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Investigating the Turing conditions for diffusion-driven instability in the presence of a binding immobile substrate

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ABSTRACT

Turing's diffusion-driven instability for the standard two species reaction–diffusion system is only achievable under well-known and rather restrictive conditions on both the diffusion rates and the kinetic parameters, which necessitates the pairing of a self-activator with a self-inhibitor. In this study we generalize the standard two-species model by considering the case where the reactants can bind to an immobile substrate, for instance extra-cellular matrix, and investigate the influence of this dynamics on Turing's diffusion-driven instability. Such systems have been previously studied on the grounds that binding of the self-activator to a substrate may effectively reduce its diffusion rate and thus induce a Turing instability for species with equal diffusion coefficients, as originally demonstrated by Lengyel and Epstein (1992) under the assumption that the bound state dynamics occurs on a fast timescale. We, however, analyse the full system without any separation of timescales and demonstrate that the full system also allows a relaxation of the standard constraints on the reaction kinetics for the Turing instability, increasing the type of interactions that could give rise to spatial patterning. In particular, we show that two self-activators can undertake a diffusively driven instability in the presence of a binding immobile substrate, highlighting that the interactions required of a putative biological Turing instability need not be associated with a self-activator–self-inhibitor morphogen pair.

1. Introduction

Alan Turing (1952) wrote his seminal paper on biological pattern formation, in which he showed that a system of chemicals (which he termed morphogens) undergoing reaction and diffusion can lead to the counter-intuitive phenomenon of diffusion-driven spatial heterogeneity. That is, a spatially uniform steady state, stable in the absence of diffusion, could be driven unstable by diffusion, evolving into a spatially heterogeneous state, a pattern. Furthermore, with non-dimensionalisation of the system equations to a fixed size domain, the diffusion coefficients acquire a domain-size dependence and hence one can deduce that Turing's instability will induce symmetry breaking from fluctuations as a domain adiabatically grows beyond a critical size. Consequently, this instability can drive the spontaneous formation of pattern, triggered simply by domain growth rather than any exquisite long-range cellular communication, and Turing proposed that this mechanism could induce a pre-pattern for cell differentiation in early developmental biology. However, this hypothesis laid largely ignored until the seminal paper of Gierer and Meinhardt (1972) 20 years later, which analysed the two chemical cases in detail. This demonstrated two ways in which pattern could arise, one of which for instance is referred to as "short-range-activation, long-range-inhibition". Further, one can readily demonstrate that the Turing instability in general for the two-species system, in the absence of a binding substrate, necessitates a short range morphogen which is a self-activator, i.e. it upregulates its own production,[1] interacting with a long range

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[1] At least in the neighbourhood of the steady state so that the corresponding diagonal entry in the Jacobian matrix evaluated at the steady state is positive. The concept of self-activation in the presence of a binding substrate is also further discussed in Section 2.

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morphogen which is a self-inhibitor and thus analogously down-regulates its own production (Murray, 2002).

Thus, implicit in the latter constraint is one of the key conditions for diffusion-driven-instability (DDI) with two chemical species, namely that their diffusion coefficients have to be different. While in principle they can be arbitrarily close to each other, this requires extensive parameter fine tuning for a Turing instability to still exist (Pearson and Horsthemke, 1989; Baker et al., 2008) and, in practice, interacting chemical molecules will typically have very similar diffusion coefficients. This led to great difficulty in identifying real Turing structures, but in 1991 they were eventually determined in a chemical system by Castets et al. (1990) and Ouyang and Swinney (1991) due to a substrate, introduced as a marker, binding to one of the chemicals and reducing its diffusion coefficient sufficiently. Furthermore, such binding dynamics have been implicated with diffusible gene-products such as fibroblast growth factor (FGF) indicating that this mechanism for inducing differential transport may potentially be active in biological systems exhibiting long range self-organisation (Miura, 2007).

We note that binding with an immobile substrate is not the only mechanism that has been highlighted as providing a means of circumventing the constraint of equal diffusion coefficients in Turing's mechanism. For finite amplitude perturbations, it is also possible for spatial patterns to arise with equal diffusion coefficients (Vastano et al., 1987). However, this is outside the scope of simple linear stability analysis and, more importantly, it is also outside the scope of fluctuation induced instability from an essentially homogeneous steady state and thus it cannot explain a core feature of Turing's instability, namely symmetry breaking from a near perfect spatial homogeneity, and is thus not considered further here. A second manner of evading the constraint of equal diffusion coefficients concerns receptor dynamics. In particular, a focussed model of hair follicle patterning (Klika et al., 2012) has also revealed that patterning can occur with equal diffusion coefficients. Coupling receptor dynamics to Turing's mechanism results in a system of coupled ordinary and partial differential equations, as also studied by Marciniak-Czochra in the context of hydra self-organisation (e.g. Marciniak-Czochra, 2003), which presents a mathematical framework with rich behaviour. Below, we do not consider the complexities associated with genuine receptor dynamics (Klika et al., 2012; Marciniak-Czochra, 2003), but instead focus on the influence of simple reversible binding with an immobile substrate such as extra-cellular matrix, representing a particularly simple class of coupled ordinary and partial differential equations with biological motivation that generalise the standard Turing model.

While this standard Turing model has many applications to pattern formation in biology (see, for example, the books by Meinhardt, 1982; Meinhardt et al., 2003; Murray, 2002) and is highly suggestive due to numerous cases of qualitative agreement with observation (e.g. Nakamasu et al., 2009; Yamaguchi et al., 2007), there is still a lot of scepticism in the biological community because the identification of Turing morphogens remains elusive. Nonetheless, there are a number of recent studies that have begun to move towards identifying possible biological components (Sick et al., 2006; Garfinkel et al., 2004; Solnica-Krezel, 2003; Chen and Schier, 2002; Hamadai, 2012; Muller et al., 2012) and even suggesting that the self-activator–self-inhibitor pair may actually be cells themselves (Yamaguchi et al., 2007; Nakamasu et al., 2009).

In this paper we first briefly revisit the original ideas of the CIMA chemical reaction used to experimentally investigate Turing's instability and, in particular, the theoretical study by Lengyel and Epstein, (1991, 1992), which was motivated by the immobile substrate in the CIMA experiments of Castets et al. (1990) and Ouyang and Swinney (1991). Lengyel and Epstein considered the equations for a Turing pair, in which one of the chemicals (the self-activator) reversibly binds to an immobile substrate and demonstrated this can be reduced using a quasi-steady approximation to a two species system with an altered effective diffusion coefficient ratio that facilities the induction of a DDI even if the two morphogens have an equal diffusion coefficient in the absence of reversibly binding to the immobile substrate. Miura presented an analogous approximation, though with piecewise continuous levels of extra-cellular matrix (ECM), for the interpretation of his experimental results (Miura, 2007), whilst Pearson (Pearson, 1992) extended Lengyel and Epstein's analysis to conditions outside the regime of the quasi-steady state approximation. All the theoretical aspects of these studies were focussed on the constraints for the morphogen diffusion coefficients associated with a DDI, though Pearson additionally assessed the relevance of such models for continuously fed reactors in observations of CIMA Turing instabilities.

However, despite the prevalence of the quasi-steady state approximation in these previous studies, there is no a priori reason to expect this approximation to be universal. For example, fluorescence recovery after photobleaching (FRAP) highlights that VEGF-ECM binding and unbinding occurs on the order of magnitude of 1000 s (Köhn-Luque et al., 2013). In contrast fast developmental events can occur on the timescale of only a few hours as illustrated by Zebrafish gene expression and fate maps for Nodal, a common putative morphogen, which demonstrate that Nodal specifies position-dependent cell fates in Zebrafish before gastrulation, i.e. under 5.25 h from fertilisation at standard conditions (Schier, 2003; Kimmel et al., 1995). Hence the timescale of fast developmental patterning, which must be significantly longer than the kinetic timescales, still need not be multiple orders higher than the timescale of ECM interaction between a diffusible signal and the extra-cellular matrix. In turn, this means that regions of parameter space where the binding-unbinding reaction rates are the same order as other kinetic interactions should not be excluded from studies. Furthermore, there is also no a priori reason to expect that any putative pair of Turing morphogens which interact with the ECM are restricted such that only one of the pair interacts with the ECM.

