**Response to Reviewer 1**

We thank the reviewer for his/her very positive and insightful remarks and detailed suggestions, including detailed comments on the Supplemental Information. They enabled us to improve our manuscript. Below, remarks of Reviewer 1 are in *italics* and are followed by our responses.

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*The manuscript by Olimpio et al. addresses the problem of representing the macroscopic dynamics of digital systems arranged on a lattice and coupled by a locally produced and diffusing signal. The basic unit of the lattice models are cells endowed with a bistable phenotype, that both secrete and sense the signal. Such spatially extended systems are usually studied by direct numerical simulation: analytically treatable low-dimensional approximations are hampered by the high number of degrees of freedom. The authors propose a scheme for deriving - under certain approximations and notably that the diffusion of the signal is much faster than the rate of switch - effective macroscopic equations for two order parameters (the fraction of 'ON' cells and the 'spatial-order parameter') that correspond to observables easily retrievable experimentally from cell cultures. Inspired by statical physic's methods, this approach is based on considering that spatial configurations can be 'lumped' into macro-states that carry the relevant information. The authors derive a Lyapunov function for the macroscopic 2-D equation (the 'multicellular Hamiltonian') and a 'landscape adhesiveness', which allow them to reproduce the time evolution - starting from a disordered configuration - of the order parameters obtained by simulation. The approximation reproduces well the macroscopic dynamics in different regimes for the cellular automaton, and in particular predicts that there are regions in the macrostate space with a higher frequency of microscopic steady-states (which cause a halt in the macroscopic trajectory). The extension of this approach to the case when noise is added to the system and when more than one signal is exchanges is presented mostly in the SI.*

*The manuscript presents a very elegant approach to solve a problem of biological significance. I think it is a very nice piece of work and I recommend it for publication.*

We thank the reviewer for this positive overall assessment.

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*I have however some comments the authors might consider in order to make the reading easier. Indeed, the main text is very compact, and many 'details' only come clear upon reading the extensive Supplementary Information. Sometimes the statistical description gets through as 'magic', whereas knowing more of the authors' thinking on why it works would just make more justice to the quality of the work.*
*I think the manuscript would gain a lot if the authors may discuss (or do it more extensively) in the main text the following points.*

**Following the reviewer's suggestion, we have now substantially revised and extended the main text.** We have done so by (1) migrating some of the text from the Supplemental Information to the main text, (2) giving more detailed explanations of how we derived the key equations and their consequences, (3) extending Discussion section to critically evaluate when our model is valid and when it is not, (4) moving the section on noisy sensing from the Supplemental Information to the main text (as a result, the manuscript now have five main figures instead of the previous four – we took the figure on noisy cellular automaton in the previous Supplemental Information and made it into a new main figure), and (5) re-arranging the main figures without changing their contents. **These changes have resulted in a longer manuscript in which we now more fully explain our thinking process, the motivation behind key mathematical definitions, intuitive ideas behind the equations, and more rigorous explanations of the equations that were previously in the Supplemental Information.**

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1. *'Spatial-order parameter'. It would be useful to have, when this order parameter is defined, some comment as to what aspects of spatial structure does the Moran I index single out, and why is this adapted to the problem at hand. This would also help to make clear what are the analogies and differences with other macroscopic approximations of spatially extended systems.*
   *Even if the authors stress this quantity should not be mistaken for common correlation statistics, I wonder if it would not be wiser for them to give it another name. The ambiguity between 'spatial-order parameter' and 'spatial order-parameter', also does not help. I think it is reasonable to assume that other people will take up this definition and apply it to other problems, so there is a risk of an avalanche of misunderstanding.*
   *(…) Moreover, it would be great if the authors can provide a qualitative explanation of why other approaches taking into account spatial autocorrelation or pair approximation would/should not allow to get to the same conclusions.*

**Following the reviewer's advice, we have changed the name of the *"I"* from "Spatial-order parameter" to "spatial index", to avoid confusion with the order parameters in physics. Furthermore, we have given a more extended explanation of the spatial index *I* and its properties in the main text (see added texts after Equations 1 and 2).** Included in the added texts is the following intuitive explanation of what the spatial index *I* measures: "A key feature that the value of the spatial index tells us is whether the lattice consists of one large, contiguous island of ON/OFF-cells (when *I* is close to one; Figure 2A - bottom row) or of many fragmented small islands of ON/OFF-cells (when *I* is close to zero; Figure 2A - top row)." Also now included (after Equation 2) is the following text that explains why the spatial index abruptly drops to zero when *p* is either zero or one: "As the spatial index approaches zero, the lattice becomes populated with more fragments of smaller islands of ON/OFF-cells. When the *p* is near zero (one), as is the case when only one cell is ON (OFF), then no clusters of ON-cells (OFF-cells) are possible since there is only one ON-cell (OFF-cell). Due to this reason and from a rigorous calculation of how the *I* changes as the *p* approaches zero or one (Supplemental Information section S2), we find that the spatial index is indeed zero when the

*p* is either zero or one." We have also updated Figure 2A to more clearly show examples of lattices with different values of *I* that are grouped into the same macrostate.

Moran's *I* is a suitable metric for spatial order because it measures the spatial autocorrelation of a system. It tells whether, on average, the value of a variable (a cell's gene-expression level in our case) at one location is correlated with that of the neighbors (gene-expression level of the neighboring cells). It is by no means the only metric capable of doing this, but has proven to be successful, over the past sixty years, in diverse fields such as geographical analysis, ecology, and econometrics. **We now cite these studies in the main text (just after Equation 1).**

As the reviewer notes, a related measure that is often used in physical systems is that of a (spatial) correlation function. For various magnetic spin systems, such a correlation function decays exponentially with distance. The exponential decay parameter sets a length scale called a "correlation length". However, simulation results for our system suggest that the correlation function does not decay exponentially (see Figure R1 below). Thus, we have decided against defining a correlation length for our cellular lattices. This in turn makes it difficult to compare correlations between different lattices.

