chi_i$ touches the threshold η. A simple computation shows that $R^2(x, 1) = (x, 1)$ for all x, *i.e.* we have a continuum of periodic orbits equivalent to the rigid rotation on a 2-dimensional torus (see Fig. 1a).

For $\epsilon > 0$, the dynamics changes drastically, as expected. Instead of a continuum of neutral periodic orbits, a single stable periodic orbit emerges which attracts all trajectories, except for the unstable periodic orbit lying on the diagonal $x_1 = x_2$ (see Fig. 1b). These are direct consequences of Lemmas 2 and 3 below. Furthermore, it can be shown that a unique and globally stable periodic orbit exists in arbitrary systems of two coupled DF oscillators with any monotonous degradation of both concentrations. Note that this result implies a somewhat unexpected corollary that two initially distinct oscillators never synchronize, and always remain distinct. As seen from Fig. 1c,d, the two oscillators in the asymptotic regime are always in anti-phase, and, for large ϵ, one oscillator remains repressed ($x_i = 0$) almost the entire

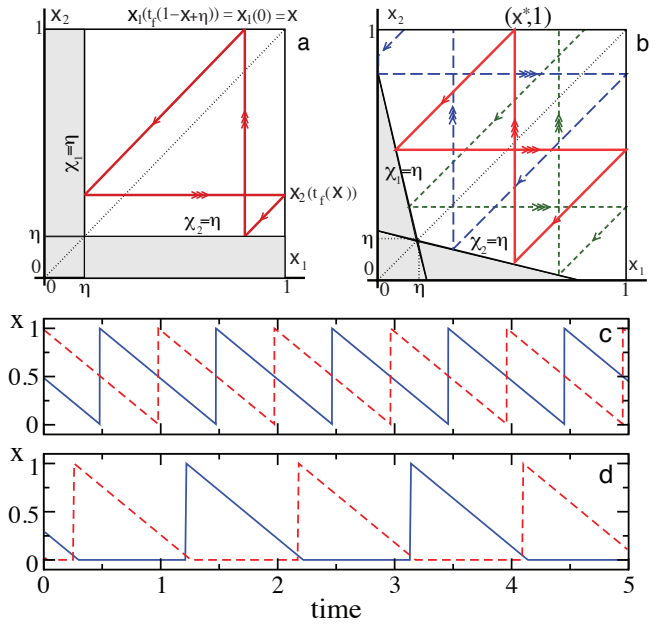

FIG. 1: (Color online) (a,b) Typical phase trajectories of two coupled DF oscillators (solid red line indicate periodic orbits and dashed blue and green lines - two transients). Simple arrows indicate motions between firings when both concentrations decay with rate 1. Triple arrows denote firings when the trajectory jumps from critical line $\chi_i = \eta$ to $x_i = 1$. (a) $\epsilon = 0$, every trajectory is periodic. (b) $\epsilon > 0$, for every initial condition except $x_1 = x_2$ the system asymptotically converges to a unique periodic trajectory passing through $(x^*, 1)$ where $x^* = 1 - 2(1-\eta)/(4-\epsilon)$ (thick red line). (c,d) time series of two coupled DF oscillators for $\eta = 0.01$ and different values of ϵ: (c) $\epsilon = 0.01$; (d) $\epsilon = 0.5$

time when the other repressor is present (more precisely, it fires when the other concentration reaches the small value $2\eta/\epsilon$), and *vice versa*.

III. MANY COUPLED DF OSCILLATORS

Now we turn to the analysis of the clustering dynamics of a population of N co-repressively coupled DF oscillators. Before we proceed with analytical results, we illustrate the typical behavior of the system numerically. Figure 2 shows the time series of 50 oscillators with initially distinct values of x for $\eta = 0.01$ and different values of the coupling parameter ϵ. For small $\epsilon = 0.01$ (Fig. 2a), all oscillators fire before they ever reach zero, and so they remain distinct (no clustering). For larger $\epsilon = 0.03$ (Fig. 2b) some oscillators reach zero, and some of them (not all) synchronize, and thus asymptotically, only 8 clusters remain. Note that this number is not universal, as it depends on specific initial conditions. For $\epsilon = 0.05$ (Fig. 2c), only two clusters remain. Their "weights" however are not equal, in this example one cluster contains 24 oscillators, and another 26 oscillators. Again, this weight distribution is non-universal. And for very large $\epsilon = 0.5$

(Fig. 2d) oscillators produce repressor protein in almost exact anti-phase, one repressor is present, the other one is absent.

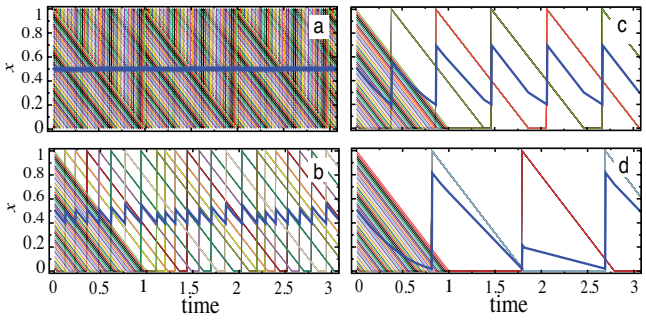

FIG. 2: (Color online) Typical time series of 50 coupled DF oscillators and of their mean value $X(t)$ (thick/blue line) for $\eta = 0.01$ and different values of ϵ: (a) 0.01; (b) 0.03; (c) 0.05; (d) 0.5.

By grouping oscillators with identical value of x_i into one cluster, the population dynamics can be described via $\{(n_k, x_k)\}_{k=1}^K$ where $n_k \in \{1, ..., N\}$ denotes the size of the cluster k and x_k the corresponding repressor concentration ($K \geqslant N$ is the total number of clusters). In this viewpoint, the cluster size distribution $\{n_k\}$ obviously remains unaffected in time unless two clusters k and k' fire together.

As before, we consider the map between consecutive firings. Any ordering in $\{(n_k, x_k)\}$ is irrelevant because of the permutation symmetry in this system. However, it is more convenient to deal with ordered values of x_k. Thus we assume that $0 < x_1 < x_2 < \cdots < x_{K-1} < x_K = 1$. Given $\{n_k\}$, this defines the state configuration set where the firing map $R \equiv R_{\{n_k\}}$ is effectively defined, *viz.* $\mathcal{T}_{\{n_k\}} = \{(x_1, \cdots, x_K) : 0 < x_1 < \cdots < x_K = 1$ and $(1-\epsilon)x_1 + \frac{\epsilon}{N}\sum_{k=1}^K n_k x_k > \eta\Big\}$. In order to maintain the ordering of protein concentrations in time, we must include cyclic permutations of indices in the firing map dynamics. The absence of clustering requires that, when starting in $\mathcal{T}_{\{n_k\}}$, the after-firing configuration has to lie in $\mathcal{T}_{\{n_{k+1}\}}$ (where all indices are understood mod K) and this should hold for each $k = 1, \cdots, K_{\rm per}$ where $K_{\rm per}$ is the minimal size distribution period. This property globally holds in state configuration sets provided that the coupling strength is small enough.

