Turing Patterning in Stratified Domains

Andrew L. Krause · Václav Klika · Jacob Halatek · Paul K. Grant · Thomas E. Woolley · Neil Dalchau · Eamonn A. Gaffney

Received: date / Accepted: date

Abstract Reaction-diffusion processes across layered media arise in several scientific domains such as pattern-forming E. coli on agar substrates, epidermal-mesenchymal coupling in development, and symmetry-breaking in cell polarisation. We develop a modelling framework for bi-layer reaction-diffusion systems and relate it to a range of existing models. We derive conditions for diffusion-driven instability of a spatially homogeneous equilibrium analogous to the classical conditions for a Turing instability in the simplest nontrivial setting where one domain has a standard reaction-diffusion system, and the other permits only diffusion. Due to the transverse coupling between these two regions, standard techniques for computing eigenfunctions of the Laplacian cannot be applied, and so we propose an alternative method to compute the dispersion relation directly. We compare instability conditions with full numerical simulations to demonstrate impacts of the geometry and coupling parameters on patterning, and explore various experimentally-relevant asymptotic regimes. In the regime where the first domain is suitably thin, we recover a simple modulation of the standard Turing conditions, and find that often the broad impact of the diffusion-only domain is to reduce the ability of the system to form patterns. We also demonstrate complex impacts of this coupling on pattern formation. For instance, we exhibit non-monotonicity of pattern-forming instabilities with respect to geometric and coupling parameters, and highlight an instability from a nontrivial interaction between kinetics in one domain and diffusion in the other. These results are valuable for informing design choices in applications such as synthetic engineering of Turing patterns, but also for understanding the role of stratified media in modulating pattern-forming processes in developmental biology and beyond.

Keywords Turing instabilities · stratified media · pattern formation · synthetic biology

1 Introduction

Since Turing’s initial insights into reaction-diffusion driven morphogenesis [78], a substantial research effort has elucidated various mathematical and biophysical aspects of such symmetry-breaking instabilities leading from homogeneity to patterned states [23, 19, 60, 49, 35, 84]. An important and well-studied aspect of these instabilities is the underlying geometry, which can influence both the stability of a homogeneous state, as well as the subsequent mode selection of emergent patterns [63]. However, one less well-studied
aspect of geometry is the coupling between layered spatial domains, which can arise in a variety of settings and is the primary object of interest in this paper.

Reaction diffusion processes arise in a diversity of layered settings, from bulk-surface membrane-cytosol interactions [38] [72] [54] [75] [21] to epithelial-mesenchymal couplings in developing skin [20] [24]. Additionally, many experiments involving bacterial pattern-formation are performed using colonies on the surface of a substrate, such as agar [9] [19]. Such systems either use natural chemotaxis mechanisms to initiate spatial pattern formation of the bacterial density itself [79], or instead use synthetic bacteria re-engineered to express additional quorum-sensing pathways that spatially coordinate patterns in gene expression [4] [76] [34]. Other examples are synthetically reconstituted protein interaction systems with bulk-membrane coupling such as the Min system [57] [54] [27], where molecular interactions [24] [31], or in vitro system geometries [85] [3] [40], are modified to stimulate changes in the observed protein patterns. Examples of particular contemporary interest include the use of bacterial colonies as exemplars of synthetic multicellular communication and self-organisation [3] [22] [69] [34] [44], for example using modified E. coli with engineered quorum-sensing signalling on the surface of an agar plate [34] [69] [12] [11]. Some of these systems take advantage of the geometry of colony growth and nutrient diffusion to influence pattern formation [69] [12] [11] while in other systems the bacteria are confined [34] [7], but the signalling molecules can diffuse into the inert agar layer below the chemically active colonies. The impact of this leaching on the prospects of a Turing instability in experimentally-relevant geometries has not been fully characterised and is a key motivation for our study.