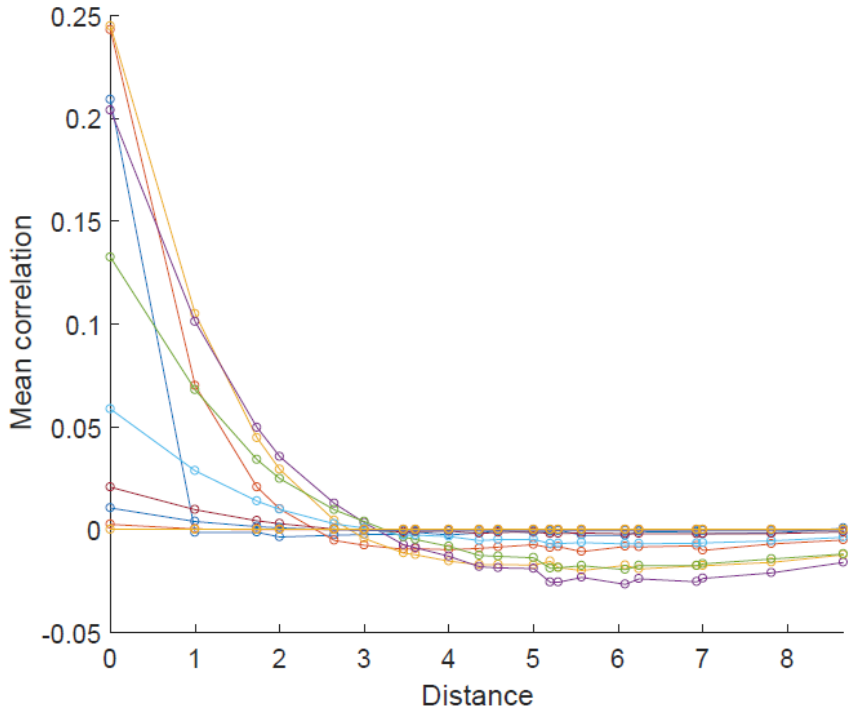


**Figure R1.** (Spatial) pair-correlation function $C(r_{ij}) = \langle X_i X_j \rangle - \langle X_i \rangle \langle X_j \rangle$ for various equilibrium configurations of the system. The averages are over all cells that are at distance $r_{ij}$ from each other (measured in units of the diffusion length $\lambda$). The function first decays sharply, but may increase at larger separations when for intermediate separation it becomes negative. The system is in the activate region of the weak interaction regime ($a_0 = 1.5$).

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2. *Analogously, I wonder if calling h a Lyapunov function rather than Hamiltonian would not respond better to the use the authors do of this quantity, when they show that*

*trajectories descend its gradient until they reach (if they do) the equilibrium for the macroscopic dynamics.*

In line with the reviewer's suggestion, we have changed the name of the "multicellular Hamiltonian" *h* to "pseudo-energy".

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3. *In the discussion, I would have expected to find a recapitulation of the hypotheses that motivate the success of the proposed approach (notably, the time-scale separation and the choice of initial configuration) and a critical assessment of the extent to which these hold/what happens when they do not. For instance, I guess that the macroscopic equations hold well as long as the microstates corresponding to a given macrostate are sufficiently disordered (and the system can be considered close to ergodic), this property being 'inherited' from the initial condition. This point is somehow touched upon in the Supplementary Information, but it would be useful to have it clarified in the text as well. Also, one wonders when the system size and the spatial scale matter.*

**We have now extended and substantially revised the Discussion section by (1) moving the relevant explanations from the Supplemental Information to the main text and (2) adding new explanations in the Discussion section. The Discussion section now (1) summarizes our main finding, (2) critically evaluates when our model is valid and when it is not (and give examples of each), (3) explains when the equation of motion (Equation 4) is a good approximation of the cellular automaton and when it is not, (4) explains how our framework is distinct from those of physics, and (5) how one might design experiments to use our model. This has resulted in a substantially more detailed and longer Discussion section.**

**The Discussion section includes an answer to the reviewer's particular question here about the macroscopic equation (i.e., equation of motion) (see third paragraph in the Discussion section).** In short, the agreement between the equation of motion and the cellular automaton is best at locations (*p, I*) where the gradient vector of the pseudo-energy is neither very close to the horizontal axis (i.e., the *p*-axis) nor the vertical axis (i.e., the *I*-axis). The gradient vector is slanted at most locations on the pseudo-energy landscape and thus the equation of motion recapitulates the main qualitative features of the particle trajectories that the cellular automaton produces at most locations. We also explain in the revised Discussion section why the equation of motion is more suitable for cells with a strong interaction (i.e., $f_N(a_0) > 1$) than cells with a weak interaction ((i.e., $f_N(a_0) < 1$)).

**The Discussion section also addresses the reviewer's comment on the system size (see end of the third paragraph in the Discussion section)**. We have not found significantly different results for widely varying system sizes (i.e., between hundreds and thousands of cells). As for the spatial scale, we note that it is taken care of by the lattice spacing $a_0$ – Changing the lattice spacing yields two qualitatively distinct regimes that we call "strong interaction" and "weak interaction" regimes in the manuscript.