Lemma 1 *$R_{\{n_k\}}\mathcal{T}_{\{n_k\}} \subset \mathcal{T}_{\{n_{k+1}\}}$ for all $k = 1, \cdots, K_{per}$ iff*

$$\epsilon_\eta \leqslant \min_{k=1,\cdots,K_{per}} \frac{N}{N - n_k - n_{k+1}} \quad \textit{where } \epsilon_\eta := \epsilon/\eta \qquad (1)$$

In particular, for $\epsilon_\eta < N/(N-2)$ which is the minimal r.h.s. in (1) over all cluster distributions $\{n_k\}$, no clustering can ever occur, independently of the initial configuration. Notice that $N/(N-2) > 1$ and $\to 1$ as $N \to \infty$. Lemma 1 is rigorously proven in Appendix A.

The non-clustering condition (1) can be intuitively understood as follows. The only way two clusters can merge is when one cluster reaches zero (collapses) and remains at zero at least until the following cluster also reaches zero. It is easy to see that in the worst case scenario two clusters 1 and 2 should be very close to zero when the other $K-2$ clusters are very close to 1. In the limit $x_{1,2} \to 0, x_3, \cdots, x_K \to 1$, after x_1 has collapsed, the value of x_2 is equal to $[N - \epsilon_\eta(N - n_1 - n_2)]/(N\epsilon_\eta)$ when χ_1 reaches η , and in order to avoid merging of n_1 and n_2, this value has to be positive. Of course, the same condition has to be satisfied for all consecutive pairs n_k, n_{k+1}, hence the minimum in inequality (1).

Independently of (1), we may ask about the fate of the trajectories for which no clustering occurs. Since the sequence of merging events is always finite, this actually would determine every possible asymptotic regime. In absence of clustering, the firing map in $\mathcal{T}_{\{n_k\}}$ becomes $R^*_{\{n_k\}}(x_1, \cdots, x_K) := (x_2 - t_f, \cdots, x_K - t_f, 1)$ where t_f still denotes the firing time. (This map is actually $K-1$-dimensional because $(R^*_{\{n_k\}}x)_K = x_K = 1$.) Since the image $R^*_{\{n_k\}}x$ belongs to $\mathcal{T}_{\{n_{k+1}\}}$, one needs to iterate further until the point returns back to $\mathcal{T}_{\{n_k\}}$; hence the dynamics to study is the return map $R^*_{\{n_{k+K_{\rm per}-1}\}} \circ \cdots \circ R^*_{\{n_{k+1}\}} \circ R^*_{\{n_k\}}$. It can be shown (see Appendix B) that this map is always a pure contraction.

Lemma 2 *For every $\{n_k\}_{k=1}^K$ and $0 < \epsilon \leqslant 1$, there is a norm in $\mathbb{R}^{K-1}$ for which $R^*_{\{n_{k+K_{per}-1}\}} \circ \cdots \circ R^*_{\{n_{k+1}\}} \circ R^*_{\{n_k\}}$ is a global contraction.*

It follows that, in absence of clustering, every trajectory initially in $\mathcal{T}_{\{n_k\}}$ must approach a unique periodic orbit (whose single element in $\mathcal{T}_{\{n_k\}}$ is the fixed point of the return map). More generally, every trajectory in a population of N oscillators must converge to the periodic orbit associated with some $\{n_k\}$. Hence, the asymptotic cluster size distributions and periodic orbits are in one-to-one correspondence. Accordingly, to get asymptotically attainable cluster distributions, it suffices to compute the existence domains of the corresponding periodic orbits. These domains are given by the following statement.

Lemma 3 *The periodic orbit in $\mathcal{T}_{\{n_k\}}$ exists iff*

$$\epsilon_\eta < \zeta(\{n_k\}) := \frac{2N^2}{N^2 - \sum_k n_k^2} \frac{1}{1 - \min_k \frac{n_k}{N - n_{k+1}}} \qquad (2)$$

This claim is proved in Appendix C where we also give the explicit expressions for the periodic orbit. Note that when $n_K = N - n_1$ (*i.e.* $K = 2$), we have $\zeta(\{n_k\}) = +\infty$, which means that every two-cluster periodic orbit exists for any ϵ. In particular, for $n_1 = n_2 = 1$ this observation implies the result for two coupled oscillators presented above.

For $K > 2$, the critical value $\zeta(\{n_k\})$ is minimal for the (equi-)distribution where all $n_k = 1$ (in this case $K = N$) and the corresponding minimum is $\zeta_c = 2N/(N-2)$. So all possible periodic orbits exist for ϵ_η up to ζ_c. For larger ϵ_η, some periodic orbits disappear - certainly the

one associated with equi-distribution - and the number of clusters K must eventually be less than N.

A closer look at the formula (2) reveals that when $\{n_k\}$ is only composed of microscopic clusters, *i.e.* when $\sum_k n_k^2 = \mathcal{O}(N)$, the corresponding critical value $\zeta(\{n_k\})$ approaches 2 in the thermodynamic limit $N \to \infty$. Therefore, such distributions do not perdure asymptotically beyond $\epsilon_\eta \simeq 2$ when N is large, and at least some of the clusters size n_k reach $\mathcal{O}(N)$ in the course of time. Moreover, distributions that contain macroscopic cluster(s) (*i.e.* $n_k \simeq \rho_k N$ with $\rho_k > 0$ for some k) all have critical value $\zeta(\{n_k\})$ larger than 2 in the thermodynamic limit. Thus, there is a sharp transition in the system at a certain $\epsilon_c = \zeta_c$ (which is close to 2η for large N), from a dynamical regime in which all kinds of cluster size distributions may exist asymptotically, to a regime where every asymptotic distribution contains at least one giant $\mathcal{O}(N)$ cluster.

IV. MAXIMAL NUMBER OF CLUSTERS

The transition at $\epsilon_\eta = \zeta_c$ can be quantitatively described by estimating the number of asymptotic clusters as a function of ϵ_η. Since the precise number actually depends on the initial condition and can be as little as 2, the appropriate quantity to compute is the maximal possible number $K_{\max}$. It can be proven (see Appendix D) that for any given $K \geqslant 3$ and $N \geqslant K$ (and $N \neq 6$ if $K = 3$), the critical value $\zeta(\{n_k\}_{k=1}^K)$ in (2) reaches its maximum for the distribution consisting of $K-1$ unitary clusters of weight $n_k = 1$ and one big cluster $n_K = N - K + 1$. In this case, we have

$$\zeta(\{n_k\}) = \zeta_{\max}(K) = \frac{2N^2(N-1)}{(K-1)(2N-K)(N-2)}$$

and therefore all distributions with K clusters exist for $0 \leqslant \epsilon_\eta < \zeta_{\max}$. This immediately yields the maximum number of clusters at a given N and ϵ_η:

$$\begin{aligned} K_{\max}(\epsilon_\eta) &= N \text{ if } \epsilon_\eta < \zeta_c \\ &= N - \left\lfloor \frac{1}{2} + \sqrt{\frac{1}{4} + N(N-1)\left(1 - \frac{\zeta_c}{\epsilon_\eta}\right)} \right\rfloor \\ &\qquad \text{if } \zeta_c \leqslant \epsilon_\eta < \zeta_{\max} \quad (3) \\ &= 2 \text{ if } \epsilon_\eta \geqslant \zeta_{\max} \quad (3) \end{aligned}$$

This result indicates that a kind of second order phase transition takes place at $\epsilon_\eta = \zeta_c$ with $K_{\max}$ that behaves like $N(1-\sqrt{1-\zeta_c/\epsilon_\eta})$ slightly above the threshold. Formula (3) implies that for each $1 \leqslant K \leqslant K_{\max}(\epsilon_\eta)$, there exists a non-empty set of initial conditions which produces a stable periodic trajectory with K clusters. However, the "typical" number of clusters emerging from an arbitrary set of initial conditions can be much less than this upper bound. We performed numerical simulations