Our first objective is to develop a two-domain model of reaction-diffusion processes coupled in a stratified bi-layer and to determine conditions for the Turing instability, on the assumption that the upper region is sufficiently substantive in the transverse direction to merit continuum modelling. Such a model is also applicable to a variety of other settings beyond multi-layered bacterial pattern formation, such as developing skin. Our second objective is to focus on the Turing instability for multi-layered bacterial systems, where signalling molecules only diffuse in the lower (agar) layer and especially where the upper layer is asymptotically thin relative to the scale of the pattern and the depth of the lower layer. The main biological motivation is to determine to what extent the diffusive bulk helps, or hinders, the ability of an engineered system to exhibit Turing-type patterning.

In terms of model development, domain-coupled reaction-diffusion systems broadly fall into three major types: instantaneously coupled, bulk-surface models and bulk-bulk models. The first type are models where the components occupy the same physical space (or the reactions occur in thin regions where a homogenisation approximation is sensible) [85] [26] [87] [28]. Such models are essentially just larger reaction-diffusion systems with linear coupling between subsystems, and amenable to block-matrix analysis in the study of Turing instabilities [13], but do not capture the spatial separation of the domains. When applied to layered media, these models effectively assume vertical transport between distinct layers (such as that of Figure 1) is instantaneous. However, considering physical scales representative of synthetic pattern formation experiments using E. coli [34] [7], and summarised below in Table 1, one has an agar block with a depth of a few millimetres, say three, and a diffusion rate on the scale of 4 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}. Thus the timescale for vertical transport is in the region of 375 minutes, which is short compared to the timescales on which experimental measurements of the equilibrated system are recorded (1500-3000 minutes, [34] [7]) but far from instantaneous. Hence, such models are inappropriate for the motivating examples here.

A second class of model considers bulk-surface coupling, where one component is confined to the boundary of the main bulk domain, and reactants flow between the two regions, such as in the case of proteins diffusing in the cytoplasm and binding on the cell membrane [72] [58] [75] [21] [68] [58] [27]. There is substantial recent interest in such models, from very theoretical results on existence and fast-reaction limiting behaviour [21] [141], to spike dynamics [33] and a myriad of applications to understanding cell polarity [77] [54] [38] [30]. One particularly well studied example is the pole-to-pole Min protein oscillation in E. coli, which has the biological function of guiding the cell division machinery to midcell [54]. Such intracellular protein patterning systems have been studied experimentally and theoretically in a wide range of system geometries, such as spherical [47] [56], elliptical [89] [85] [30], and planar membrane geometries [40]. A striking feature of these examples is that the geometry itself has a major impact on pattern formation and pattern selection, which has been confirmed experimentally [85] [8]. More generally, the Turing instability has also been studied in the context of membrane-cytosol models [72] [58]. Overall, linear stability analysis (as used by Turing) is highly applicable to such membrane-cytosol systems because the nonlinear interac-
tions are typically restricted to the lower-dimensional membrane surface. The dynamics in the extended bulk are typically linear such that a general solution (or a good approximation) can be obtained analytically and used to satisfy the linearised reactive boundary condition. This is justified because the membrane can be considered as a surface with no transverse extent, and so transverse gradients only play a role in the cytosolic layer close to the membrane surface. However, in multi-layered cellular systems the transverse length scales are at least that of many cells and hence transverse gradients cannot be neglected a priori, and thus should be accommodated in the modelling. Models accounting for this represent the final class, with two separated spatial domains with an interface and suitable coupling boundary conditions. From the perspective of pattern formation, this kind of model has only been subject to recent numerical exploration \[83\], though it is used in the derivation of the second class – bulk-surface models – given appropriate distinguished limits and scaling assumptions (for example, \[15\] \[29\]).