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4. *The Supplementary Information presents a large amount of material that is covered to a small extent in the main text. I think that the two sections on the role of noise and on the generalization of the formalism would stand on their own as publications in a physics journal. This way, they are sort of marginalized, and require to go through an amount of supplementary reading that seems disproportionate. In my opinion, the paper would be more streamlined if it only dealt with the deterministic equation for the macrostates and the 'adhesiveness'.*

**We have now expanded the section on the role of noise in the main text by moving both the supplemental text and the supplemental figure (now Figure 5) on noisy sensing to the main text.** We have done this because the section on noise is important for establishing that the particles that are adhered to the landscape at locations where the $p$ is neither zero nor one represent metastable spatial configurations. Since noise is an important aspect of secrete-and-sense cells, we believe that including this material is indispensable and increases our manuscript's value and impact. We have also kept the material on the generalization of the formalism because it involves a relatively short series of mathematical steps (this is referred to in the Discussion section). We also think that it is important for the reader to know that the framework is extendable to more than one type of cells that use multiple types of signaling molecules. This also increases our work's impact and thus we have kept this section in the Supplemental Information.

**We have moved several other materials that were in the Supplemental Information to the main text and streamlined the organization of the Supplemental information.** But in the end, we still kept a large portion of the Supplemental Information because they are detailed, step-by-step derivations of the equations in the main text, and details of numerical simulations that are part of the "Transparent methods" section, which should be a detailed description of all the methods that we have used in our work according to the journal's guidelines (methods would be step-by-step calculations in our case). Moving these exhaustive derivations to the main text would detract the reader from the main storyline. Thus we have kept these in the Supplemental Information.

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*Some additional minor observations:*

*Abstract:*
*It should be said early on that this work describes cells with a bistable intracellular dynamics: there are other 'secrete-and-sense' cases when the intracellular dynamics is different, for instance a limit cycle, and whose description would require a different class of models. It would also be useful that the hypothesis of time-scale separation is mentioned in the abstract.*

We have made this change.

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*Abstract and discussion:*
*'how and why': to a biologist, 'why' evokes evolutionary explanations, that are not found in the paper*.

We eliminated the "why" in the abstract and the Discussion section.

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*Pages 5 & 6:*
*the equation for f(r_{ij}) is misspelled.*

We have fixed the typo.

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*Page 5:*
*It might be useful to introduce early on the quantity f(r_{ij}), recalling what is R and giving a qualitative explanation of what f measures. This would allow to define more transparently f_N (a_0) as its (scale-dependent) average.*

*It would be useful to have the names of the order parameters and of 'particles' in italics in the text, in order to make it easier to go back to the definitions.*

We now introduce $f(r_{ij})$ earlier in the text, before introducing $f_N(a_0)$. Whenever possible, we also restate the names of the variables again throughout the main text to remind the reader what the variables (e.g., *p, I, h*) mean. For instance, instead of simply writing "*I*", we now write "spatial index *I*" at several locations throughout the manuscript to remind the reader what the *I* stands for.

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*Page 7:*
*Reference to fig. 2b:*
*It is not clear what is depicted in the left-hand panels of fig. 2b, and what do the green and red lines mean. This should be explained either in the main text or in the figure caption.*
*I think the description of the trajectories in Fig. 2b could be expanded in the main text, differentiating the different phases, and introducing explicitly the notion of 'endpoint', which is not entirely clear from the figure caption*.

We have revised the figure caption for Figure 2B (and the captions for all the other figures) and expanded our description of it in the main text as suggested by the reviewer.

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*4 lines from the end of the first paragraph: 'Thus, we must interpret …'. Besides referring to the SI, it would be useful if the authors could expand a bit on this point here.*

We now give a more detailed explanation of this in the main text (see added text after Equation 2 and our response to point 1).

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*Figures:*

*Figure 3: explain the color-code.*

*Figure 4: the color-code does not seem to be consistent with that used in Fig. 2 or 3.*

The color code is now explained in the caption for Figure 3.

The color code in Figure 4 is different from that of Figure 3 and is now explained properly in the caption for Figure 4.

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*Typos:*

*page 7: 'which microstate is represented BY the moving particle'*
*caption figure 2: 'views of SOME trajectories'*

*SI: there are a few little typos, but without the line numbering it would be too long to list them.*
*SI page 22: title 'This configuration' → 'The trapping configuration'*

We have fixed these typos. We have also gone through the Supplemental Information and corrected all the other typos.

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We thank Reviewer 1 for his/her positive overall assessment and insightful comments that have improved our manuscript.

**Response to Reviewer 2**

We thank the reviewer for his/her positive remarks and suggestion. Below, remarks of Reviewer 2 are in *italics* and are followed by our responses.

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*Olimpio et al. presented a statistical mechanics approach to address how communicating cells can coordinate their gene expression to form spatial patterns. They focused on studying the "secrete and sense cell", a system that Dr. Youk had worked on previously. This is a very interesting approach to modeling cell communication and provides a method to gain insights into the systems without requiring all the detail kinetic parameters of the system. It also provides a rare analytical framework for studying multicellular behavior. It deserves to be published in iScience. I have one suggestion that may improve the manuscript.The analytical framework presented ultimately need to be verified experimentally. The authors describe briefly mentioned tagging the output with fluorescent protein as a way to verify the model. More discussion on this will help readers design experiment. What other parameters would you need to measure or calculate from microscopy images?*

**We have extended the Discussion section to explain in more detail how one can apply our work to experiments (see last paragraph in the Discussion section).** In particular, we now mention how optogenetics can be used to engineer cells, arrange these cells in a monolayer, and then use sculpted light (e.g., through a digital mirror device) to sculpt a field of cells with a desired initial pattern (thus initializing the values of $p$ and $I$). **We have also extended the Discussion section to explain what conditions must be obeyed by the cells for our model to be applicable for them (see second paragraph in the Discussion section).** This discussion can help the experimentalist in first checking if their system meets these conditions. As a guide, we have also listed in the Discussion section and Table S1 some well-studied systems that meet either all or some of the conditions of our model.