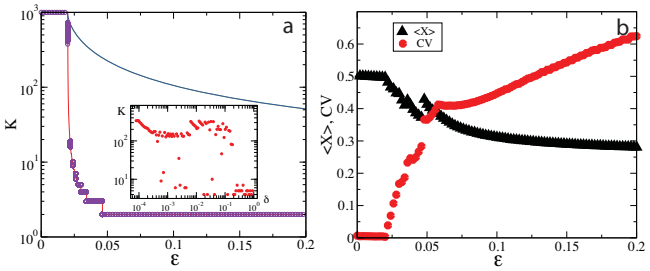

FIG. 3: (Color online) (a) Number of clusters in the asymptotic regime for 1000 coupled oscillators with $\eta = 0.01$ and 1000 different random initial conditions for each ϵ. The solid blue line indicates the upper bound (3), and the solid red line shows the number of clusters $K_e(\epsilon)$ for the uniform initial distribution. *Inset:* the number of clusters as a function of the initial distribution width δ for $\epsilon = 0.03$; (b) time-averaged mean field $\langle X \rangle$ and the coefficient of variation CV as a function of ϵ for $\delta = 0.01$ and firing noise $\eta = 0.05$ for 10000 oscillators and 500 periods.

of the full model with initial conditions randomly selected from the $[0,1]^N$ hypercube. Figure 3 shows that the typical number of clusters for a given ϵ can be well approximated by the number K_e corresponding to the uniformly distributed initial condition $x_i = i/N$. However, the number of asymptotic clusters depends strongly on the width of the distribution of initial states. We performed simulations for initial conditions x_i equi-distributed between $1-\delta$ and 1, and found that the number of asymptotic clusters strongly increases as the width of the initial distribution δ gets smaller, and approaches the values close to the upper bound $K_{\max}$ (Fig. 3a, inset).

We addressed the robustness of the clustering transition against noise. DF oscillators are most sensitive to noise during the firing, so we modeled this by resetting x_i to a random number uniformly distributed in $[1-\eta, 1+\eta]$ (η characterizes the noise strength). In this case, the clusters are not "exact", since after firing identical x_i become different, however for small noise groups of oscillators remain closely correlated. We can characterize the degree of correlation by the coefficient of variation of the mean field $X(t)$, $CV = \left[\langle X^2 \rangle - \langle X \rangle^2\right]^{1/2} / \langle X \rangle$. CV is small in the non-clustered regime, but increases rapidly as soon as the clustering occurs (Fig. 3b).

V. CONCLUDING REMARKS

We presented an analytically solvable model of co-repressively coupled degrade-and-fire oscillators in a strongly nonlinear regime. At a certain coupling strength this model exhibits a phase transition from a regime when any cluster distributions including the non-clustered state can be attained to the regime of strong clustering characterized by the appearance of at least one giant $\mathcal{O}(N)$ cluster. This phenomenology is reminiscent of the synchronization in the Kuramoto model of globally coupled oscillators [18] or populations of integrate-and-fire neurons [14–17], however the details of the transition are different. In particular, in the Kuramoto and IF

models with identical oscillators synchronization occurs for any positive coupling strength, while in the array of co-repressively coupled DF oscillators the non-clustered regime is stable for sufficiently small coupling strength.

Our model neglected the variability in parameters of individual oscillators. It also ignores the spatial localization of coupling or the time delays in exchange of the repressor concentrations among the oscillators. It is well known that these effects may play significant role in the synchronization phenomena [17, 19, 20]. We plan to address these effects in our future work. The experimental realization of coupled gene oscillators [13] incorporated a different (co-excitatory) mechanism of coupling through a quorum-sensing mechanism. However, we believe that co-repressive coupling may also be realized experimentally, if for example oscillators produce an enzyme that degrades a freely diffusing inducer or the quorum sensing molecule activates a repressor protein. Our results demonstrate that a strong clustering can be expected in this case, and large out-of-phase synchronized clusters are likely to emerge.

Acknowledgements. LT acknowledges useful discussions with Jeff Hasty and William Mather. He is also grateful to CPT for hospitality during his stay at the University of Provence, Marseille. BF thanks Lai-Sang Young for comments and suggestions and the Courant Institute (NYU) for hospitality. This work was supported by EU Marie Curie fellowship PIOF-GA-2009-235741 and NIH grants RO1-GM69811-01 and P50-GM085764.

Appendix A: Proof of Lemma 1

Recall that $R_{\{n_k\}}$ denotes the mapping in the ordered configuration set $\mathcal{T}_{\{n_k\}}$ that defines the new (ordered) configuration after firing. Recall also that $\epsilon_\eta := \epsilon/\eta$.

We first prove that (1) is a sufficient condition. Assume that initially $\{x_k\}_{k=1}^K \in \mathcal{T}_{\{n_k\}}$. Prior to firing, we have $\chi_1(t) = (1-\epsilon)x_1 + \frac{\epsilon}{N}\sum_{k=1}^K n_k x_k - t$. Accordingly, the quantity χ_1 reaches η at time t_f defined by

$$t_f := \chi_1(0) - \eta = x_1 + \frac{\epsilon}{N}\sum_{k=2}^K n_k(x_k - x_1) - \eta.$$

The quantity t_f is the actual firing time provided that $x_1(t_f) \geqslant 0$, *viz.* $t_f \leqslant x_1$. The latter is equivalent to

$$\epsilon_\eta \sum_{k=2}^K n_k(x_k - x_1) \leqslant N \tag{A1}$$

Using that $0 \leqslant x_k - x_1 \leqslant 1$ for state configurations in $\mathcal{T}_{\{n_k\}}$, a sufficient condition for this inequality is $\epsilon_\eta \leqslant \frac{N}{N-n_1}$. Applying cyclic permutations, we conclude that, for every configuration in any of the $\mathcal{T}_{\{n_{k+\ell}\}}$, the lowest cluster of oscillators fires before (or exactly when) it reaches 0 if

$$\epsilon_\eta \leqslant \min_{i=1,\cdots,K} \frac{N}{N-n_i} \tag{A2}$$

When this condition is violated, cluster configurations with all coordinates x_k, $k \geqslant 2$, sufficiently close to 1 and x_1 sufficiently close to 0 satisfy the inequality $\chi_1(x_1) = \frac{\epsilon}{N}\sum_{k=2}^K n_k(x_k - x_1) \geqslant \eta$. Accordingly, the concentration x_1 reaches 0 before χ_1 reaches η and, to prevent clustering we have to make sure that $\chi_2(t_f) > \eta$ *i.e.* $t_f < x_2$ if t_f still denotes the time when x_1 fires. For $t \geqslant x_1$, we have $\chi_1(t) = \frac{\epsilon}{N}\sum_{k=2}^K n_k(x_k - t)$. The condition $\chi_1(t_f) = \eta$ defines the firing time as follows $t_f = \frac{1}{N-n_1}\left(\sum_{k=2}^K n_k x_k - N\eta_\epsilon\right)$ where $\eta_\epsilon = \eta/\epsilon$. Now, the inequality $t_f < x_2$ turns out to be equivalent to

$$\sum_{k=3}^K n_k x_k - N\eta_\epsilon < (N - n_1 - n_2)\, x_2 \tag{A3}$$

which is certainly satisfied when $\sum_{k=3}^K n_k x_k - N\eta_\epsilon \leqslant 0$. Solving this inequality for all permutations $\{n_{k+\ell}\}$ gives the desired condition (1) on the coupling parameter.