Hence, we revisit the full system for a Turing pair in the presence of reversible morphogen binding to an immobile substrate, without any quasi-steady approximations, and also briefly consider the system where both diffusing morphogens reversibly bind to the immobile substrate. This modelling framework will reduce to the standard model in the limit of negligible interactions with the immobile substrate so, in particular, our objective is to assess whether the introduction of a mobile substrate allows a *relaxation* of the conditions for a Turing instability. However, our main focus is fundamentally different from the previous work that clearly demonstrated that the 2-species requirement of equal diffusion coefficients needs no longer apply in the presence of reversible binding. In particular, there has been no study of whether it is still necessary to enforce other characteristics of the 2-species DDI, for instance the need to pair a self-activator with a self-inhibitor. Thus, rather than considering diffusive aspects of the diffusion-driven instability, we explore how the presence of a DDI constrains the *kinetics* of interacting morphogens given the presence of reversible binding to an immobile substrate. Furthermore, the diffusible gene-products Nodal and Lefty are the subject of intensive investigation concerning whether they fulfil the criteria of a Turing morphogen pair (e.g. Solnica-Krezel, 2003; Chen and Schier, 2002; Hamadai, 2012; Muller et al., 2012). Thus, more generally we are investigating whether one should refine or generalise the interactions that Nodal and Lefty, or indeed any prospective Turing pair, undertake in order to verify, at the molecular level, that the conditions for Turing's mechanism are satisfied given at least one of the morphogens undergoes reversible binding with an immobile substrate.

2. Diffusion-driven instability and morphogen binding to an immobile substrate

The classical Turing instability or diffusion-driven instability (DDI) occurs within a system of two reaction–diffusion equations that describe the time evolution for concentrations of two chemicals that diffuse and chemically interact. In order to distinguish the instability caused by diffusion from other types of instabilities, we consider the following linear system of first order ordinary differential equations:

$$u_t = f_u u + f_v v,$$
$$v_t = g_u u + g_v v, \tag{R}$$

where subscript t denotes a time derivative and f_u, f_v, g_u, g_v are constants. These equations capture the dynamics of perturbed concentrations u and v of two reacting, well-mixed, chemicals at least sufficiently close to the homogeneous steady state, which is simply $(0,0)$ as we are considering perturbed concentrations. In particular, for a DDI, we require that the homogeneous steady state $(0,0)$ is locally asymptotically stable, so that in the absence of diffusion there is no instability. Thus

$$\operatorname{tr} J_2 = f_u + g_v < 0 \quad \text{and} \quad \det J_2 = f_u g_v - g_u f_v > 0, \tag{1}$$

where

$$J_2 := \begin{pmatrix} f_u & f_v \\ g_u & g_v \end{pmatrix}, \tag{2}$$

which is evaluated at the homogeneous steady state when non-linear kinetics are considered. Note that the assumption $\det J_2 > 0$ guarantees regularity of the matrix J_2 and consequently uniqueness of the (trivial) equilibrium of the system (R). Therefore we can indeed speak about stability or instability of the system, meaning stability or instability of its trivial equilibrium. This is a standard convention that we use throughout the whole text.

Let Δ_x denote the Laplacian with respect to $x \in \Omega$ and consider the following system of reaction–diffusion equations:

$$u_t = D_u \Delta_x u + f_u u + f_v v,$$
$$v_t = D_v \Delta_x v + g_u u + g_v v \quad \text{in } \Omega$$
$$\frac{\partial u}{\partial n} = \frac{\partial v}{\partial n} = 0 \quad \text{at } \partial\Omega, \tag{RD}$$

where Ω is generally a domain in $\mathbb{R}^n$, whereas in the example in Section 5 we restrict ourselves to the real line. If the system (RD), that was generalised from (R) by adding diffusion terms, is unstable, we say that the system (RD) exhibits a Turing bifurcation or DDI. The set of parameter values that permit a Turing instability, so that for a suitable choice of domain the system exhibits a DDI, is often referred to as the Turing parameter space, or simply the Turing space. One can also readily show that, evaluated at the homogeneous steady state, one must have one of the following sign structures for J_2 (Murray, 2002):

$$J_2 := \begin{pmatrix} f_u & f_v \\ g_u & g_v \end{pmatrix} \sim \underbrace{\begin{pmatrix} + & + \\ - & - \end{pmatrix}}_{\text{cross}}, \quad \underbrace{\begin{pmatrix} - & - \\ + & + \end{pmatrix}}_{\text{cross}}, \quad \underbrace{\begin{pmatrix} + & - \\ + & - \end{pmatrix}}_{\text{pure}}, \quad \underbrace{\begin{pmatrix} - & + \\ - & + \end{pmatrix}}_{\text{pure}}. \tag{3}$$

The first two possible sign structures are commonly referred to as cross kinetics and the latter two as pure kinetics (Dillon et al., 1994), and we adopt this notation (though there are others, for instance cross kinetics are often referred to as a substrate depletion model (Gierer and Meinhardt, 1972). More importantly, denoting u (respectively v) as a self-activator if $f_u > 0$ ($g_v > 0$) at the homogeneous steady state since it upregulates its own production during the initiation of an instability, and a self-inhibitor if $f_u < 0$ ($g_v < 0$), as it downregulates its own production during the initiation of an instability, we see that the Turing instability necessitates the pairing of a self-activator with a self-inhibitor.

In this paper we consider a generalization of the system (RD) where we let one of the chemicals bind to an immobile substrate, for example extra-cellular matrix, which yields equations with the structure studied by Lengyel and Epstein (1991, 1992) and Pearson (1992). To write down these equations, we first need to distinguish the two states of the binding chemical, namely the concentration of the unbound state, u, and the concentration of the bound state, w. The second chemical is not allowed to bind and has concentration denoted by v.

With first order kinetics for the binding reactions, the corresponding system of reaction–diffusion equations reads

$$u_t = D_u \Delta_x u + (f_u - h_u) u + f_v v - h_w w,$$
$$v_t = D_v \Delta_x v + g_u u + g_v v,$$
$$w_t = h_u u + h_w w \quad \text{in } \Omega$$
$$\frac{\partial u}{\partial n} = \frac{\partial v}{\partial n} = 0 \quad \text{at } \partial\Omega, \tag{RDB}$$

where $h_u > 0$, $h_w < 0$.

We restrict ourselves to linear reaction kinetics, allowing the use of simple algebraic tools for stability analysis. However, the system (RDB) can also be derived by linearisation about a positive-definite homogeneous steady-state for a system with kinetics given by non-linear smooth functions $f(u,v,w)$, $g(u,v)$ and the linear first-order reaction rate function

$$h(u,w) = h_u u + h_w w, \quad h_u > 0, \quad h_w < 0$$

or a generalisation thereof with the same linearisation. For this reason we keep the notation f_u, g_u etc. that is typically used when studying a linearisation of a reaction–diffusion system with non-linear reactions. Further, below we refer to self-activation as positive auto-regulation, whereby the morphogen induces its own production on interacting with cells in the surrounding tissue so that, for example, the self-activation of u implies $f_u > 0$ with an analogous definition of self-inhibition, in terms of negative autoregulation.