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We thank Reviewer 2 for his/her positive assessment and helpful suggestion that has improved our manuscript.

**Reviewer 3 - Editor's note**

"I heavily edited the report from Ref. #3, as the tone of his/her original report was inappropriate."

**Response to Reviewer 3**

We thank the reviewer for his/her comments. **While we value the criticisms, particularly on points that were unclear in the manuscript, we think that most of the reviewer's major criticisms arise from the reviewer erroneously believing that our framework should be exactly identical to the conventional physics frameworks. Our modelling framework is not derived from nor is it identical to existing frameworks of physics. We did *not* use *any* quantities from physics, thus there is no reason why anything in our work should follow the conventional rules of statistical mechanics and thermodynamics.** Specifically, there is no reason why terms from physics such as temperature, entropic forces, real Hamiltonians, and real energy should apply in our framework. In fact, they do not apply to our framework nor do they readily describe gene-regulations in multicellular systems such as the one that we have studied here. Inventing a new framework given this challenge is precisely the purpose of our work. We understand that some of the confusion may have stemmed from us giving names that sound similar to those of physics to key variables. To avoid these confusions, we have now changed the names of several key parameters – the "spatial-order parameter" is now renamed as "spatial index" and the "multicellular Hamiltonian" is now renamed as "pseudo-energy". We have also substantially extended the Discussion section, including how our framework is distinct from those of physics (second last paragraph in the Discussion section).

Below, the reviewer's remarks are in *italics* and are followed by our responses.

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*This manuscript looks at the dynamics of cells communicating through a secrete/sense mechanism and how this can lead to the formation of ordered states on a cellular lattice. The authors define the problem, then suggest two macroscopic observables, p and I (fraction active cells and spatial order, respectively), and consider the evolution of a "macrostate" defined as an "ensemble" of lattice microstates with the same p,I values. The macrostate is hypothesized to behave as a single "particle" moving in a phase space. They then proceed to define microscopic dynamics (which they call a "multicellular Hamiltonian") which resemble Ising-like dynamics and attempt to deduce that trajectories in p,I space correspond to flows along the gradient of the "Hamiltonian". Finally, some stochasticity is included and its role is vaguely discussed.*

*I find this a subject of interest and importance and so I consider the question as interesting and important. My personal view is that a fresh and "out of the box" approach to this question is welcome. And so, I was very excited and curious to review this article. Unfortunately, my impression is that in its current form it suffers from several issues which I outline below. Some of these issues are quite grave and may undermine the science behind this study to the point that I suspect it is at least partially wrong. Alas, without a substantial revision and major changes in content and presentation I cannot see how this work could be published, neither in iScience nor in any other journal for that matter. Such changes would be in effect a complete rewrite*

*of the manuscript after much careful thought and reading of relevant literature.*

**As stated above, our goal was not to build a framework that is fully consistent with existing frameworks of (non-equilibrium) thermodynamics and statistical physics. Thus, the fact that our framework does not agree with the conventional physics frameworks does not undermine and invalidate our work nor does it mean that our work is, to quote the reviewer, "*wrong*".** We have revealed concepts that help us to better understand multicellular systems, and in doing so, we have indeed used an "*out of the box*" approach to build a modelling framework that has some mathematical resemblance with the established frameworks of statistical physics but is, in the end, different. Precisely because our framework does not match those of established physics, our approach is "*out of the box*". To better explain which facets are similar and which are distinct between our framework and those of conventional physics, we have expanded the Discussion section (see 2nd last paragraph of the Discussion section). We admit these points were insufficiently discussed in the previous version of the manuscript. We thank the reviewer for hinting to this. We also note here that we are aware of and have already sufficiently cited the literature that is relevant to our work.

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1. *My biggest concern is that no attention is given to the number of microstates that are lumped together in a single (p,I) macrostate. I am baffled by this, since it directly corresponds to entropy, and resulting entropic forces. Entropy is off-handedly mentioned in the Suppl material, but then promptly ignored. Moreover, this has implications for points (2) and (3) below.*

**We can calculate the number of microstates for a given (p, I) (see below and Fig. R2) but it is irrelevant for our work**. In fact, it does not lead to a useful definition of thermodynamic entropy in the way that the reviewer suggests. Hence there are also no entropic forces of the kind found, for instance, in colloidal systems. All of this is because we did not use any quantities from physics to derive our framework. Even the idea of thermodynamic equilibrium does not apply to steady-states of our system. This is because the transitions between our microstates are dictated by the rules of the cellular automaton rather than by detailed balance as in a thermodynamic equilibrium. Hence, there is no mechanism that drives the system towards macrostates with a higher "entropy". On the contrary, as we show below, if we indeed define the "entropy" to be the total number of states for a given (p, I) as the reviewer suggests, the cellular lattice tends to evolve towards states of lower "entropy", such as a state in which all cells are either ON or OFF – this is in completely *opposite* of what the reviewer suggests.

As for the entropy mentioned in the Supplemental Information, this is a quantity called "entropy of population" that we defined in an earlier work (T. Maire and H. Youk, *Cell Systems* (2015)). It is an estimate of the total number of steady states for fixed values of $K$ and $C_{ON}$. **This is different from the quantity the reviewer is referring to and is also irrelevant for the present work.** Thus, we have omitted it, except for a brief mention in the Supplemental Information to say that our previous work considered it for a different purpose.