We now show that $R_{\{n_k\}}$ maps $\mathcal{T}_{\{n_k\}}$ into $\mathcal{T}_{\{n_{k+1}\}}$ when (1) holds. This consists in checking three conditions.

- First, we have $x_2 - t_f > 0$ either because $x_1 - t_f \geqslant 0$ and $x_2 > x_1$ or simply because of (A3) when (A2) fails.

- Then, the strict ordering $x_2 < \cdots < x_{K-1} < 1$ implies a similar ordering for the coordinates of $R_{\{n_k\}}x$ where $x = (x_1, \cdots, x_K)$.

- Finally, we have

$$\begin{aligned}
&(1-\epsilon)(R_{\{n_k\}}x)_1 + \frac{\epsilon}{N}\sum_{k=1}^K n_{k+1}(R_{\{n_k\}}x)_k \\
&= (1-\epsilon)(x_2 - t_f) + \frac{\epsilon}{N}\Big(\sum_{k=1}^{K-1} n_{k+1}(x_{k+1} - t_f) + n_1\Big) \\
&= (1-\epsilon)(x_2 - t_f) + \frac{\epsilon}{N}\Big(\sum_{k=2}^{K} n_k(x_k - t_f) + n_1\Big) \\
&> (1-\epsilon)(x_1 - t_f) + \frac{\epsilon}{N}\sum_{k=1}^K n_k(x_k - t_f) = \eta
\end{aligned} \tag{A4}$$

  where we used $x_2 > x_1$ and $1 \geqslant x_1 - t_f$ to obtain the inequality. Thus the last inequality in the definition of $\mathcal{T}_{\{n_{k+1}\}}$ is satisfied for $R_{\{n_k\}}x$ and the proof is complete.

Finally, that (1) is a necessary condition is easy to check. Indeed, when $\epsilon_\eta > \frac{N}{N-n_1-n_2}$, initial conditions in $\mathcal{T}_{\{n_k\}}$ with x_1 and x_2 sufficiently close to 0 and the other x_i close enough to 1 simultaneously violate (A1) and (A3). Accordingly, the clusters n_1 and n_2 fire simultaneously, and a K'-cluster distribution results with $K' \leqslant K-1$. In particular, the image $R_{\{n_k\}}x$ cannot belong to $\mathcal{T}_{\{n_{k+1}\}}$. The proof is complete.

Appendix B: Proof of Lemma 2

Recall that $R^*_{\{n_k\}}(x_1,...,x_K) := (x_2 - t_f, ..., x_K - t_f, 1)$ denotes the firing map acting in $\mathcal{T}_{\{n_k\}}$ in absence of clustering. The proof of Lemma 2 essentially consists in showing that, due to the dissipative nature of the coupling, all eigenvalues of the linear parts of each map $R^*_{\{n_k\}}$ lie inside the unit circle.

We first prove contraction for the individual map $R^*_{\{n_k\}}$. Its form depends upon whether the initial condition $x = (x_1, \cdots, x_K)$ satisfies (A1) or not.

Stability in the domain where x satisfies (A1). When only considering the first $K-1$ coordinates, we obtain

$$\begin{aligned}(R^*_{\{n_k\}}x)_k &= x_{k+1} - t_f \\ &= \eta + x_{k+1} - x_1 - \frac{\epsilon}{N}\sum_{\ell=2}^{K}\alpha_\ell(x_\ell - x_1),\end{aligned}$$

for all $k = 1, \cdots, K-1$. Applying the change of variable $x \mapsto y$ defined by

$$\begin{aligned}y_k &= \begin{cases} x_k - x_{k+1} & \text{if } k = 1, \cdots, K-2 \\ x_{K-1} & \text{if } k = K-1 \end{cases} \qquad \Longleftrightarrow \\ x_k &= \sum_{\ell=k}^{K-1} y_\ell, \ k = 1, \cdots, K-1\end{aligned}$$

the linear part of the previous expression becomes $y \mapsto L_{\{n_k\}}y$ where

$$(L_{\{n_k\}}y)_k = \begin{cases} y_{k+1} \quad \text{if} \quad k = 1, \cdots, K-2 \\ -\sum_{\ell=1}^{K-1} y_\ell(1 - \frac{\epsilon}{N}\sum_{j=\ell+1}^{K} n_j) \text{ if } k = K-1 \end{cases}$$

(We used $\sum_{j=2}^{K-1} n_j \sum_{\ell=1}^{j-1} y_\ell = \sum_{\ell=1}^{K-2} y_\ell \sum_{j=\ell+1}^{K-1} n_j$.) The corresponding $(K-1)\times(K-1)$-matrix $L_{\{n_k\}}$ is a companion matrix whose characteristic polynomial $P_{\{n_k\}}$ immediately follows by reading the bottom line, namely

$$P_{\{n_k\}}(\lambda) = \sum_{\ell=0}^{K-1}\lambda^\ell\left(1 - \frac{\epsilon}{N}\sum_{j=\ell+2}^{K} n_j\right)$$

The polynomial coefficients $1 - \frac{\epsilon}{N}\sum_{j=\ell+2}^{K} n_j$ are positive and decaying (as ℓ decreases) when $0 < \epsilon \leqslant 1$. By a classical result in numerical analysis (see *e.g.* [21], p.116), this property implies that $P_{\{n_k\}}$ is Schur stable, *viz.* all its roots lie inside the unit disk.

Stability in the domain where x does not satisfy (A1). In this case, the map reads

$$(R^*_{\{n_k\}}x)_k = x_{k+1} - \frac{N}{N-n_1}(\sum_{\ell=2}^{K} n_\ell x_\ell - \eta_\epsilon)$$

for all $k = 1, \cdots, K-1$. After applying the same change of variable as before the corresponding linear part becomes

$$(L'_{\{n_k\}}y)_k = \begin{cases} y_{k+1} \ \text{ if } k = 1, \cdots, K-2 \\ -\frac{1}{N-n_1}\sum_{\ell=2}^{K-1} y_\ell \sum_{j=2}^{\ell} n_j \ \text{ if } \ k = K-1 \end{cases}$$

now based on $\sum_{j=2}^{K-1} n_j \sum_{\ell=j}^{K-1} y_\ell = \sum_{\ell=2}^{K-1} y_\ell \sum_{j=2}^{\ell} n_j$. The associated characteristic polynomial in this case reads

$$P'_{\{n_k\}}(\lambda) = \frac{1}{N-n_1}\sum_{\ell=1}^{K-1}\lambda^\ell\sum_{j=2}^{\ell+1} n_j$$

As before, the polynomial $\lambda^{-1}P'_{\{n_k\}}(\lambda)$ has positive and decaying coefficients; hence all roots of $P'_{\{n_k\}}(\lambda)$ lie inside the unit disk.