It should be noted that linear analysis need not capture the non-linear system's behaviour and this can be particularly observed in higher spatial dimensions. For instance, Ermentrout (1991) demonstrated that the selection of stripes or spots in two-dimensional patterning depends on the nonlinear terms and cannot be discerned from the linearised model. It also follows that linear systems, as opposed to physical systems, allow unbounded growth of solutions (Page et al., 2003). However, numerical simulations suggest that from linear stability analysis one can generally deduce essential properties of the system, in particular the existence of Turing instability (Murray, 2002). Further details on the relationship between the linear and nonlinear dynamics are widely documented (Schnakenberg, 1979; Flach et al., 2007a,b), for instance in the case of one spatial dimension, as here, the structure of the pattern in the nonlinear regime is generally very similar to the prediction from the linear analysis as illustrated in many examples (Murray, 2002).

3. Lengyel and Epstein's reduction

As mentioned above, a system of the form (RDB) was first introduced by Lengyel and Epstein (1992) and we briefly consider the simplifying asymptotic approximation they invoke for fast binding dynamics to reduce the original system (RDB) to a system of two equations. This, in turn, differs from the system (RD) only by a scaling of the parameters. Nevertheless, this reduced system allows one to relax some of the standard restrictions of a

diffusively driven instability, in particular the lack of an instability given equal diffusion coefficients.

In detail, with u, v denoting the concentration of the self-activator and self-inhibitor respectively, so that $f_u>0$ and $g_v<0$, and with $|h_w|\sim h_u$ sufficiently large, a regular perturbation expansion reduces system (RDB) to

$$\begin{aligned}&(1+K)u_t = D_u\Delta_x u+f_u u+f_v v,\\ &v_t = D_v\Delta_x v+g_u u+g_v v \quad \text{in } \Omega\\ &\frac{\partial u}{\partial n}=\frac{\partial v}{\partial n}=0 \quad \text{at } \partial\Omega,\end{aligned} \tag{RDBr}$$

Here, $K:=-h_u/h_w>0$ and $w=-h_u/h_w\,[u+(1/h_w)u_t+\mathrm{O}(1/h_w^2)]$. At leading order, $h_u u+h_w w=0$ and hence this is referred to as a quasi-steady state approximation. The above system differs from the standard reaction–diffusion system without binding due to the factor $1+K$ in the equation for u. In fact, we can regard the change between the standard system (RD) and the system (RDBr) only as rescaling of the parameters by a positive constant $1+K$. Hence, this modification cannot change the sign of the parameters and consequently alter the classification of the reactants into self-activator and self-inhibitor, but it does effectively reduce the diffusion rate of the species that is allowed to bind, which here is the self-activator u.

Indeed, the standard necessary conditions for the diffusively driven instability corresponding to (RD), i.e. the condition for asymptotic stability of the system (R):

$$f_u+g_v<0 \tag{4}$$

$$f_u g_v-g_u f_v>0 \tag{5}$$

and the necessary conditions for instability of the spatial system (RD):

$$D_u g_v+D_v f_u>2\sqrt{D_u D_v(f_u g_v-f_v g_u)}>0 \tag{6}$$

now become

$$\frac{f_u}{1+K}+g_v<0, \tag{4*}$$

$$\frac{f_u}{1+K}g_v-g_u\frac{f_v}{1+K}=\frac{1}{1+K}(f_u g_v-g_u f_v)>0, \tag{5*}$$

$$\frac{1}{1+K}(D_u g_v+D_v f_u)>\frac{1}{1+K}2\sqrt{D_u D_v(f_u g_v-f_v g_u)}>0. \tag{6*}$$

The scaling $f_u\to f_u/(1+K), f_v\to f_v/(1+K), D_u\to D_u/(1+K)$ does not change the interval for admissible modes k,

$$k^2\in\left(\frac{A-\sqrt{A^2-B}}{2D_u D_v},\frac{A+\sqrt{A^2-B}}{2D_u D_v}\right),$$

where $A=D_v f_u+D_u g_v$ and $B=4D_u D_v\det J_2$, and hence the sufficient conditions are left unaffected.

Notice that the conditions (5) and (6) are identical with the conditions (5*) and (6*). In other words, it is precisely the condition involving the trace of the Jacobian that can possibly be relaxed in the case with binding. Equations (4*) and (6*) imply that f_u and g_v indeed need to have opposite signs as in the standard case, but, as opposed to the set of conditions (4)–(6), the new conditions (4*)–(6*) are not in contradiction with the identity $D_u=D_v$. Thus, a diffusely driven instability can occur in the system (RDB) even if the chemicals diffuse at the same rates. We refer to Appendix C for more details.

4. Analysis of the full three-dimensional model

This section is devoted to the analysis of the full system

$$\begin{aligned}&u_t = D_u\Delta_x u+(f_u-h_u)u+f_v v-h_w w,\\ &v_t = D_v\Delta_x v+g_u u+g_v v,\\ &w_t = h_u u+h_w w \quad \text{in } \Omega\\ &\frac{\partial u}{\partial n}=\frac{\partial v}{\partial n}=0 \quad \text{at } \partial\Omega,\end{aligned} \tag{RDB}$$

that has been introduced in Section 3. In this section, however, we do not specify *a priori* the type of the reactions, that is u and v can stand for two self-activators, two self-inhibitors or a self-activator and a self-inhibitor, while it remains unspecified which one of them is permitted to bind to the substrate. Hence, the corresponding matrix of reaction coefficients is going to have one of the following sign structures:

$$\begin{pmatrix}+ & & +\\ & + & 0\\ + & 0 & -\end{pmatrix},\quad \begin{pmatrix}+ & & +\\ & - & 0\\ + & 0 & -\end{pmatrix},$$
$$\begin{pmatrix}- & & +\\ & + & 0\\ + & 0 & -\end{pmatrix},\quad \begin{pmatrix}- & & +\\ & - & 0\\ + & 0 & -\end{pmatrix},$$

where the blank positions can be occupied by a positive, negative or zero coefficient.

Again, we shall assume asymptotic stability of the system

$$\begin{aligned}&u_t = (f_u-h_u)u+f_v v-h_w w,\\ &v_t = g_u u+g_v v,\\ &w_t = h_u u+h_w w.\end{aligned} \tag{RB}$$

as required for a diffusively driven instability.

4.1. The method for linear stability analysis

Let us denote by $p:=(D_u, D_v, f_u, f_v, g_u, g_v, h_u, h_w)$ the vector of parameters. Using the standard approach to linear stability analysis we formulate the eigenvalue problem corresponding to (RDB),

$$\begin{aligned}&\lambda u = -k^2 D_u u+(f_u-h_u)u+f_v v-h_w w,\\ &\lambda v = -k^2 D_v v+g_u u+g_v v,\\ &\lambda w = h_u u+h_w w.\end{aligned} \tag{7}$$

The solvability condition for (7), typically called the dispersion relation, is of the form

$$\lambda^3+A(\kappa,p)\lambda^2+B(\kappa,p)\lambda+C(\kappa,p)=0, \tag{DR}$$

with $\kappa:=k^2$ and

$$A(\kappa,p):=\kappa(D_u+D_v)-\operatorname{tr} J_3, \tag{8}$$

$$\begin{aligned}B(\kappa,p):=&\kappa^2 D_u D_v+\kappa(-D_v f_u-D_u g_v+D_v h_u-D_u h_w-D_v h_w)\\ &-f_v g_u+f_u g_v-g_v h_u+f_u h_w+g_v h_w,\end{aligned} \tag{9}$$

$$C(\kappa,p):=\det(\kappa D_3-J_3), \tag{10}$$

where D_3 is a diagonal matrix containing the diffusion coefficients and J_3 the Jacobian matrix of the system (RB),

$$D_3:=\begin{pmatrix}D_u & 0 & 0\\ 0 & D_v & 0\\ 0 & 0 & 0\end{pmatrix},\quad J_3:=\begin{pmatrix}f_u-h_u & f_v & -h_w\\ g_u & g_v & 0\\ h_u & 0 & h_w\end{pmatrix}. \tag{11}$$

The explicit expression for $C(\kappa,p)$ reads

$$C(\kappa,p)=h_w(-\kappa^2 D_u D_v+\kappa(D_v f_u+D_u g_v)-\det J_2), \tag{12}$$

where J_2 is the two-dimensional Jacobian matrix, see Eq. (2).