**Although it is irrelevant for the storyline, we followed the reviewer's comment and calculated the number of states for a given (p, I)** – we will denote this as $\Omega(p, I)$. To do so, we modified the well-established, Wang-Landau algorithm for calculating the density of states in statistical mechanics (Figure R2) (F. Wang and D. P. Landau, *Phys. Rev. Lett.* (2001)). Our calculation shows that $\Omega(p, I)$ is the highest when ($p \approx 0.5,\ I \approx 0$) and that it decreases sharply as $p$ moves closer to the boundaries (i.e., *p=0* and *p=1*). This makes sense as the number of states for a given $p$ is binomially distributed and there are typically more disordered states than highly ordered states for a given $p$. If we define entropy as $S(p, I) = \Omega(p, I) \log \Omega(p, I)$ (which is what the reviewer seems to refer to), the "entropy" would generally *decrease* over time rather than increase, as the trajectories move towards either $p = 0$ or $p = 1$. **Therefore, although this may be an interesting observation, the "entropy" $S(p, I)$ does not match the property of thermodynamic entropy from physics in contrast to the referee's suggestion (and thus the referee's "entropic force" also does not make sense in our system). More importantly, "entropy" is not essential or useful for understanding our work**. For these reasons, we have not defined and included "entropy" in the manuscript.

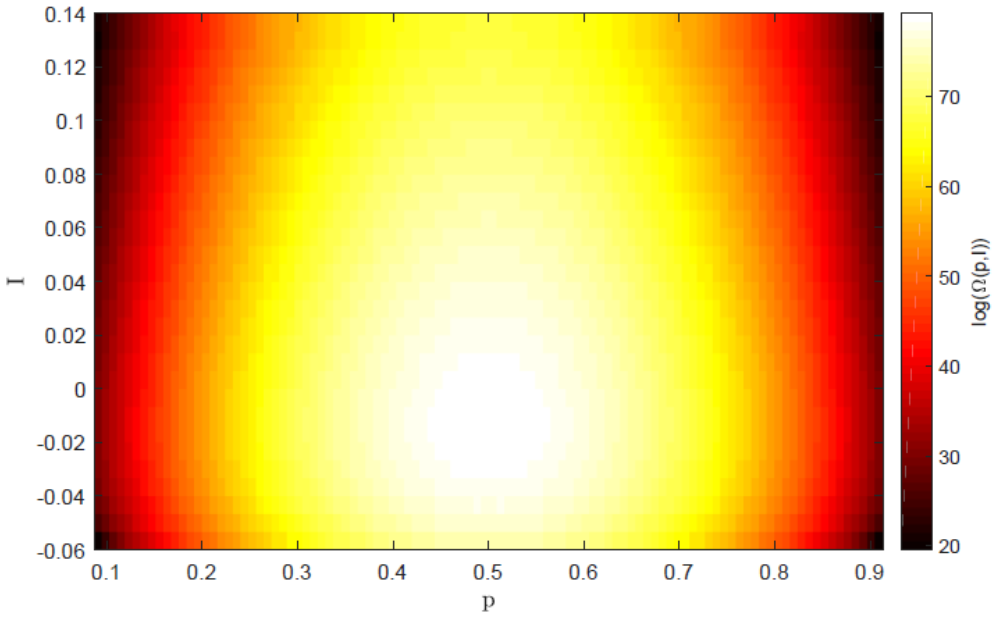


**Figure R2.** Estimate of the number of states for a given (p,I) - denoted $\Omega(\mathrm{p}, \mathrm{I})$ - using a modified version of the Wang-Landau algorithm (F. Wang and D. P. Landau, *Phys. Rev. Lett.* (2001)). Note that this has been done for only a small window of phase space. Outside this window the "entropy", defined as $S(p, I) = \Omega(p, I) \log \Omega(p, I)$, takes lower values.

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2. *A little below Eq. 3, (which is essentially an Ising model), appears the statement "... it competes with the cell-cell interaction term in Eq. 3...", referring to the "signal field" B (the equivalent of magnetic field in the Ising model). Again - I am baffled by this statement. The coupling vs. magnetic field is not the source of order-disorder competition in Ising systems. Indeed, both high coupling and strong magnetic field lead to breaking of up-down (here: active/inactive) symmetry, (spontaneously and explicitly, respectively). In this system, there is no active/inactive symmetry per se, however, the two terms should still work together and not in competition.*

*Rather - it is the competition between coupling (first term in Eq. 3) and *temperature* (which is canonically conjugate to the entropy) which leads to the order-disorder Ising transition. Admittedly, this notion of temperature is an equilibrium notion, however it is the authors themselves who invoke the Ising model. Regardless, entropy and an effective temperature are relevant quantities in non-equilibrium systems also.*

*I am left to conclude that as in point (2) above, since entropy has not been properly accounted for, temperature (effective, or otherwise) also appears to be neglected. Neglecting these quantities and their effect certainly requires explicit discourse and, in the process, may undermine the entire premise of the paper.*

**As in point 1, we think that the reviewer is confused here because he/she erroneously believes that our system should be exactly identical to a conventional physics system – an Ising model in this case. Our system is *not* an Ising system; we have a field of cells, *not* spins.** The "multicellular Hamiltonian" (now renamed as "pseudo-energy") is not the Hamiltonian from physics – it does *not* follow the same rules as in physics. Our system also does *not* follow the Boltzmann statistics. But the reviewer is correct that, in order to decrease *h*, the two terms in our pseudo-energy *h* do not act in competition. We have now clarified this explanation in the manuscript.