Hence, we will develop models of the latter type, with an exploration of the conditions for the Turing instability, and their detailed study in the context of a stratified model with an inert underlying agar layer. Turing instabilities of reaction-diffusion systems have been studied on a variety of complex spatial domains such as compact manifolds \[81\] \[14\], networks \[2\] \[43\] \[64\], and many of the aforementioned complex system geometries \[39\] \[47\] \[49\]. The primary difficulty in such cases, compared to the textbook example of a continuous line, is determining the corresponding set of eigenfunctions and eigenvalues of the spatial transport operators, which for some system geometries do not need to coincide between domains (e.g. in the surface-bulk elliptical case \[39\]). In such cases approximate solutions for the system’s eigenfunctions need to be derived that are orthogonal in the patterning layer. Examples that deviate even further from the classical case are growing domains \[18\] \[70\] \[52\] \[73\] and spatially heterogeneous reaction-diffusion processes \[5\] \[66\] \[67\] \[37\] \[48\], for which the canonical approach does not work. In such cases, novel approaches to pattern-forming instabilities have recently been developed for growth \[59\] \[80\] and heterogeneity \[53\] under certain simplifications, but such analyses are quite different to the classical case. In a similar direction, as part of our objective in exploring the Turing instability for layered reaction-diffusion systems, we will aim to demonstrate a much richer diversity of structure in the resulting dispersion relations (and hence instability conditions), compared to classical counterparts.

As an outline, in Section 2 we present a two-domain layered model, where each domain consists of closed two-dimensional rectangular regions, coupled through a single shared boundary (See Figure 1) and briefly discuss how it can be reduced to a variety of other models. We focus on a special case of a two-domain model where we assume linear coupling and no reactions in the second (bulk) region, but note that our analysis can be applied with relatively simple modifications to more general cases. In Section 3 we develop an approach to linear stability analysis of homogeneous states. In Section 4 we derive a variety of asymptotic results regarding our dispersion relation, especially considering limits that are of particular relevance for synthetic pattern formation in E. coli colonies. We further explore these results and other parameter regimes numerically in Section 5. Finally we discuss our results in Section 6.

2 Two-Region Model

We consider a layered two-domain model where each domain is governed by a different reaction-diffusion system. We consider several interacting species in these two domains, which we write as \( \Omega = \Omega_S \cup \Omega_B \) where we refer to \( \Omega_B = [0, L] \times [0, H] \) as the bulk region, and \( \Omega_S = [0, L] \times [H, H + H_e] \) as the surface region (see Figure 1). We write \( \hat{u}_B \in \mathbb{R}^n \) for the concentrations of reactants in the bulk, and \( \hat{u}_S \in \mathbb{R}^n \) for the concentrations of reactants in the surface region. For simplicity, we consider a simple one-dimensional lateral geometry (orthogonal to the direction of the coupling condition), but note that the geometric details in the lateral direction(s) can be easily extended to much more complicated geometries, as long as eigenfunctions of the Laplacian in these directions are separable from the transverse coordinate \( y \). We only consider reactions on the surface layer and assume the bulk only permits diffusion.

We have the following equations for the species concentrations in the bulk and surface regions:

\[
\frac{\partial \hat{u}_S}{\partial t} = D_S \nabla^2 \hat{u}_S + \hat{f}_S(\hat{u}_S), \quad \hat{x} \in [0, L], \quad \hat{y} \in [H, H + H_e], \tag{1}
\]

\[
\frac{\partial \hat{u}_B}{\partial t} = D_B \nabla^2 \hat{u}_B, \quad \hat{x} \in [0, L], \quad \hat{y} \in [0, H], \tag{2}
\]
where $\hat{D}_S$, $\hat{D}_B$ are positive definite diagonal matrices. We further specify Neumann (no-flux) boundary conditions on the outer boundaries as,

$$\frac{\partial \hat{u}_S}{\partial \hat{x}} = \frac{\partial \hat{u}_B}{\partial \hat{x}} = 0, \text{ for } \hat{x} = 0, L,$$

(3)

and lastly coupling conditions on the interior boundary which conserve fluxes and take the form,

$$\hat{D}_S \frac{\partial \hat{u}_S}{\partial \hat{y}} = \hat{\eta} \hat{g}(\hat{u}_S, \hat{u}_B), \quad \hat{D}_B \frac{\partial \hat{u}_B}{\partial \hat{y}} = \hat{\eta} \hat{g}(\hat{u}_S, \hat{u}_B), \text{ for } \hat{y} = H,$$