Proof that the return map is a contraction. Given that both spectral radii $r(L_{\{n_k\}})$ and $r(L'_{\{n_k\}})$ are less than 1, the end of the proof is quite standard. Take any norm $|\cdot|$ in $\mathbb{R}^{K-1}$. We have $r(L_{\{n_k\}}) = \lim_{t\to\infty} |L^t_{\{n_k\}}|^{1/t}$ (see e.g. [22]). Take $\delta > 0$ sufficiently small so that $r(L_{\{n_k\}}) + \delta < 1$ and let t_δ be large enough so that

$$|L^t|^{\frac{1}{t}} \leqslant r(L_{\{n_k\}}) + \delta, \quad \forall t \geqslant t_\delta$$

Consider the analogous t'_δ for the operator $L'_{\{n_k\}}$ and take $s_\delta = \max\{t_\eta, t'_\eta\}$ (this requires electing δ so that both inequalities $r(L_{\{n_k\}}) + \delta < 1$ and $r(L'_{\{n_k\}}) + \delta < 1$ simultaneously hold). By choosing $\|x\| := |x^{s_\delta}|^{1/s_\delta}$ in $\mathbb{R}^{K-1}$, we conclude that the linear parts of $R^*_{\{n_k\}}$ have norm $\|\cdot\|$ less than 1, *viz.* $R^*_{\{n_k\}}$ is a global contraction.

Finally, the return map $R^*_{\{n_{k+K_{\text{per}}-1}\}} \circ \cdots \circ R^*_{\{n_{k+1}\}} \circ R^*_{\{n_k\}}$ will be contracting for the norm $\|x^s\|^{1/s}$ where s is any integer larger than each s_δ of the $R_{\{n_{k+\ell}\}}$ and δ is such that all these maps are contracting. The proof is complete.

Appendix C: Proof of Lemma 3

Lemma 3 is a consequence of existence conditions for the periodic orbit associated with each cluster size distribution $\{n_k\}$. This analysis of these conditions directly follows from the explicit computation of periodic orbits coordinates.

Without loss of generality, we study the solution in $\mathcal{T}_{\{n_k\}}$ of the equation

$$R^*_{\{n_{k+K-1}\}} \circ \cdots \circ R^*_{\{n_{k+1}\}} \circ R^*_{\{n_k\}}(x) = x \qquad \text{(C1)}$$

for any K-cluster configuration $\{n_k\}_{k=1}^K$. For the sake of notation, we use the symbol R^ℓ instead of $R^*_{\{n_{k+\ell-1}\}} \circ \cdots \circ R^*_{\{n_{k+1}\}} \circ R^*_{\{n_k\}}$ and $R^0 = \text{Id}$.

Periodic orbits have a property that remarkably simplifies their analysis; namely all coordinates fire from the

same value. In particular, this implies that they either all fire before reaching 0 or all reach 0 before firing. To see this, recall that $t_f(x)$ denotes the firing time for x. The firing map definition obviously implies $(R^K x)_K = 1$, but also $(R^K x)_{K-1} = 1 - t_f(R^{K-1}x)$ since $(R^{K-1}x)_{K-1} = 1$ and $t_f(R^{K-1}x)$ is the firing time of the configuration $R^{K-1}x$. By repeating the argument for the other coordinates $(R^K x)_k$, we easily obtain the following expression

$$(R^K x)_k = 1 - \sum_{\ell=k}^{K-1} t_f(R^\ell x), \quad k = 1, \cdots, K. \qquad \text{(C2)}$$

Together with the periodicity assumption $R^K x = x$ and the firing map definition, this expression implies

$$\begin{aligned}(Rx)_1 - t_f(Rx) &= (R^K x)_2 - t_f(Rx) - t_f(x) \\ &= (R^K x)_1 - t_f(x) = x_1 - t_f(x).\end{aligned}$$

By induction, we conclude that successive firing levels $(R^\ell x)_1 - t_f(R^\ell x)$ do not depend on $\ell = 0, \cdots, K-1$ and the announced alternative follows. To proceed, we consider both cases separately.

Case 1: All coordinates fire before reaching 0. In this case, we assume that $t_f(R^\ell x) \leqslant (R^\ell x)_1$ for all $\ell = 0, \cdots, K-1$ and we accordingly solve the equation (C1), *i.e.* $R^K(x) = x$.

Using the notation $\tau_k = x_{k+1} - x_k$, it is convenient to rewrite the periodic orbit coordinates as follows

$$x_k = 1 - \sum_{\ell=k}^{K-1} \tau_\ell, \quad k = 1, \cdots, K$$

which in particular yields $x_K = 1$. In addition, periodicity condition (C1) and (C2) imply

$$t_f(R^k x) = \tau_k, \quad k = 1, \cdots, K-1 \qquad \text{(C3)}$$

To proceed, we use the assumption $t_f(x) \leqslant x_1$ to rewrite $t_f(x) = x_1 + \frac{\epsilon}{N}\sum_{\ell=2}^K n_\ell(x_\ell - x_1) - \eta$ as follows

$$t_f(x) = 1 - \sum_{\ell=1}^{K-1} \tau_\ell - \Delta_0$$

where

$$\Delta_k = \eta - \frac{\epsilon}{N}\sum_{\ell=2}^K n_{\ell+k}((R^k x)_\ell - (R^k x)_1),\ k = 0, \cdots, K-1.$$

Accordingly, the definition of the firing map implies $(Rx)_k = x_{k+1} - t_f(x) = \sum_{\ell=1}^k \tau_k + \Delta_0$ for all $k = 1, \cdots, K-1$ and then $t_f(Rx) = (Rx)_1 + \frac{\epsilon}{N}\sum_{\ell=2}^K n_{\ell+1}((Rx)_\ell - (Rx)_1) - \eta = \tau_1 + \Delta_0 - \Delta_1$ since we are assuming $t_f(Rx) \leqslant (Rx)_1$. Repeating this argument inductively, we obtain

$$(R^\ell x)_k = \sum_{j=\ell}^{k+\ell-1} \tau_j + \Delta_{\ell-1},\ k = 1, \cdots, K-\ell \qquad \text{(C4)}$$

and

$$t_f(R^\ell x) = \tau_\ell + \Delta_{\ell-1} - \Delta_\ell,\ \ell = 1, \cdots, K-1 \qquad \text{(C5)}$$

From equation (C3), it results that $\Delta_{\ell-1} = \Delta_\ell$ for all $\ell = 1, \cdots, K-1$ which is equivalent to

$$\sum_{j=2}^K n_{j+\ell-1}((R^{\ell-1}x)_j - (R^{\ell-1}x)_1) = \sum_{j=2}^K n_{j+\ell}((R^\ell x)_j - (R^\ell x)_1)$$

Using again the firing map definition we have

$$(R^\ell x)_k = \begin{cases} (R^{\ell-1}x)_{k+1} - t_f(R^{\ell-1}x) & \text{if } 1 \leqslant k < K \\ 1 & \text{if } k = K \end{cases}$$

and, together with the relation $\sum_{j=1}^K n_{j+\ell-1} = N$, the previous relation thus simplifies to

$$N((R^\ell x)_2 - (R^\ell x)_1) = n_{\ell+1}(1 - (R^\ell x)_1 + t_f(R^\ell x)),$$

for $\ell = 0, \cdots, K-2$. For $\ell = 1, \cdots, K-2$, we can use relations (C4) and (C5) to get $(R^\ell x)_2 - (R^\ell x)_1 = \tau_{\ell+1}$ and $(R^\ell x)_1 = \tau_\ell + \Delta_{\ell-1}$. Accordingly, the previous relation results in

$$\tau_\ell = \frac{n_\ell}{N}(1 - \Delta_\ell), \quad \ell = 2, \cdots, K-1.$$

For $\ell = 0$, using the expressions of x_1 and $t_f(x)$ the previous equality becomes

$$\tau_1 = \frac{n_1}{N}(1 - \Delta_0)$$

Since all the $\Delta_\ell = \Delta$ are equal, we finally get the condensed expression $\tau_\ell = \frac{n_\ell}{N}(1-\Delta)$ for all $\ell = 1, \cdots, K-1$.