A standard result is that the Hopf bifurcation does not occur for a diffusively driven instability of the standard two species model system (RD), from its homogeneous steady state (Murray, 2002). Furthermore, in the weakly restricted context of self-organisation during metazoan development, oscillations for putative Turing morphogen pairs are typically not observed (for example, see Pearson and Horsthemke, 1989) and thus Hopf

bifurcations with frequencies greater than fluctuation growth rates can be excluded from consideration. Moreover, we show in Appendix A that Hopf bifurcations cannot occur with equal diffusion coefficients for the three component model, but more generally a Hopf bifurcation has the potential to induce patterning in a non-linear model such that fluctuation growth occurs and is stabilised by non-linearities before an oscillation prevails. While this is an interesting direction, the prospect of assessing such dynamics in general is beyond the scope of the current study and we restrict ourselves to real eigenvalues below.

At a Turing bifurcation point we therefore have $\lambda = 0$ which happens if and only if $C(\kappa, p) = 0$. Moreover, the solutions κ of this equation do not depend on the parameters h_u, h_w that quantify the rate of binding and unbinding, as $C(\kappa, p)$ is of the form $C(\kappa, p) = h_w \tilde{C}(\kappa, \tilde{p})$ with $\tilde{p} = \{f_u, f_v, g_u, g_v\}$. This is true for an arbitrary number of chemicals, regardless of how many of them bind to the substrate, at least given first order binding and unbinding kinetics. The proof of this statement is a simple application of linear algebra: for a system with n differential equations we have $C(\kappa, p) = \det(\kappa D_n - J_n)$ and the matrix $\kappa D_n - J_n$ is of a special form for which all the unbinding parameters can be factored out of the expression for the determinant, while all the binding parameters cancel each other.

Moreover, if $D := D_u = D_v$, then $C(\kappa, p)$ reads

$$C(\kappa, p) = h_w(-\kappa^2 D^2 + \kappa D \operatorname{tr} J_2 - \det J_2). \tag{13}$$

The dispersion relation, Eq. (DR), is cubic in λ and thus always has at least one real root. As motivated above, we can focus on real roots and thus the emergence of a stationary pattern that is caused by a real eigenvalue crossing the imaginary axis.

According to Vieta's formulae $C(\kappa, p) = -\lambda_1\lambda_2\lambda_3$, where λ_1, λ_2 and λ_3 are the three, in general complex, roots of (DR). Thus, the fact that a real eigenvalue crosses the imaginary axis precisely when $C(\kappa, p) = 0$ gives us a simple tool to detect non-oscillatory (in time) diffusively driven bifurcations. The existence of two distinct positive roots κ_1 and κ_2 of the equation $C(\kappa, p) = 0$ ensures existence of another κ^* for which the dispersion relation (DR) has a positive real solution. This follows from the following:

1. For $k = 0$ parameters *are taken* to be such that the system (RB) is locally asymptotically stable and all the eigenvalues must have negative real part. In generality, there is a range of parameters that satisfy this constraint, as illustrated in the examples below.
2. The left hand-side of (DR) is a cubic polynomial with real coefficients, with a unit coefficient for the highest power. Thus, once the coefficient of the lowest power, i.e. $C(\kappa, p)$, is negative continuity ensures that there is a least one positive real root, and thus instability for the associated square wavenumber κ.
3. The negativity of h_w implies local asymptotic stability of the system for $k \to +\infty$. For a proof of this statement see Klika et al. (2012). Hence we have $C(\infty, p) > 0$.
4. $C(\kappa, p)$ is a quadratic polynomial in κ. Hence, for any set of parameters p the curve $(\kappa, C(\kappa, p))$ is a quadratic curve in the plane that intersects the line $C(\kappa, p) = 0$ at most twice. We have the existence of precisely two solutions $\kappa_1 < \kappa_2$ given positivity of the discriminant for the value of κ associated with $C(\kappa, p)$. We additionally require that these roots are positive since $\kappa := k^2 \geq 0$. Once these conditions are satisfied, asymptotic stability for $k \to +\infty$ (see point (3)) implies negativity of $C(\kappa, p)$ for $\kappa \in (\kappa_1, \kappa_2)$ and thus instability, via the reasoning of point 2.

Furthermore, Eq. (12) implies that the set of parameters $\{D_u, D_v, \tilde{p}\}$ is a solution of the equation $C(\kappa, p) = h_w \tilde{C}(\kappa, \tilde{p}) = 0$ if and only if $\tilde{C}(\kappa, \tilde{p}) = 0$. Thus, the stability properties of the system (17) are independent of the parameters h_u, h_w and only contingent on the diffusion constants D_u, D_v and the parameters of the chemical reaction f_u, f_v, g_u, g_v.

To summarize, for any set of parameters p and a suitable choice of the domain size the homogeneous steady state of the system (RDB) is unstable provided the equation $C(\kappa, p) = 0$ has two distinct positive real solutions κ_1 and κ_2. As demonstrated in Appendix B, two self-inhibitors cannot undergo a non-oscillatory diffusively driven instability. In contrast, there is no a priori restriction on the prospect of a self-activator pair inducing a Turing instability and hence, informed by the need for two positive roots of $C(\kappa, p) = 0$, we find and explore possibilities for pairs of self-activators to induce Turing patterning in the sections below.

5. Examples and sensitivity analysis

Using the method from Section 4.1 one can rather easily find examples of (linear) reaction kinetics that lead to DDI for the full 3-species model (RDB) even when $D_u = D_v$. We single out one example of a system with two self-activators ($f_u > 0$, $g_v > 0$), one of which binds to a substrate, such that DDI occurs for the same diffusivities ($D_u = D_v$)

$$\begin{aligned} &f_u = 3/2, \quad f_v = 1, \quad g_u = -41/128 \sim -0.32, \\ &g_v = 5/16 \sim 0.31, \quad h_u = 9/4, \quad h_w = -1/64 \sim -0.016. \end{aligned} \tag{14}$$

With kinetic parameter values given by (14) and $D_u = D_v = 10^{-2}$, Fig. 1 shows a plot of dispersion relation (DR) in the $(\kappa, Re\,\lambda)$−plane with a close up of the instability domain. This highlights that a pair of self-activators can induce patterning in the presence of an immobile substrate in distinct contrast to the need for self-activator–self-inhibitor pairs for the two species Turing instability, as explicitly illustrated in Fig. 2a for random initial conditions and homogeneous Neumann boundary conditions.

There is no standard self-activator Turing pair kinetics in the literature with which to compare results; the only example reported is theoretical and considered by Madzvamuse et al. (2010) in the context of domain growth, whereby a self-activator Turing pair was observed to induce patterning in the presence of growth rather than an immobile substrate. These kinetics are given by

$$\begin{aligned} &u_t = D_u \Delta_x u + \delta(u-1) + (v-1) + 2\delta(v-1)^3 - h_w w, \\ &v_t = D_v \Delta_x v - a(u-1) + b(v-1) + (v-1)^2 - (v-1)^3, \\ &w_t = h_u u + h_w w \quad \text{in } \Omega \\ &\frac{\partial u}{\partial n} = \frac{\partial v}{\partial n} = 0 \quad \text{at } \partial\Omega, \end{aligned} \tag{15}$$

with a linearisation corresponding to $\delta = f_u$, $a = -g_u$, $b = g_v$. With parameters again taken from (14), $D_u = D_v = 10^{-2}$, homogeneous Neumann boundary conditions and random initial conditions, pattern once more emerges, as illustrated in Fig. 2b.