(5)

where $\hat{g}$ is a given function determining the transport between the surface and the bulk region, and $\hat{\eta}$ is a rate of transport across the boundary. Essentially, all of the forthcoming analysis can be carried out with a general $\hat{g}$, as linearisation will also linearise this function. For brevity and concreteness, we will henceforth assume a linear transport law, so that we have

$$\hat{g} = \hat{u}_S - \hat{u}_B.$$  

(6)

We non-dimensionalise the above model via concentration, time and length scales corresponding to the reaction kinetics and a unit lengthscale $\hat{L}$, respectively. Specifically, we define $\hat{u}_S = U u_S$, $\hat{u}_B = U u_B$, where $U$ is a diagonal matrix of concentration scalings. Equally, we set $\hat{t} = \tau t$, where $\tau$ is the timescale of the fastest reaction in the surface and bulk, and $\hat{x} = \hat{L} x$. The dimensional scalings are then chosen such that

$$\hat{f}_S(\hat{u}_S) = (1/\tau) U f_S(u_S), \quad \hat{g}(\hat{u}_S, \hat{u}_B) = U g(u_S, u_B).$$

(7)

We define new dimensionless groupings $h = H/\hat{L}$, $\epsilon = h/\hat{L}$, $D_S = \tau \hat{D}_S/(\hat{L}^2)$, $D_B = \tau \hat{D}_B/(\hat{L}^2)$, $\hat{L} = L/\hat{L}$ and $\eta = \tau \hat{\eta}/\hat{L}$. The nondimensional system is written as

$$\frac{\partial u_S}{\partial \hat{t}} = D_S \nabla^2 u_S + f_S(u_S), \quad x \in [0, \hat{L}], \quad y \in [0, h + \epsilon],$$

(8)

$$\frac{\partial u_B}{\partial \hat{t}} = D_B \nabla^2 u_B, \quad x \in [0, \hat{L}], \quad y \in [0, h],$$

(9)
pattern formation with E. coli

There are several distinguished limits of the nondimensional system \([3]-[12]\) that reduce the model to different cases already present in the literature. In the limit \(\varepsilon \to 0\), one can consider either scaling \(\eta \sim O(\varepsilon)\) or scaling \(f_S \sim O(\varepsilon^{-1})\) in order to reduce the system to a bulk-surface model, which is well-studied in the literature (though primarily in radial geometries) \([56, 72, 71, 58, 21, 33, 68]\). The second scaling, indicating that the surface timescale is rapid, can be related to assumptions regarding rapid surface reactions used to justify reactive boundary conditions from the microscopic viewpoint \([15]\). Finally another limit is the case of infinite permeability, \(\eta \to \infty\), wherein the concentrations and fluxes are continuous across the interface. In this case, the system can be seen as a single domain model with a step function heterogeneity, which has been studied extensively as an example of spatially heterogeneous reaction-diffusion systems \([5, 66, 51]\). Nonetheless, pattern formation in the system above, as well as several other distinguished limits, has not been analysed yet in the literature.