In order to compute Δ we introduce the latter into the definition of Δ_0 via the coordinates x_k. This gives a first order equation for Δ whose solution is

$$\Delta = \frac{\eta - \epsilon\Sigma_K}{1 - \epsilon\Sigma_K},$$

where $\Sigma_K = \frac{1}{N^2}\sum_{k=2}^K n_k \sum_{\ell=1}^{k-1} n_\ell$. Direct combinatorics implies

$$N^2 = \sum_{k=1}^K n_k \sum_{\ell=1}^K n_\ell = 2N^2\Sigma_K + \sum_{k=1}^K n_k^2$$

showing that $\Sigma_K = \frac{N^2 - \sum_k n_k^2}{2N^2}$. Notice that Δ is well-defined when $\epsilon < 1$ since $\Sigma_K < 1$. Moreover, we have $\Delta < 1$ when $\eta < 1$. From the expression of Δ, we immediately get $\tau_k = \frac{n_k(1-\eta)}{N(1-\epsilon\Sigma_K)}$.

To conclude the analysis in the present case, it remains to check the conditions on parameters for which we have

$$t_f(x) \leqslant x_1 \quad \text{and} \quad R^\ell x \in \mathcal{T}_{\{n_{k+\ell}\}}, \quad \ell = 0, \cdots, K-1.$$

Using (C4) and (C5), the first inequality amounts to $\Delta > 0$, *viz.* $\epsilon_\eta \leqslant \frac{1}{\Sigma_K}$. As for the second conditions, the only non trivial restriction is $(R^\ell x)_1 > 0$ for all $\ell = 0, \cdots, K-1$, since the other ones are automatically satisfied from the definition of R and the non-clustering assumption. Again, (C4) and (C5) show that $(R^\ell x)_1 = \tau_\ell + \Delta > 0$ for all $\ell = 1, \cdots, K-1$. For $\ell = 0$, we have $\sum_{k=1}^{K-1} \tau_k = (1 - \frac{n_K}{N})(1 - \Delta) < 1$, *i.e.* $x_1 > 0$. Summarizing, the periodic orbit passing $\mathcal{T}_{\{n_k\}}$ exists with all coordinates firing before they reach 0 iff

$$\epsilon_\eta \leqslant \frac{1}{\Sigma_K} = \frac{2N^2}{N^2 - \sum_k n_k^2}$$

Case 2: All coordinates reach 0 before firing. This case can only occur when $\epsilon_\eta > \frac{1}{\Sigma_K}$. We claim that the solution reads

$$x_k = \frac{1}{N}\left(\sum_{\ell=0}^{k-1} n_\ell - (1 - \frac{\eta_\epsilon}{\Sigma_K}) \sum_{\ell=k+1}^{K} n_\ell\right) \tag{C6}$$

for all $k = 1, \cdots, K-1$ and obviously $x_K = 1$. To check this assertion, according to (C2), it suffices to verify that

$$t_f(R^\ell x) = \frac{1}{N}\left(n_\ell + (1 - \frac{\eta_\epsilon}{\Sigma_K}) n_{\ell+1}\right), \quad \ell = 1, \cdots, K-1.$$

We shall indeed show by induction that this relation holds for all $k = 0, \cdots, K-1$. Since we now assume that $t_f(x) > x_1$ we have $t_f(x) = \frac{1}{N-n_1}\left(\sum_{k=2}^{K} n_k x_k - N\eta_\epsilon\right)$. Using (C6) and the definition of Σ_K, we obtain

$$\begin{aligned}
N\left(\sum_{k=2}^{K} n_k x_k - N\eta_\epsilon\right) &= \sum_{k=2}^{K} n_k \sum_{\ell=0}^{k-1} n_\ell - \sum_{k=2}^{K} n_k \sum_{\ell=k+1}^{K} n_\ell \\
&\quad + \left(\frac{\sum_{k=2}^{K} n_k \sum_{\ell=k+1}^{K} n_\ell}{\Sigma_K} - N^2\right)\eta_\epsilon \\
&= \sum_{k=2}^{K} n_k \sum_{\ell=0}^{k-1} n_\ell - \sum_{k=3}^{K} n_k \sum_{\ell=2}^{k-1} n_\ell \\
&\quad + \left(\frac{\sum_{k=3}^{K} n_k \sum_{\ell=2}^{k-1} n_\ell - \sum_{k=2}^{K} n_k \sum_{\ell=1}^{k-1} n_\ell}{\Sigma_K}\right)\eta_\epsilon \\
&= (N - n_1)(n_0 + n_1 - \frac{n_1}{\Sigma_K}\eta\epsilon)
\end{aligned}$$

which gives the desired result for $k = 0$. The other cases proceed similarly by induction.

The existence conditions now become

$$x_1 < t_f(x), \quad t_f(R^\ell x) \leqslant (R^\ell x)_2$$

and

$$R^\ell x \in \mathcal{T}_{\{n_{k+\ell}\}}, \quad \ell = 0, \cdots, K-1.$$

The inequalities $x_k < x_{k+1}$ for all $k = 1, \cdots, K-1$ are equivalent to the following ones

$$\eta_\epsilon < (1 + \frac{n_k}{n_{k+1}})\Sigma_K, \quad k = 1, \cdots, K-1$$

which certainly hold when $\epsilon_\eta > \Sigma_K^{-1}$. Moreover, $x_1 > 0$ is equivalent to $\eta_\epsilon > (1 - \frac{n_K}{N-n_1})\Sigma_K$ and naturally, we obtain $x_1 < t_f(x)$ iff $\eta_\epsilon < \Sigma_K$.

For $\ell > 0$, the only non trivial constraint in $R^\ell x \in \mathcal{T}_{\{n_{k+\ell}\}}$ is $(R^\ell x)_1 > 0$, *i.e.* $(R^{\ell-1}x)_2 > t_f(R^{\ell-1}x)$, since all other constraints follow from the definition of the firing map and the non-clustering assumption. Therefore, all what remains to be checked is $t_f(R^\ell x) \leqslant (R^\ell x)_2$ for all $\ell = 0, \cdots, K-1$.

By induction we get $(R^\ell x)_2 - t_f(R^\ell x) = x_{\ell+2} - \sum_{j=0}^{\ell} t_f(R^j x)$. Using the explicit expression above, we obtain

$$(R^\ell x)_2 - t_f(R^\ell x) = -(N - n_{\ell+2} - n_{\ell+1}) + \frac{\eta_\epsilon}{\Sigma_K}(N - n_{\ell+2}),$$

for all $\ell = 0, \cdots, K-1$. Altogether, we conclude that the existence condition in the case when all coordinates reach 0 before firing, reduces to

$$\begin{aligned}
\frac{1}{\Sigma_K} < \epsilon_\eta &< \min_{k=1,\cdots,K} \frac{1}{\Sigma_K}\frac{N - n_k}{N - n_k - n_{k+1}} \\
&= \frac{2N^2}{N^2 - \sum_k n_k^2}\frac{1}{1 - \min_k \frac{n_k}{N - n_{k+1}}}
\end{aligned}$$

Appendix D: Maximising configurations

Lemma 4 *Given $K \geqslant 3$ and $N \geqslant K$ (and $N \neq 6$ if $K = 3$), the K-cluster configuration that maximizes the critical value $\zeta(\{n_k\})$ is the one defined by*

$$n_k = \begin{cases} 1 & \text{if } k = 1, \cdots, K-1 \\ N - K + 1 & \text{if } k = K \end{cases} \tag{D1}$$

This result is not as obvious as it may first appear. Indeed, collapsing extensive clusters together and splitting off unitary clusters in a way to keep the total number K constant, increases the left term in the expression of $\zeta(\{n_k\})$ (see (2)) but simultaneously decreases the right term; so the overall shift of the critical value needs to be carefully evaluated. In addition, Lemma 4 does not hold in the case $K = 3$ and $N = 6$ since one can check that $\zeta(\{1, 1, 4\}) < \zeta(\{2, 2, 2\})$.