Noting that a parameter fine-tuning is required in Madzvamuse et al. (2010), as detailed further in the discussion, we consider whether parameter fine-tuning is required to achieve a DDI in the self-activator–self-activator mechanism introduced in this paper, keeping the diffusion coefficients equal as we anticipate many cases where the morphogen pair have very similar diffusion rates. For this purpose we plot the Turing parameter space for selected pairs of reaction kinetics, perform a simple sensitivity analysis and also plot derivatives of λ with respect to a chosen parameter.

Fig. 3 shows two-dimensional slices of the Turing parameter space in the planes (f_u, g_v), (f_u, h_u), (f_u, h_w) and (h_u, h_w), respectively, that contain the point given by (14) for the linear system (RDB). Furthermore, for each of the parameters we find the

one-dimensional slice of the Turing space containing the point (14) is given by the ranges,

$$f_u \in [1.46, 1.9], \quad f_v \in [0.94, 1.08], \quad g_u \in [-0.32, -0.26],$$

$$g_v \in [-0.2, 0.35], \quad h_u \in [1.9, 2.34], \quad h_w \in [-0.022, 0], \tag{16}$$

and remark that the relative size of each of the intervals measured with respect to its centre varies approximately between 13% and 750%.

Finally, in Fig. 4 we successively plot the derivative of the real eigenvalue λ with respect to f_u, f_v, f_w, g_u, g_v and h_u holding the other parameters fixed in a neighbourhood of the example (14). While the derivatives are relatively small, one must recall that λ around this point is small too, $\lambda \sim 4 \times 10^{-4}$ from Fig. 1. Hence relative changes in growth rates can be sensitive to parameters. However the derivatives are signed demonstrating that, *ceteris paribus*, one can impose order one relative changes in a parameter, at least in one direction, without leaving the Turing parameter space, illustrating that parameter fine tuning is not required to find an example of DDI in such a setting even if the growth rates are sensitive. Of course the observation that identical diffusivities or a self-activator–self-activator type kinetics can induce patterning further emphasises that the Turing space is not subject to excessive constraints in the presence of an immobile substrate.

In summary, we conclude that for the system (RDB), the diffusively driven instability arises in a more robust manner than in (15) and does not require fine tuning of certain parameters.

6. Both species allowed to bind

The reduction proposed by Lengyel and Epstein relaxes the DDI conditions in favour of a diffusively driven instability only when the activator binds to a substrate. Conversely, if only the inhibitor binds, the DDI conditions are even stricter; regardless, there is no a priori reason why both morphogens do not interact with the ECM. Moreover, experimental studies suggest that differential binding might be a general feature of reaction–diffusion based patterning (Muller et al., 2012; Hamada, 2012). Thus, we briefly numerically investigate the more complex general case of both morphogens interacting with the ECM to assess whether the special cases considered to this point are robust to the possibility that the second morphogen also binds.

Let us therefore adjust the notation accordingly - now let v stand for the concentration of the unbounded second chemical and z for the concentration of its bound version. Then, once more assuming first order kinetics for the binding and unbinding, the time evolution of the concentrations is governed by the system

$$\begin{aligned} u_t &= D_u \Delta_x u + (f_u - h_u)u + f_v v - h_w w, \\ v_t &= D_v \Delta_x v + g_u u + (g_v - s_v)v - s_z z, \\ w_t &= h_u u + h_w w, \\ z_t &= s_v v + s_z z, \end{aligned} \tag{17}$$

with $s_v > 0 > s_z$.

6.1. Asymptotic approximation

Next we show that in the most natural setting, where both morphogens are binding to a substrate, the behaviour of binding

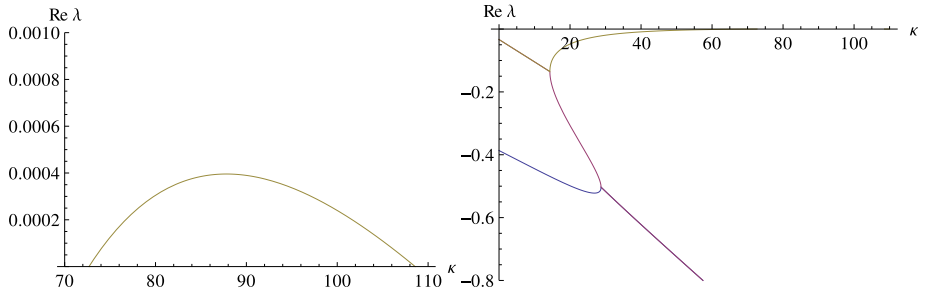

Fig. 1. Real parts of the eigenvalues of (RDB) with self-activator–self-activator type kinetic parameters given in (14) and $D_u = D_v = 10^{-2}$ plotted against κ. This figure, and all other presented figures, have been generated using Wolfram Mathematica (Wolfram Research, Inc., 2010).

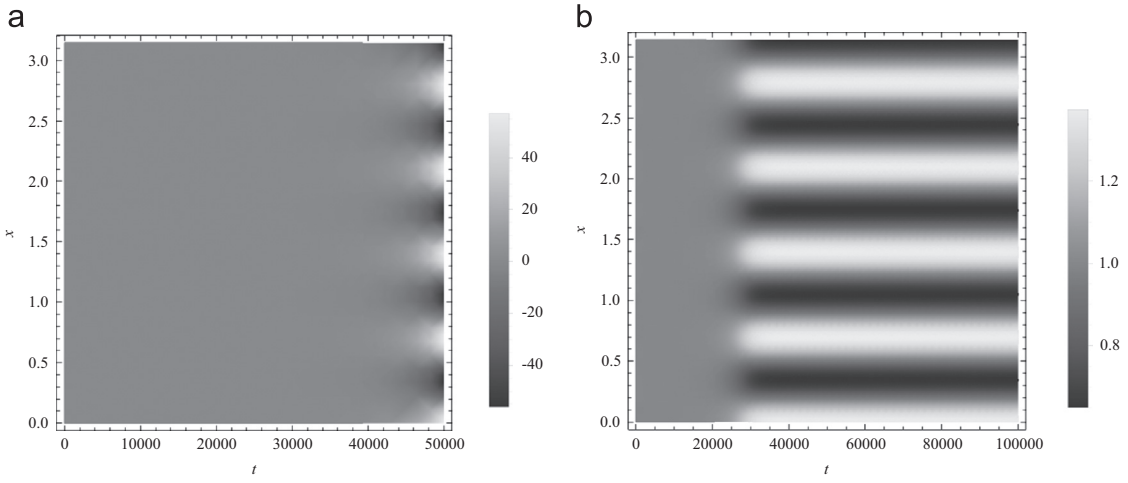

Fig. 2. Numerical solution u of (a) the linear system (RDB) and (b) the nonlinear system (15) with a random initial condition of order at most 10^{-3} with self-activator–self-activator type kinetic parameters given in (14), $D_u = D_v = 10^{-2}$ and homogeneous Neumann boundary conditions.