In Table 1, we give the dimensional parameter scales to be considered in our framework, taken from the key motivating example of synthetic patterning in E. coli bacterial colonies on an agar substrate \([34, 7]\). While such experiments can be conducted with a variety of settings, an overall restriction on the variation of these parameters is motivated by the range of the physical scales in these studies. Here, bacteria are plated in squares of about 1 mm (Methods, \([7]\)) with patterning cells considered in an \(8 \times 8\) grid in one study (Supplementary Information, \([34]\)) and more generally the patterning fields are observed across about 22 such squares (Fig 5B, \([7]\) and Fig 3E \([34]\)). Thus we consider a range of \(L \sim 8 - 22\) mm. For the diffusion matrices, the infinity (max) norm \(\| \cdot \|_\infty\) is presented, i.e. the maximum value of the matrix’s components. From Grant et al., (Supplementary Material, Tables S8, S9, \([34]\)) diffusion coefficients have been estimated in the range \(\|\hat{D}_S\|_\infty \sim 10^{-10} \text{ m}^2\text{s}^{-1} - 10^{-9} \text{ m}^2\text{s}^{-1}\) by model fitting to exemplar results. As this is also the scale of diffusion (or slightly more than the scale) for the signalling molecule EGF in water \([65]\), the same scale is used for \(\|\hat{D}_B\|_\infty\). Similarly, in the parameter fitting by Grant et al., a reaction timescale on the scale of the faster reactions is such that \(1/\tau \sim 8.4 \times 10^{-3} \text{ s}^{-1} - 10^{-3} \text{ s}^{-1}\), with the range arising from the use of different model kinetics in parameter fitting. We further assume 10-50 layers of bacteria, with an \(E. coli\) bacterium size scale of about \(10^{-6} \text{ m} - 2 \times 10^{-6} \text{ m}\) \([55]\), and hence a surface depth on the scale of \(H_c \sim 10^{-5} \text{ m} - 10^{-4} \text{ m}\). Finally, the depth of the bulk is highly variable and easily changed upwards from the millimetre scale and so \(H\) is taken with the range of \(1 - 10\) mm; estimates for the interfacial permeability, \(\hat{\eta}\), are currently unavailable. These dimensional parameter estimates generate the non-dimensional scales of Table 2, which will guide the asymptotic and numerical investigations presented below.

| Parameter | Range | Justification |
|-----------|-------|---------------|
| \(L\) | \(8 \times 10^{-3} \text{ m} - 2.2 \times 10^{-2} \text{ m}\) | See text |
| \(\|\hat{D}_S\|_\infty, \|\hat{D}_B\|_\infty\) | \(10^{-10} \text{ m}^2\text{s}^{-1} - 10^{-9} \text{ m}^2\text{s}^{-1}\) | Table S8, \([34]\) |
| \(1/\tau\) | \(8.4 \times 10^{-3} \text{ s}^{-1} - 1.0 \times 10^{-3} \text{ s}^{-1}\) | Tables S8, S9, \([34]\) |
| \(H_c\) | \(10^{-5} \text{ m} - 10^{-4} \text{ m}\) | See text |
| \(H\) | \(10^{-2} \text{ m} - 10^{-2} \text{ m}\) | See text |
| \(\hat{\eta}\) | Unknown | – |

Table 1 Numerical scales of various dimensional parameters and parameter groupings in SI units, based on patterning in synthetic pattern formation with \(E. coli\) bacterial colonies, using physical scales motivated by the studies of Grant et al. \([34]\) and Boehm et al. \([7]\).
3.1 Spatially homogeneous perturbations

We now consider the appropriate generalisation of stability in the absence of transport, as is typically assumed in a Turing-type analysis. However, unless the reaction kinetics are the same in both domains, which is not true in our setting, then spatially homogeneous perturbations are not consistent with equations (13)-(15). Such perturbations will not remain homogeneous under time evolution due to the coupling condition.

| Parameter | Typical Value/Range |
|-----------|---------------------|
| $\epsilon = H_s/L_s$ | $4.5 \times 10^{-4} - 1.3 \times 10^{-2}$ |
| $h = H_s/L_s$ | $0.045 - 1.3$ |
| $L_s = L_s/L_s$ | 1 |
| $\epsilon_s = \epsilon|D_s^{-1}f_s|^{1/2}$ | $1.1 \times 10^{-3} - 0.87$ |
| $h_s = h|D_s^{-1}f_s|^{1/2}$ | $0.11 - 87$ |
| $\epsilon^2 / 3$ | $4.0 \times 10^{-7} - 0.3$ |
| $\|D_s\|_\infty = \tau|D_s|_\infty / L^2$ | $2 \times 10^{-4} - 0.2$ |
| $\|D_s\|_\infty \sim |D_s|_\infty$ | $2 \times 10^{-4} - 0.2$ |
| $\eta = \tau h / L$ | Unknown |