The reviewer insists on defining entropy and temperature for our system. As mentioned in our previous response, "entropy" is not a relevant quantity for our study. As for temperature, if a term like temperature could be defined for our system, then it would only be relevant in the case in which noise is involved in the secretion or sensing of the signal. But as we show in the manuscript (last subsection of the Results section), we are able to describe stochastic cellular automaton, in which cells experience noise in their sensing, without defining temperature. We instead define the "noise strength" of the system. When noise of low strengths are introduced, the system is unperturbed whereas when noise that is stronger than a critical strength $\xi_{min}$ (defined in the main text and in Supplemental Information section 7), the cellular lattice undergoes significant changes (Figs. 3F and S9). One could think of the noise strength as an "effective temperature". But other than giving the noise strength a different name, it still would not lead to a thermodynamic entropy, which would be required to build a framework that is identical to established frameworks of thermodynamics. **Aside from physical temperature and entropy either not making sense or being irrelevant for our system, our goal is not to extend the physics of thermodynamics to include a description of our systems. Therefore, we do not see the necessity of defining such an "effective temperature".**

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*3. At the bottom of page 8, the authors write that "We discovered that the particles closely follow the streamlines dictated by the gradient field." (with reference to Figs. 4a-d). However, I believe Figs. 4a-d tell a very different story. Certainly near p~0.5 (large entropy) the trajectories resemble the field lines (though even this could and should be quantified better). Crucially, though, near p~0 or p~1 (small entropy) the trajectories are mostly perpendicular to the field lines ! This apparent paradox (movement perpendicular to force !) is acknowledged through the mechanism of Imax(p), but still gravely undermines the usefulness of the approach. What we have,*

*is a statement about the field lines and the trajectories which is appears incorrect, and is not quantified or rigorously argued.*

**We have now extended the Discussion section to explain in more detail the successes and limitations of the equation of motion (previously this discussion was in the Supplemental Information).** Our goal was to obtain a *phenomenological description* that recapitulates the main *qualitative* features of the particle trajectories. In this regard, as seen in Figs. S4-7, the equation of motion provides an accurate overall description of the particle motion (i.e., producing distinct shapes of trajectories depending on the behavioral phases as well as where the particle gets stuck). The deviations that occur for values of *p* near zero and one result from the equation of motion not recognizing the boundaries of the phase space (i.e., nothing in Eq. 4 restricts the value of *p* to be between zero and one). To account for this, we force the particles that attempt to escape the phase space to go to $p = 0$ or $p = 1$, which is indeed what happens in the cellular automaton (Figs. 4A-D and Figs. S4-S5).

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*4. There is a sizable body of literature on going from a Hamiltonian system to a stochastic Langevin description such as Eq. 4. Often cited is the seminal review by PC Hohenberg, BI Halperin - Reviews of Modern Physics, 1977. This literature should be acknowledged when writing down Eq. 4, and this substantial body of knowledge about these systems should be capitalized on, in the manuscript. By properly acknowledging the literature the authors will find that (i) Eq. 4 is trivial and (ii) ill-defined quantities in their analysis eg. \delta are well studied and are well defined (iii) this section of the manuscript does not appear to make any obvious contribution to the literature. This also relates to comment (5) below.*

**As in points 1 and 2, we think that the reviewer is confused here because he/she erroneously believes that our system should be exactly identical to a conventional physics system – a Hamiltonian system of the type found in conventional physics in this case. We have never claimed to have found such a description for our system and our system indeed is not a conventional Hamiltonian system** because we have not described our microstates in terms of positions and momenta of particles. Moreover, the macrostate variables, *p* and *I,* are not conjugates of each other. Thus, the temporal evolution of our system is not dictated by the Hamilton's equations of motion, neither at the microstate level nor at the macrostate level. Thus, the results mentioned in Hohenberg and Halperin's review that the reviewer cites and in other works of non-equilibrium thermodynamics do not apply to our system. It also then follows that our equation of motion is not "trivial", in contrast to the reviewer's suggestion, since it *cannot* be derived from the established results that the reviewer mentions – Once again, the established results from conventional statistical physics have nothing to with our framework since our framework has not used any quantities from physics.

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*5. Whereas, in principle, I am fully in favor of defining new observables and analytic frameworks which may yield insight into complex systems, I find that the authors have abused this notion. Throughout the manuscript, the authors make vague statements eg., "Langevin-type", "strikingly similar to... the Hopfield network", "akin to a situation in physics", "... the spatial-order parameter is different from the order parameters of physics".*
*- It appears that the authors hint at concepts from physics, saying they are similar to what they do, without outlining precisely in what way they are similar or different, and where precisely is the novelty in their approach.*

**We have now extended the Discussion section to give a detailed analysis of where and how our framework differs from the established frameworks of physics such as statistical mechanics and thermodynamics (see 2nd last paragraph in the Discussion section).** We have also renamed the two main variables of our theory – We renamed "Spatial-order parameter" to "spatial index" and "multicellular Hamiltonian" to "pseudo-energy" – in order to prevent readers from erroneously thinking that these quantities are identical to those of physics. Indeed, the spatial index *I* is not the same as the order parameters in physics (and thus has properties that are different from those of order parameters in physics) and the "pseudo-energy" *h* is not the same as the Hamiltonians in physics.

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*- The authors define the "spatial-order parameter" I in Eq. 1 and proceed to state that, by construction it obeys 0<= |I| <= 1. Then, it appears they plot in their figures only the interval 0<= I <= 1 and make no mention of the fact that in Eq. S15, "I" actually becomes negative.*

The value of the spatial index *I* is, by definition, between -1 and 1. A positive value of *I* indicates that cells of the same type are clustered (e.g., ON-cells form clusters), whereas a negative value of *I* indicates "anti-clustering" (e.g., alternating ON-cell and an OFF-cell from one place to the next, as in a checkerboard). We now clarify this in the manuscript right after we introduce the *I* (Equation 1). In this work, we investigate how an initially disordered spatial configuration ($I = 0$) evolves over time. We have shown that the *I* increases over time, and thus the *I* cannot become negative, if the *p* is not near zero or one due to the positive "coupling" in the cell-cell interaction term in the pseudo-energy *h* (see the next response for what happens to *I* when the *p* is initially zero or one). Even when *p* is near zero or one, we show that the fact that *I* starts at zero means that *I* cannot become negative. Thus, we do not find any stable patterns with a negative spatial index forming if we start with $I \sim 0$. Therefore, we consider only positive values of *I* in this work.