Proof. To show that the configuration (D1) minimizes the denominator in $\zeta(\{n_k\})$, we can assume without loss of generality that $\{n_k\}$ is a permutation of the configuration $\{q_k\}_{k=1}^K$ where $1 \leqslant q_k \leqslant q_{k+1}$ and $\sum_{k=1}^K q_k = N$. Let $\ell \leqslant K-1$ be the largest index for which $q_k = 1$. We separate the cases $\ell \geqslant \lfloor \frac{K}{2} \rfloor + 1$ and $\ell \leqslant \lfloor \frac{K}{2} \rfloor$.

If $\ell \geqslant \lfloor \frac{K}{2} \rfloor + 1$, then we have $\ell > K - \ell$, *i.e.* the configuration $\{q_k\}$ has more clusters with a single unit than it has clusters with more than 1 unit. Thus every permutation $\{n_k\}$ must have two consecutive clusters with a single unit. In this case, the quantity

$$S = \min_{k=1,\cdots,K} \frac{n_k}{N - n_{k+1}} = \frac{1}{N-1}$$

reaches its global maximum. Moreover, adding units to the largest cluster q_K by taking off units from smaller clusters q_k has the effect to increase the quantity $\sum_k q_k^2$. Thus the configuration (D1) minimizes the denominator in $\zeta(\{n_k\})$ over all permutations of increasing configurations $\{q_k\}$ with $\ell \geqslant \lfloor \frac{K}{2} \rfloor + 1$. The corresponding minimum is

$$Q = (K-1)(2N-K)\frac{N-2}{N-1}$$

To deal with the case $\ell \leqslant \lfloor \frac{K}{2} \rfloor$, we begin by showing that for every K-cluster configuration we have $1 - S \geqslant \frac{K-2}{K-1}$. Consider the following alternative. Either all $n_k \leqslant \lceil \frac{N}{K} \rceil$ or there is a pair (n_k, n_{k+1}) for which $\max\{n_k, n_{k+1}\} > \lceil \frac{N}{K} \rceil$. In the second case, by contradiction there must be another pair $(n_{k'}, n_{k'+1})$ where $\min\{n_{k'}, n_{k'+1}\} < \lceil \frac{N}{K} \rceil$ (otherwise we would have $\sum_k n_k > N$ which is impossible) implying that

$$S \leqslant \frac{\lceil \frac{N}{K} \rceil - 1}{N - \lceil \frac{N}{K} \rceil} \leqslant \frac{1}{K-1}$$

In the first case, if $\lceil \frac{N}{K} \rceil = \frac{N}{K}$, then we have

$$S \leqslant \frac{\frac{N}{K}}{N - \frac{N}{K}} = \frac{1}{K-1}.$$

Otherwise there must be k such that $n_k \leqslant \lceil \frac{N}{K} \rceil - 1$ (otherwise we would again have $\sum_k n_k > N$) and then again

$$S \leqslant \frac{\lceil \frac{N}{K} \rceil - 1}{N - \lceil \frac{N}{K} \rceil} \leqslant \frac{1}{K-1}$$

Thus in all cases, we have $S \leqslant \frac{1}{K-1}$ which implies the desired inequality.

Still for $\ell \leqslant \lfloor \frac{K}{2} \rfloor$, the configuration that maximizes the sum $\sum_k q_k^2$ is

$$q_k = \begin{cases} 1 & \text{if } k = 1, \cdots, \ell \\ 2 & \text{if } k = \ell+1, \cdots, K-1 \\ N - 2(K-1) + \ell & \text{if } k = K \end{cases}$$

(Notice that the assumption that $\{q_k\}_{k=1}^K$ has exactly ℓ sites for which $q_k = 1$ implies that $N - 2(K-1) + \ell \geqslant 2$.) Altogether we conclude that when $\ell \leqslant \lfloor \frac{K}{2} \rfloor$, the denominator of $\zeta(\{n_k\})$ is certainly not smaller than

$$Q_\ell = [(2(K-1)-\ell)(2N-2(K-1)+\ell) - 4(K-1) + 3\ell]\frac{K-2}{K-1}, \quad \ell = 0, \cdots, \lfloor \frac{K}{2} \rfloor.$$

Now, the quantity $Q_\ell - (K-1)(2N-K)$ increases with N provided that $(2(K-1)-\ell)\frac{K-2}{K-1} \geqslant K-1$. This inequality holds for every $\ell \leqslant \lfloor \frac{K}{2} \rfloor$ when $K \geqslant 5$. Moreover, the quantity $(K-1)\frac{2N-K}{N-1}$ also increases with N for $K \geqslant 2$. Thus the quantity $Q_\ell - Q$ increases with N and to ascertain that Q is a global minimum, it suffices to check that each $Q_\ell - Q$ is non-negative for $N = 2K - \ell$. This amounts to verify that the following inequalities hold

$$2K(K-1)(2(2K-1) - 3\frac{(K-1)^2}{K-2}) \geqslant \ell(c(K) + b(K)\ell + \ell^2), \quad \ell = 0, \cdots, \lfloor \frac{K}{2} \rfloor$$

where

$$c(K) = 12K^2 - 14K + 3 - \frac{(K-1)^2}{K-2}(7K-4)$$

and

$$b(K) = 2(\frac{(K-1)^2}{K-2} - 3K + 2).$$

The r.h.s. in the inequality above is a third order polynomial in ℓ that is decreasing between 0 and $\lfloor \frac{K}{2} \rfloor$ when $K \geqslant 5$. Moreover, it is easy to check that

$$2(2K-1) - 3\frac{(K-1)^2}{K-2} \geqslant 0$$

for every $K \geqslant 4$, *i.e.* we indeed have $Q_\ell \geqslant Q$ for every $\ell \leqslant \lfloor \frac{K}{2} \rfloor$ when $K \geqslant 5$.

For the cases $K = 4$ and $K = 3$, the proof needs to be improved because the quantity $Q_{\lfloor \frac{K}{2} \rfloor} - Q$ actually decreases with N when this integer is large. We begin with assuming $K = 4$ and check separately cases $j = 0, 1,$ and 2.

For $K = 4$ and $j = 0$, the inequality $2(K-2) \geqslant K-1$ shows that $Q_0 - Q$ increases with N. Moreover, we have just checked in the general case that $Q_0 \geqslant Q$ for all $N \geqslant 2K$ when $K = 4$; thus Q is also a global minimum in this case.

For $K = 4$ and $j = 1$, since $(2(K-1)-1)\frac{K-2}{K-1} \geqslant K-1$, we still have $Q_1 - Q$ increases with N. By numerically computing the values Q_1 and Q for $N = 7 = 2K-1$, we conclude that Q remains a global minimum in this case.