Table 2 Numerical scales of various non-dimensional parameters and parameter groupings, motivated by the physical scales of synthetic pattern formation with E. coli bacterial colonies, in the studies of Grant et al. [34] and Boehm et al. [7]. For matrices, the infinity (max) norm $\| \cdot \|_\infty$ is used, which is the modulus of the matrix component with largest magnitude. For the non-dimensional matrix Jacobian, this norm is taken to be of order unity as the timescale is non-dimensionalised relative to $\tau$, a representative timescale associated with a fast reaction in the system. The non-dimensional lengthscale, $L$, is retained symbolically throughout the presentation to facilitate determining the impact of this scale, though it is unity for these scalings. The parameter scales $\epsilon$ and $h_s$ as well as the range of $\epsilon^2 / 3$ are presented as they will be important in the asymptotic analyses below.

**3 Linear Stability Analysis**

For a linear stability analysis of homogeneous equilibria of (8)-(12), we require the steady states to this system, which arise from specifying

$$f_S(u_S^s) = g(u_S^s, u_B^s) = u_S^s - u_B^s = 0,$$

so that the surface reactions determine the spatially-homogeneous steady state concentration in both regions, and our simple constitutive choice of $g$ implies that these concentrations must be equal. We will focus exclusively on the case of an absence of reactions in the bulk, as motivated by the underlying inert agar layer in synthetic pattern formation with E. coli bacterial experiments [34, 7] and which requires only a root of the surface kinetics for there to be a steady state.

We proceed by considering perturbations to this steady state of the form

$$u_S = u_S^s + \sigma w_S(x, y, t), \quad u_B = u_B^s + \sigma w_B(x, y, t),$$

where $|\sigma| \ll 1$, and in general the bulk and surface perturbations are $n$-dimensional functions, where $n$ is the number of species. We substitute these perturbations into equations (8)-(12) to find, from equations (8)-(12), that the perturbations will satisfy

$$\frac{\partial w_S}{\partial t} = D_S \nabla^2 w_S + J_S w_S, \quad x \in [0, L], \quad y \in [h, h + \epsilon],$$

$$\frac{\partial w_B}{\partial t} = D_B \nabla^2 w_B, \quad x \in [0, L], \quad y \in [0, h],$$

where the Jacobian, $J_S = \partial f_S / \partial u_S \in \mathbb{R}^{n \times n}$, is evaluated at the steady state concentrations. We also have the coupling condition from equation (12) given by,

$$D_S \frac{\partial w_S}{\partial y} = \eta (w_S - w_B), \quad -D_B \frac{\partial w_B}{\partial y} = \eta (w_B - w_S), \text{ for } y = h.$$
except in the mathematically fine-tuned case where the homogeneous surface perturbation is along an eigenvector of $J_S$ with eigenvalue 0.

Previous studies of more complex systems (beyond those considered in textbook Turing models) also highlight that, when generalising the conditions that arise from the stability of the homogeneous steady state with respect to spatially homogeneous perturbations, one must also consider a perturbation with respect to the zero mode(s) of the transport operator \[16\]. However, given the assumption that separable solutions in $x, y$ and $t$ span the space of possible solutions, as generally used in linear stability theory, the existence of zero modes of the transport operator also requires mathematical fine tuning. In particular, with $\nabla^2 u^0_S = 0$ for the zero mode of the transport operator acting in the surface layer, $u^0_S$, one has
\[
u^0_S = A \cos(k_q x) \cosh(k_q(y - (h + \epsilon))), \quad k_q = q\pi/L,
\]
for a general $A$ and $q$ a natural number on enforcing the zero flux boundary conditions. There is a directly analogous expression for the zero mode of the transport operator within the bulk region. However, after rearrangement, the interfacial condition at $y = h$ requires
\[
D_S D_B \sinh(k_q \epsilon) \sinh(k_q h) = \eta \left(D_B \cosh(k_q \epsilon) \sinh(k_q h) - D_S \sinh(k_q \epsilon) \cosh(k_q h)\right).
\]

One possible solution occurs for $k_q = 0$, which generates a spatially homogeneous mode that has already been considered above. Satisfying this equation for other $k_q$ requires mathematical fine tuning as $k_q$ is already constrained to a set of zero measure and all other parameters are either geometrical or biophysical in origin.