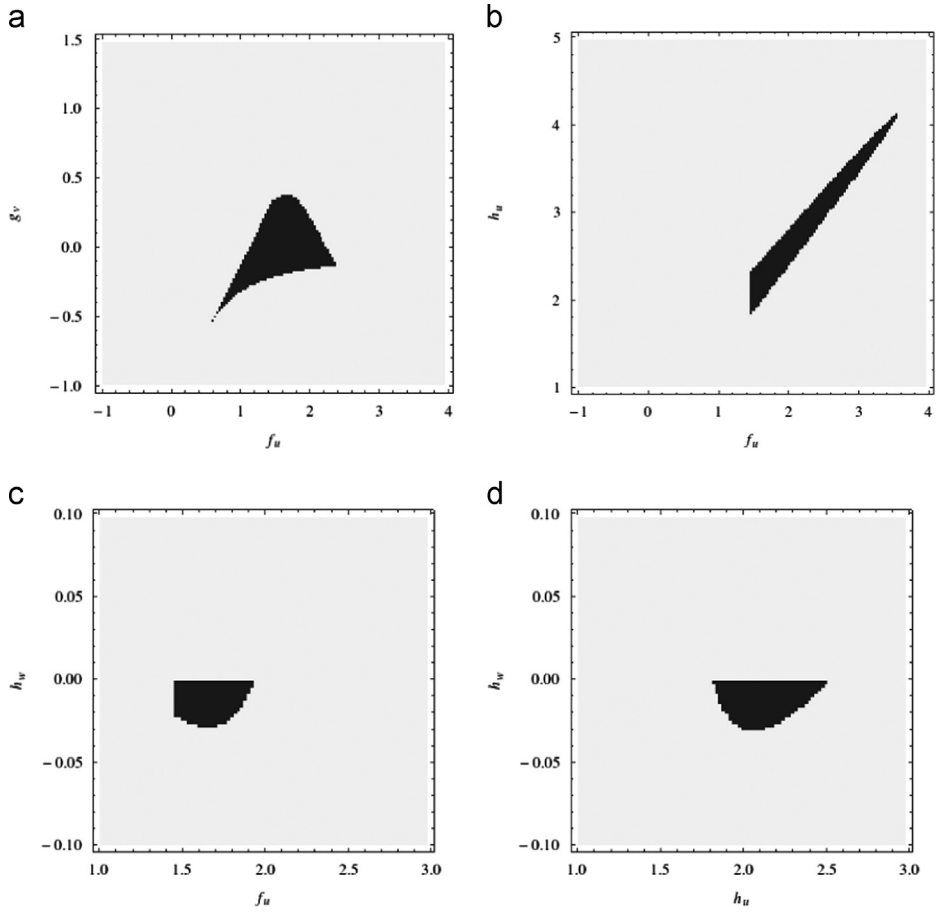

Fig. 3. A slice of the Turing space in the (a) (f_u, g_v)−plane, (b) (f_u, h_u)−plane, (c) (f_u, h_w)−plane, (d) (h_u, h_w)−plane in the neighbourhood of the example (14) with $D_u = D_v = 10^{-2}$ for the linear system (RDB).

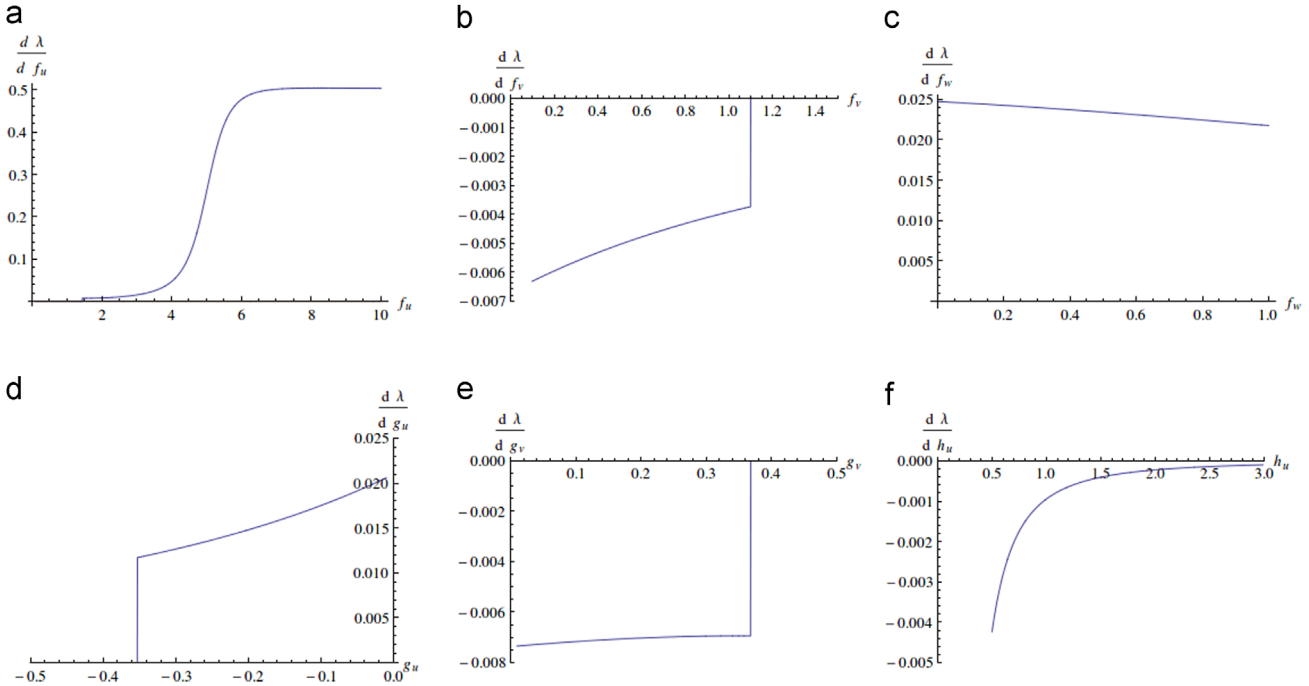

Fig. 4. Plot of (a) $d\lambda/df_u$ against f_u, (b) $d\lambda/df_v$ against f_v, (c) $d\lambda/df_w$ against f_w, (d) $d\lambda/dg_u$ against g_u, (e) $d\lambda/dg_v$ against g_v and (f) $d\lambda/dh_u$ against h_u, in the neighbourhood of the example (14). A curve is shown only when $\lambda \in \mathbb{R}$ and $\lambda > 0$.

morphogens can be again described by a standard two-species reaction–diffusion model with a single scaling parameter σ that is positive but not necessarily greater than one (which is the case in the Lengyel and Epstein, 1992 paper).

By repeating the approach of Section 3 we can reduce the system (17) to a two-dimensional system

$$(1+K_u)u_t = D_u\Delta_x u + f_u u + f_v v,$$
$$(1+K_v)v_t = D_v\Delta_x v + g_u u + g_v v, \tag{18}$$

where $K_u = -h_u/h_w$ and $K_v = -s_v/s_z$. If we further rescale time by the factor of $(1+K_v)$ and recycle the notation for time and parameters, we obtain

$$\sigma u_t = D_u\Delta_x u + f_u u + f_v v,$$
$$v_t = D_v\Delta_x v + g_u u + g_v v, \tag{19}$$

with $\sigma = (1+K_u)/(1+K_v)$. Note that σ is positive, but not necessarily larger than one.

Obviously, the system (19) also allows pattern formation even if the diffusion coefficients are identical. This is apparent from the mathematical point of view, given that the systems (RDBr) and (19) differ only by the factor $(1+K)$ or σ, respectively. Hence, under certain conditions on the model parameters, a Turing instability can occur in a system with identical diffusion coefficients and both the self-activator and the self-inhibitor binding to a substrate. More precisely, the rescaled diffusion constants D_u/σ and D_v from Eq. (19) need to satisfy the standard conditions on DDI (see, for example, Murray, 2002) and therefore there is a constraint on the relation of the rates of diffusion, binding and unbinding. Roughly speaking, if the self-inhibitor diffuses too slowly, one can slow down the self-activator accordingly by letting it bind at a very fast rate and unbind slowly. However, the reduced system (19) would not permit DDI with a different type of chemical reaction, for example with two self-activators. This, again, follows from positivity of the factor σ and the standard conditions for DDI, namely the condition $f_u/\sigma + g_v < 0$ that is necessary for the stability of the system without diffusion.

6.2. Analysis of the full system

As discussed in Section 4.1, we omit Hopf bifurcations from our analysis and concentrate on a situation when the linear system does not exhibit temporal oscillations.