For $K = 4$ and $j = 2$, we claim that given an increasing configuration $(1, 1, q, N-q-2)$ with $2 \leqslant q \leqslant \lfloor \frac{N}{2} \rfloor - 1$, the minimum S is maximal for the permutation $(1, N-q-2, 1, q)$. Indeed, up to a cyclic permutation, there are 3 distinct permutations, and $(1, N-q-2, 1, q)$ is the only one that has non-consecutive '1'. For $(1, N-q-2, 1, q)$, we have $S = \frac{1}{N-q}$ thanks to the assumptions on q, and the corresponding denominator of $\zeta(\{n_k\})$ reads

$$2(N(q+2) - q^2 - 2q - 3)\frac{N-q-1}{N-q}$$

This quantity is convex for q between 2 and $\lfloor \frac{N}{2} \rfloor - 1$ (as a product of two positive functions, one increasing with decreasing derivative and the other one decreasing with decreasing derivative). Thus we only have to check that the values for $q = 2$ and $q = \lfloor \frac{N}{2} \rfloor - 1$ are not smaller than the value for $q = 1$. Direct calculations reveal that the former quantity eventually grows with N faster than

the latter when N is large enough. A numerical check shows that these values for $q = 2$ and $q = \lfloor \frac{N}{2} \rfloor - 1$ are not smaller than the value for $q = 1$ provided that $N \geqslant 6$ (which is the minimal N for $j = 2$), and so the proof is complete for $K = 4$.

For $K = 3$, there are two types of configurations to consider, namely the ordered ones (q_1, q_2, q_3) and those of the form (q_1, q_3, q_2). For (q_1, q_2, q_3), thanks to the assumption $q_i \leqslant q_{i+1}$, the minimum S is given by $S = \frac{q_1}{N-q_2}$. For (q_1, q_3, q_2), depending on the sign of $q_2^2 - q_1 q_3$, the minimum S is either $\frac{q_2}{N-q_1}$ or $\frac{q_1}{N-q_3}$. Since both these quantities are never smaller than $\frac{q_1}{N-q_2}$, the non-ordered configuration always minimizes the denominator of $\zeta(\{n_k\})$.

Using that $S \leqslant \frac{q+p}{N-q}$ for the configuration $(q, N-2q-p, q+p)$ (where $0 \leqslant p \leqslant \lfloor \frac{N-3q}{2} \rfloor$ and $1 \leqslant q \leqslant \lfloor \frac{N}{3} \rfloor$), we get that the denominator of the critical value is at least

$$\left(2N(2q+p) - (2q+p)^2 - (q+p)^2 - q^2\right) \frac{N-2q-p}{N-q} \tag{D2}$$

Using similar arguments as above, one concludes that this quantity is convex for p between 0 and $\lfloor \frac{N-3q}{2} \rfloor$. Therefore, it reaches its minimum at the boundaries of this interval.

For $p = 0$, the quantity (D2) becomes

$$2q(2N-3q)\frac{N-2q}{N-q}$$

which is again convex with q between 1 and $\lfloor \frac{N}{3} \rfloor$. Moreover, the value for $q = \lfloor \frac{N}{3} \rfloor$ grows faster with N than the value for $q = 1$ does. A numerical investigation reveals that the former is not smaller than the former provided that $N \geqslant 7$, *i.e.* $\zeta(1, N-2, 1) \leqslant \zeta(q, N-2q, q)$ for all $q \leqslant \lfloor \frac{N}{3} \rfloor$ when $N \geqslant 7$.

For $p = \lfloor \frac{N-3q}{2} \rfloor$, (D2) becomes

$$\left(2N\lfloor \frac{N+q}{2} \rfloor - \lfloor \frac{N+q}{2} \rfloor^2 - \lfloor \frac{N-q}{2} \rfloor^2 - q^2\right) \frac{\lceil \frac{N-q}{2} \rceil}{N-q}$$

Using once again the convexity argument, we obtain that this quantity reaches its minimum for either $q = 1$ or $q = \lfloor \frac{N}{3} \rfloor$. In the first case we want to check the inequality

$$\left(2N\lfloor \frac{N+1}{2} \rfloor - \lfloor \frac{N+1}{2} \rfloor^2 - \lfloor \frac{N-1}{2} \rfloor^2 - 1\right) \frac{\lceil \frac{N-1}{2} \rceil}{N-1} \geqslant 2(2N-3)\frac{N-2}{N-1}$$

Again, as N increases, the l.h.s grows faster than the r.h.s., and numerics show that the inequality holds for all $N \geqslant 10$. For $q = \lfloor \frac{N}{3} \rfloor$, one can prove that the corresponding inequality holds for all $N \geqslant 7$. Therefore, we have shown that $(1, 1, N-2)$ maximizes $\zeta(n_1, n_2, N-n_1-n_2)$ provided that $N \geqslant 10$. For N between 4 and 9, we have listed all 3-cluster configurations and checked that the property remains valid, except for $N = 6$, as stated above.

[1] U. Alon, *An introduction to systems biology: design principles of biological circuits* (CRC Press, 2007).
[2] J. Stelling, U. Sauer, Z. Szallasi, F. Doyle III, and J. Doyle, Cell **118**, 675 (2004).
[3] S. Panda, J. B. Hogenesch, and S. A. Kay, Nature **417**, 329 (2002).
[4] M. B. Elowitz and S. Leibler, Nature **403**, 335 (2000).
[5] J. Stricker, S. Cookson, M. R. Bennett, W. H. Mather, L. S. Tsimring, and J. Hasty, Nature **456**, 516 (2008).
[6] M. Tigges, T. Marquez-Lago, J. Stelling, and M. Fussenegger, Nature **457**, 309 (2009).
[7] W. Mather, M. R. Bennett, J. Hasty, and L. S. Tsimring, Phys. Rev. Lett. **102**, 068105 (2009).
[8] N. MacDonald, J. Theor. Biol. **67**, 549 (1977).
[9] R. D. Bliss, P. R. Painter, and A. G. Marr, J. Theor. Biol. **97**, 177 (1982).
[10] M. C. Mackey and I. G. Nechaeva, Phys. Rev. E. **52**, 3366 (1995).
[11] D. Bratsun, D. Volfson, L. S. Tsimring, and J. Hasty, Proc. Natl. Acad. Sci. USA **102**, 14593 (2005).
[12] R. Coutinho, B. Fernandez, R. Lima, and A. Meyroneinc, J. Math. Biol. **52**, 524 (2006).
[13] T. Danino, O. Mondragon-Palomino, L. Tsimring, and J. Hasty, Nature **463**, 326 (2010).
[14] H. Tuckwell, *Introduction to Theoretical Neurobiology: Linear cable theory and dendritic structure*, vol. 1 (Cambridge, 1988).
[15] R. Mirollo and S. Strogatz, SIAM J. Appl. Math. **50**, 1645 (1990).
[16] S. Bottani, Phys. Rev. E **54**, 2334 (1996).
[17] U. Ernst, K. Pawelzik, and T. Geisel, Phys. Rev. Letters, **74**, 1570 (1995).
[18] Y. Kuramoto, *Chemical Oscillations, Waves and Turbulence* (Springer, Berlin, 1984).
[19] M. Timme, F. Wolf, and T. Geisel, Phys. Rev. Lett. **89**, 258701 (2002).
[20] M. Barahona and L. M. Pecora, Phys. Rev. Lett. **89**, 054101 (2002).
[21] E.I. Jury, *Theory and applications of the z-transform method*, Wiley (1964)
[22] M. Reed and B. Simon, *Functional analysis I*, Academic Press (1980)