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*- More on "I": At the bottom of page 7, the authors state that "we must interpret I carefully near the extreme values of p... The spatial order parameter is different from*

*conventional order parameters from physics in this sense." The authors should kindly explain why they would insist on using such a parameterization of the system that is not to be trusted, and if so, should certainly establish clear bounds on where it can be trusted vs. where it can not be trusted. Simply putting dashed black lines in Fig.3 to denote the values of Imax(p) is not enough. If I is small in those regions, is it because it's dominated by Imax(p) but the state is ordered, or because the state is disordered ? Given these limitations, why is "I" a useful and informative "order parameter"? This ambiguity prevails to Fig. 4, where the authors do not plot explicitly Imax(p), leaving the interpolation from Fig. 3 to Fig. 4 to the reader's imagination. This tells me that "I" is simply not a good choice of parameterization. Granted - finding the right order parameter for a system is difficult. However, I see no justification why "I", with all its disadvantages, is useful.*

**As in points 1, 2 and 4, we think that the reviewer is confused here because he/she erroneously believes that our framework is using terms that should be exactly identical to those of conventional physics systems – an order parameter from physics in this case. The spatial index *I* is not an order parameter from physics and thus it does not need to (and indeed does not) follow the properties that order parameters from physics obey.** We have revised the description of the spatial index *I* in the manuscript to avoid this confusion and added a more detailed explanation of why the spatial index becomes zero when *p* is either zero or one (see added text after Equation 2). To restate this explanation here: The spatial index goes to zero when *p* goes to zero or one. This makes sense because the spatial index is a measure of whether or not the cellular lattice consists of a large, contiguous island of ON/OFF-cells. As the spatial index approaches zero, the lattice becomes populated with more fragments of smaller islands of ON/OFF-cells. When the *p* is near zero (one), as is the case when only one cell is ON (OFF), then no clusters of ON-cells (OFF-cells) are possible since there is only one ON-cell (OFF-cell). Due to this reason and from a rigorous calculation of how the *I* changes as the *p* approaches zero or one (Supplemental Information section S2), we find that the spatial index is indeed zero when *p* is either zero or one. The fact that $I_{max}$(p) also approaches zero at *p=0* and *p=1*, follows from this explanation.

In fact, as suggested by the reviewer, we could use a parameter other than the *I* - we can replace the *I* by $\Theta$ (defined in Equation 2 and Equation S14). Since the *I* and the $\Theta$ are related through an algebraic transformation, they are equivalent (except at *p*=0 or *p*=1, but we explained above that the value of *I* is irrelevant for these cases). However, there are several major drawbacks. The major one is that unlike the *I*, the $\Theta$ is not normalized. In other words, we do not know the maximum and minimum values of $\Theta$ for a given *p* (without using $I_{max}$). Another drawback is that the value of $\Theta$ does not directly tell us whether the lattice is disordered or not (whereas $I = 0$ tells us that the lattice is completely disordered). Thus, knowing just $(p, \Theta)$ is not sufficient to tell us whether a lattice is highly ordered in space or not. For this reason, we use (*p, I*) instead.

**Finally, we point out that the spatial index is based on a well-established class of spatial metrics called Moran's *I*, which has now been in use for over fifty years in various fields.** We now state in the main text (see right after Equation 1) that Moran's *I* is widely used in spatial analysis in fields such as geographical analysis, ecology, and econometrics (we also now cite representative works from these fields). Our work illustrates how one can also use Moran's *I* to analyze the dynamics of spatial-pattern generating cells.

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*6. I do not see the logic in jumping from microscopic (Eq. 1), to macroscopic (Eq. 2), back to microscopic (Eq. 3), then back to macroscopic (Eq. 4). Jumping back and forth like this is confusing, and obscures your contribution and the reasoning leading to it. This zig-zag, riddled with multiple references to the supp. material for essential derivations, tells me the manuscript has been poorly constructed and needs significant rewrite. This relates to points (7) and (8) below.*

**The manuscript is structured in a way that presents the new ingredients one by one and shows the tight connection between the microstate and macrostate pictures, rather than jumping between microscopic and macroscopic pictures.** For example, we first introduce the spatial index *l* (Eq. 1 – shows how a macroscopic variable is defined in terms of microstate variable), then we introduce the pseudo-energy *h* (Eq. 3 and the text below it – shows the macroscopic-microscopic connection rather than focusing on one), and finally we introduce stochasticity. **Our goal is to show a tight connection between micro and macro throughout the manuscript and the current organization allows us to do so.**

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*7. The manuscript comes with 36 pages of supplemental information. Despite the authors' claim that the manuscript can be understood without it, I beg to differ. I find the suppl. material comprises a mixture of details that should be in the main body of the manuscript and details that rightfully belong in the supplement. I find that this makes reading the manuscript difficult (with multiple references to essential derivations in the supplement). The suppl. section is not organized as a logical progression of discourse, but is, rather, a collection of unrelated items. To make matters worse, these 36 pages of supplement are loosely referred to throughout the manuscript without being specific about where precisely to look.*
*If the authors choose to resubmit the paper, I would strongly encourage them to improve on this point. I acknowledge that it is very hard to strike the right balance between readability and rigor, especially so when submitting to a multi-disciplinary journal with a broad readership. However, I believe the solution they had arrived at can and should be improved upon.*