Since the Routh–Hurwitz conditions for asymptotic stability of the system (17) without diffusion terms are rather complicated and lengthy, we only present the analogue to the equation $C(\kappa, p) = 0$ that was introduced in Section 4.1. Once more a Turing instability occurs whenever there are two distinct positive roots of the lowest power in the dispersion relation, $C(\kappa, p)$, for parameter values which also enforce stability in the absence of diffusion. We find this coefficient is explicitly given by

$$C(\kappa, p) = h_w s_z(-\kappa^2 D_u D_v + \kappa(D_v f_u + D_u g_v) - \det J_2). \tag{20}$$

Again, for $D := D_u = D_v$ we obtain

$$C(\kappa, p) = h_w s_z(-\kappa^2 D^2 + \kappa D \operatorname{tr} J_2 - \det J_2). \tag{21}$$

Notice the similarity of Eqs. (13) and (21) and the fact that even in the case when both species bind to a substrate, the conditions for instability are independent of the binding and unbinding rates. Furthermore, the presence of two positive roots for κ of the quadratic equation $C(\kappa, p) = 0$ facilitates finding regions of parameter space where a Turing instability occurs, which is non-empty for $s_v = s_z = 0$ by the results of previous sections and thus, by continuity, the Turing space is non-empty for non-zero s_v, s_z.

7. Discussion

As previously reported by Lengyel and Epstein together with Pearson (Lengyel and Epstein, 1991, 1992; Pearson, 1992), the presence of an immobile substrate, such as an extracellular matrix, can fundamentally alter the parameter space constraints required for diffusively driven instability. However, these reports explored circumventing the standard requirement that a Turing morphogen pair must have different diffusion coefficients, which in practice entails a large ratio of diffusivities to avoid parameter fine tuning. While we observe such effects too, we have instead focussed on the impact of binding to an immobile substrate for the constraints associated with the kinetics of morphogens capable of presenting a diffusively driven instability.

In particular, while two self-inhibitors cannot undergo a diffusively driven non-oscillatory instability, two self-activators can generate a pattern in the presence of binding with an immobile substrate; in other words the Turing instability can occur when both f_u and g_v are positive. Examples of such systems with two self-activators have been presented in Section 5 together with a numerical study that illustrates the instability behaviour. We also observed that the conditions for a non-oscillatory DDI in the full three-species model when $D_u = D_v$ are independent of the magnitude of D_u. More precisely, the existence of $\kappa > 0$ and κ^* such that $C(\kappa, p) = 0$ and $C(\kappa^*, p) < 0$ is independent of the value of D_u as $\kappa(D_u, p) = 1/D_u\tilde{\kappa}(p)$. The same holds for the remaining conditions for DDI. Thus, once an example of reaction kinetics leading to DDI is found for a given D_u, the DDI conditions are met for any value of D_u. Hence, the Turing parameter space of a system that allows DDI for the same diffusion constants necessarily contains a semi-infinite line $D_u = D_v$. More generally, this reflects our observations that the parameter space where a diffusively driven non-oscillatory instability can occur does not require any particular fine-tuning, as illustrated in Fig. 4 where one can see that the eigenvalues of the dispersion relation vary in a fixed direction with changes in parameters.

Whilst we have previously shown that two self-activators can undergo a Turing instability on a slowly growing domain (Madzvamuse et al., 2010), this is subject to parameter fine tuning. In particular an asymptotically slow growth timescale, relative to all other timescales in the model, is assumed in the analysis of Madzvamuse et al. (2010) and more generally a growth timescale multiple orders of magnitude smaller than other timescales in the model is required for the emergence of patterning (Crampin et al., 1999). However, self-activating pairs will not induce a diffusively driven instability for zero growth for the standard model, but the Turing conditions are only linearly and quadratically perturbed by the *small* growth parameter (Madzvamuse et al., 2010). Hence, in the context of a self-activator pair undergoing diffusively driven instability on a growing domain but with no binding to an immobile substrate, the kinetics have to be fine-tuned to ensure that the introduction of the additional, small growth, parameters into the Turing conditions is sufficient to bring the system into the Turing space. For instance, in the example presented in Madzvamuse et al. (2010) one had $f_u = 10^{-3}$, $g_v = 1$ emphasising that the self-activation properties for one of the morphogens *has* to be fine-tuned to be only weakly self-activating. In contrast, in the present paper we show that the presence of an immobile substrate can induce DDI with two self-activators and, because we are not constrained to asymptotic limits within parameter space, we can demonstrate that such DDIs do not require parameter fine tuning.

The possible implication of this work concerns the increased prospect of finding Turing's mechanism in biological systems and the fact that patterning can occur via this mechanism with fewer constraints on the kinetics given the presence of binding to an

immobile substrate such as the extracellular matrix. Due to the enormous literature on morphogen reaction–diffusion patterning, we focus on a specific candidate for a Turing morphogen pair, Nodal and Lefty, which have been extensively studied (Chen and Schier, 2002; Solnica-Krezel, 2003; Hamadai, 2012; Muller et al., 2012) and are considered to interact via cross kinetics (whereby Nodal upregulates both itself and Lefty with the latter, in turn, downregulating Nodal, though the comments below would generalise to pure kinetic morphogen pairs). In particular, it is commonly reported in the biological literature that Nodal and Lefty are required to fulfil the following constraints if they are to exhibit a Turing pattern (e.g. Chen and Schier, 2002; Solnica-Krezel, 2003 who refer to Meinhardt and Gierer, 2000): (i) the activator, Nodal, is a self-activator, (ii) the activator activates the inhibitor, Lefty, (iii) the inhibitor blocks the auto-activation of the activator, (iv) the inhibitor acts at long range to restrict the effective pattern formation. However, such constraints arise from the properties of the two-component reaction–diffusion equations representing the interactions of Nodal and Lefty (e.g. Gierer and Meinhardt, 1972; Murray, 2002) and thus are subject to the standard constraints of the Turing instability. This additionally necessitates that the inhibitor molecule, here Lefty, must be self-inhibitory according to the standard model, equation (RD), as illustrated by Eq. (3).

Thus the above list of constraints, (i)–(iv), for a Turing instability is incomplete. Strictly, there would be a need to confirm that any putative inhibitor such as Lefty must, *independently of activator activity*, downregulate its own production sufficiently near the homogeneous steady state to ensure that one is dealing with a diffusively driven instability, at least for the standard model. This requirement is generally overlooked in discussions of what is required to demonstrate that Nodal and Lefty undergo a diffusively driven instability (e.g. Chen and Schier, 2002; Solnica-Krezel, 2003; Hamadai, 2012) and it could also be anticipated to be very demanding to empirically demonstrate in a highly coupled system of interacting morphogens. However, the results of this paper show that the need for self-inhibition in the standard model in fact need not be necessary given the presence of binding to an immobile substrate such as extracellular matrix. In turn, by relaxing the interaction constraints required for a diffusively driven instability without the need for parameter fine-tuning, these results offer the prospect of reducing the difficulties and the verifications required for an unambiguous demonstration that a diffusively driven instability occurs at the molecular level in biological tissue. In particular, an enhanced binding of Nodal to matrix is speculated from recent studies of Nodal and Lefty transport (Muller et al., 2012). Thus in this context, and contingent on the same speculation of Mueller et al, our results show that the self-inhibition of Lefty need not be a requirement for an unambiguous demonstration at the molecular level that Nodal and Lefty undergo a Turing instability, in contrast to the predictions of the standard model.

Appendix A. A Hopf bifurcation cannot occur for equal diffusion coefficients

Let us consider one real λ_R and a complex conjugate pair $\lambda_C=\mu+i\nu$ $(\nu\neq 0)$ as roots of the dispersion relation. A Hopf bifurcation can occur if $\Re(\lambda_C)=\mu$ can become positive while $\lambda_R<0$.

1. By comparing the dispersion relation with the polynomial $(\lambda-\lambda_R)(\lambda-\lambda_C)(\lambda-\overline{\lambda_C})=0$ where we have employed the knowledge of roots of the dispersion relation, we can show that (from comparison of coefficients of the quadratic terms)

$$\lambda_R=-2\mu-k^2(D_u+D_v)+\mathrm{tr}\,J_3. \tag{A.1}$$

Therefore, if a Hopf bifurcation occurs, $\mu>0$, the real root of the dispersion relation is negative as $\mathrm{tr}J_3<0$ due to the requirement of stability without diffusion.

2. As both λ_C and $\overline{\lambda_C}$ are roots of the dispersion relation, we have

$$P(\mu,\nu,k^2):=\mathrm{dispRel}|_{\lambda=\lambda_C}-\mathrm{dispRel}|_{\lambda=\overline{\lambda_C}}=0 \tag{A.2}$$

which is a quadratic polynomial in μ but containing the unknown imaginary part $\nu\neq 0$ as well. Again, by comparing the dispersion relation with the polynomial $(\lambda-\lambda_R)(\lambda-\lambda_C)(\lambda-\overline{\lambda_C})=0$ we can also obtain $\nu=\nu(\mu)$ and substitute it into the polynomial P retrieving again a cubic polynomial for the unknown μ. Note that $\mu\in\mathbb{R}$ and that Hopf instability occurs when μ changes sign as k^2 changes. Thus Hopf instability requires existence of $k^*>0$ such that the cubic polynomial $P(\mu,\nu(\mu),k^{*2})=0$ has a root for $\mu=0$, i.e. the absolute term of P has to vanish for $k=k^*$. With equal diffusion coefficients, one can show that this absolute term is a polynomial in k^2 with all coefficients being positive and therefore, due to Descartes' rule of signs, there is no $k^*>0$ such that the absolute term would vanish. Thus the Hopf bifurcation cannot occur.

The results of Appendix A were obtained using the computing software *Wolfram Mathematica* (Wolfram Research, Inc., 2010), see the Supplementary Material for further details.

Appendix B. Two self-inhibitors cannot undergo a non-oscillatory diffusively driven instability

Adopting the notation of (4), we have for two self-inhibitors that $f_u,g_v<0$ and hence

$$\mathrm{tr}\,J_2=f_u+g_v<0,\quad \mathrm{tr}\,J_3=f_u+g_v+h_w-h_u<0,\quad D_vf_u+D_ug_v<0, \tag{B.1}$$

noting that $h_u>0>h_w$. Now consider roots of $C(\kappa,p)=0$ i.e. solutions in terms of κ for

$$C(\kappa,p):=|h_w|[\kappa^2D_uD_v-\kappa(D_vf_u+D_ug_v)+(f_ug_v-f_vg_u)]=0.$$

Given $D_vf_u+D_ug_v<0$ and neglecting the possibility of mathematical fine tuning parameters to obtain double roots, which is not relevant in practice, we have two possibilities: (i) $C(\kappa,p)=0$ has one positive real root for κ or (ii) $C(\kappa,p)=0$ has no positive real roots for κ.

Case (i): in conjunction with point (2) of (4.1) above, namely that stability is guaranteed at large κ and therefore $C(\infty,p)>0$, it follows that $C(0,p)<0$. Hence the dispersion relation, Eq. (DR),

$$\lambda^3+A(\kappa,p)\lambda^2+B(\kappa,p)\lambda+C(\kappa,p)=0,$$

has at least one positive real root for λ at $\kappa=0$, excluding the possibility of a diffusively driven instability.

Case (ii): The absence of positive real roots, combined with $C(\infty,p)>0$, implies that $C(\kappa,p)>0$ for $\kappa\geq 0$. Thus

$$(f_ug_v-f_vg_u)>-\kappa^2D_uD_v+\kappa(D_vf_u+D_ug_v)$$

for $\kappa\geq 0$. We also have, noting the above inequality together with $\kappa\geq 0, f_u<0, g_v<0, h_u>0, h_w<0$, the following:

$$A(\kappa,p):=\kappa(D_u+D_v)-\mathrm{tr}\,J_3>0, \tag{B.2}$$

$$\begin{aligned}B(\kappa,p):=&\kappa^2D_uD_v+\kappa(-D_vf_u-D_ug_v+D_vh_u-D_uh_w-D_vh_w)\\&-f_vg_u+f_ug_v-g_vh_u+f_uh_w+g_vh_w,\end{aligned} \tag{B.3}$$

$$>\kappa(D_vh_u+|h_w|(D_u+D_v))-|h_w|(f_u+g_v)-h_ug_v>0. \tag{B.4}$$

Hence we have $A(\kappa,p)$, $B(\kappa,p)$, $C(\kappa,p)>0$ for $\kappa\geq 0$; thus the dispersion relation has no positive real roots by Descartes rule of signs, again excluding a non-oscillatory diffusively driven instability.

Appendix C. Inferring the conditions of a DDI from the asymptotically reduced systems in Section 3

There are many possible limits that give rise to the reduced system (RDBr). There is (i) the asymptotics used by Lengyel and Epstein, with $|h_w|\sim h_u$ asymptotically large, or instead, (ii) taking $|h_w|$ *only* as asymptotically large, which can be deduced by writing the solution for w implicitly as an integral equation and using integral asymptotics (i.e. Laplace's method based on Watson's lemma).

However, the requirements for a DDI are not independent of the choice of this limit. For instance, in case (i) detailed symbolic algebraic calculations show that the Routh–Hurwitz conditions corresponding to the full system (RDB) reduce to the constraints (4*)–(6*) which emerge from an analysis of the reduced system. In contrast, for case (ii) the Routh–Hurwitz conditions corresponding to the full system (RDB) can be written in the form

$$-\mathbf{det}\,\mathbf{J_2}\,\mathbf{h_w}>0 \tag{C.1}$$

$$\mathbf{tr}\,\mathbf{J_2}\,\mathbf{h_w}-g_v h_u+\det J_2>0 \tag{C.2}$$

$$-\mathbf{h_w}+h_u-\mathrm{tr}\,J_2>0 \tag{C.3}$$

$$-\mathbf{tr}\,\mathbf{J_2}\,\mathbf{h_w^2}+(f_u+2g_v)h_u h_w-g_v h_u^2 +(g_v^2+f_v g_u)h_u-(\mathrm{tr}\,J_2)^2 h_w-\det J_2\,\mathrm{tr}\,J_2>0 \tag{C.4}$$

which clearly requires $\mathrm{tr}(J_2)<0$ (for large h_w only the bold terms play a role). However, this condition does not emerge from the conditions for a DDI in the reduced system (unless considering only $|h_w|\to\infty$). Further symbolic algebraic calculations also show conditions (4*)–(6*) hold which, in combination with $\mathrm{tr}\,(J_2)<0$, reduce to the naive Turing model conditions, Eqs. (4)–(6). In contrast, in case (i), with $|h_w|\sim h_u$ asymptotically large, a DDI is associated with different constraints from the naive Turing model and thus one can escape the constraint that the activator must diffuse more slowly, for example in case (i) but not case (ii).

Given the information about the reduction is only implicit in parameter values, one must be cautious about inferring the conditions for a DDI in the full model by inferring conditions from reduced models. Nonetheless, such subtle difficulties do not emerge in the study presented as we do not rely on asymptotic regimes within parameter space; furthermore one cannot deduce the possibility of patterning without self-inhibition from either reduction (i) or (ii) above, necessitating the consideration of the full system in our study without asymptotic approximations.

Appendix D. Supplementary data

Supplementary data associated with this paper can be found in the online version at http://dx.doi.org/10.1016/j.jtbi.2014.11.024.

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