**We have revised the manuscript so that all references to the Supplemental Information now refer to the specific supplemental sections and supplemental figures. We have also substantially revised the main text to include more extensive descriptions (by migrating relevant material from the Supplemental Information to the main text), without including too many mathematical details that would obscure the main message.** The result is a main text that is much longer than the previous version. We have also revised the order of the supplemental sections so that they agree with the order in which the ideas that they support are introduced in the main text. Even after these changes, the Supplemental Information, including the supplemental figures, is still ~30 pages but this is due to the fact that we have included step-by-step derivations of all the equations and simulation procedures

used in the main text. Showing all these steps is in line with the journal's policy of including all the necessary methods (i.e., calculations and simulation procedures) as part of the "Transparent Methods" section in the Supplemental Information.

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*8. The authors make several bold, unsupported claims, eg.,*

*- "We have done this to show that it is possible to build physics-type frameworks for complex multicellular..."*
*The authors are not the first to build "physics-type" approaches for complex multicellular systems.*

**This quote is taken out of context.** The full sentence in the manuscript is: "*We have done this to show that it is possible build physics-type frameworks for complex multicellular systems that are governed by chemical signals, gene-regulatory networks, and multiple cells.*" We do not claim, in this sentence or anywhere else, that no one else has built a physics-type framework for any multicellular system. What the reviewer seems to be referring to is that other researchers have indeed built physics-type models for multicellular systems that involve mechanical, motile, or electrical parts, for which quantities from physics such as force, stress, and energy are valid. A notable example of this is the Cellular Potts Model (F. Graner, J. Glazier*, Phys. Rev. Lett.* (1992)). But such multicellular systems are distinct from the one that we study: Communicating cells that are not motile, mechanical, or electrical, and for which gene networks and diffusing signals are the key parameters. To our knowledge, such systems, for which quantities of physics do not seem to readily apply, have been lacking the kind of physics-type approach that our work presents.

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*- "... it is useful to focus on statistically describing how certain classes of spatial order arise... as we have done here"*
*- "... our framework and its extensions may help in predicting, based on a limited knowledge... how the spatial configuration of the cells evolves over time..."*

*However, the constant need for the microscopic formulation in their discourse (see point (6) above), with its very explicit (and somewhat unreasonable) set of constraints and assumptions, speaks otherwise.*

**We have extended the Discussion section to explain why our set of assumptions is not unrealistic and describe which biological systems they agree with (this was previously in the Supplemental Information but we have migrated it to the main text).** We also point out that in the original and the revised manuscript, Table S1 lists specific biological systems and cites specific papers that our model applies to.

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> *How would the authors predict the dynamics of spatial order in a real system? eg., one of their underlying assumptions is that the cells are a 2-dimensional sheet, with periodic boundary conditions (ie., a torus), embedded in a 3-dimensional tissue, but with an isotropic diffusion coefficient. (Explained just under Suppl. Eq. S1). How would this situation be amenable to the microscopy experiments they propose in the discussion section? Apart from the notion of increasing order as a particle rolling down a hill - hardly a new idea, how precisely are their ideas actionable in the way they claim them to be? How could these ideas be used to analyze one of the numerous experimental datasets available?*

**First, we note that the periodic boundary condition in a two-dimensional sheet of secrete-and-sense cells has been verified to match experimental results in some biological systems**: For example, hair-follicles on mouse skin that secrete-and-sense a molecule with an isotropic diffusion coefficient and are arranged in a triangular lattice (as in our study) – we cited this study, mentioned it in the original manuscript, and listed in Table S1. As in this experimental study, our framework is suitable for two-dimensional sheet of cells, such as epithelial sheet of cells or a monolayer of cells. The microscopy experiment that we mention in the discussion would involve a monolayer of cells, which one can observe when viewing micro-colonies of yeasts, bacteria, and mammalian cells on a microscope slide. **We have also extended the Discussion section to carefully explain when the assumptions of our model hold, when they do not, and for what systems they are or are not applicable.**

**Finally, as in points 1, 2, 4, and 5, we think that the referee's criticism that a particle rolling down an energy landscape is "*hardly a new idea*" stems from he/she erroneously believing that our framework is using terms that should be exactly identical to those of conventional physics systems.** In physics, a particle rolls down an energy landscape. But our framework involves no actual energy landscape. It also does not involve actual particles that have a physical position, momentum, and any other quantities associated with physics. Instead, we have defined a pseudo-energy landscape (not based on conventional physics), shown that we can think of an entire lattice of cells as a single particle (not from physics), showed how to calculate the shape of the landscape in terms of the position (*p, I*) (again, not from physics), and then showed this abstract particle rolls down and sticks on the pseudo-energy landscape. **None of these exist in conventional physics.** In the end, what we find here – a pseudo-particle rolling down on a pseudo-energy landscape – all makes an intuitive sense and are reminiscent of familiar notions from physics of an actual particle rolling down an actual energy landscape. That is the appeal of our framework and is the reason why we call it a "physics-type" framework. But none of these elements come from conventional physics and thus we respectfully disagree with the reviewer – they are indeed new ideas.

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We thank Reviewer 3 for his/her criticisms that have improved our manuscript. We hope that the substantially revised writing clarifies the reviewer's confusion, from which the bulk of his/her criticisms appear to have originated. Our work does not use established frameworks of physics. Our work presents a new framework that does not use any quantities from physics. We apologize if the original manuscript did not make this clear. We have substantially revised the manuscript to deliver this message.