Hence to summarise, in contrast to the textbook Turing case, unless the surface and bulk kinetics are the same, constraints on the parameters do not arise from the constraint of stability to homogeneous perturbations; instead this stability always holds, at least in the absence of a mathematical fine tuning of parameters and the possibility of such fine-tuning is neglected below.

### 3.2 Spatially inhomogeneous perturbations

To proceed, we assume a separable solution in $x, y$ and $t$ for the linearised system \[13\]–\[15\]. With the usual assumption of a uniform (across all components) temporal growth rate $\lambda$, the perturbation ansatz for a separable solution is
\[
w_S = \sum_{\lambda \in \mathbb{I}_\lambda} \sum_{q=0}^{\infty} e^{\lambda t} s(y) \cos(k_q x), \quad w_B = \sum_{\lambda \in \mathbb{I}_\lambda} \sum_{q=0}^{\infty} e^{\lambda t} b(y) \cos(k_q x),
\]
where $k_q = q\pi/L$ for $q$ a natural number (including 0) and we do not know \textit{a priori} what the set of growth rates, $\mathbb{I}_\lambda$, is. We note that for each $q$, there may be many distinct $\lambda$, and corresponding to each distinct pair of $(q, \lambda)$ we will have possibly different eigenfunctions $s$, and $b$. We will suppress this dependence in the following, but it is important to keep in mind that the following analysis applies for a given pair $(q, \lambda)$.

As we are looking for modes which grow in time, leading to instability, we will impose $\Re(\lambda) > 0$ in the following. Substituting these expansions into \[13\]–\[14\], we find that a given mode satisfies,
\[
\lambda s = D_S (-k^2_q + \partial_y^2) s + J_S s, \quad y \in [h, h + \epsilon],
\]
\[
\lambda b = D_B (-k^2_q + \partial_y^2) b, \quad y \in [0, h].
\]

After multiplying these equations by the inverse of the diffusion matrices and rearranging, we find
\[
s'' = (k^2_q I_n + D_S^{-1} (\lambda I_n - J_S)) s, \quad y \in [h, h + \epsilon],
\]
\[
b'' = (k^2_q I_n + \lambda D_B^{-1}) b, \quad y \in [0, h],
\]
where $'$ denotes the ordinary derivative with respect to $y$. These spatial functions are required to satisfy the external boundary conditions $b'(0) = s'(h + \epsilon) = 0$ and the coupling conditions which read,
\[
D_S s' = \eta(s_q - b_q), \quad D_B b_q = \eta(s_q - b_q), \text{ for } y = h.
\]
To find suitable \((\lambda, q)\) that solve the coupled problem \([19]-[21]\), we will make use of the matrix-valued function defined by \(\cosh(M) = (\exp(M) + \exp(-M))/2\), for some matrix \(M\), as well as \(\sinh(M) = (\exp(M) - \exp(-M))/2\). We recall the differentiation identity \(\cosh(\mu M)' = M \sinh(\mu M)\), which follows from this definition. We now seek to take the square-root of the matrices on the right-hand side of equations \([19]\) and \([20]\) and thus define
\[
M^2_\alpha = (k^2 h M + D^{-1}_S (\lambda I_n - J_S)), \quad M^2_B = (k^2 h M + \lambda D^{-1}_B).
\]

We next consider solutions to equations \([19]\) and \([20]\) via hyperbolic matrix functions. As we will observe (e.g. equation \([26]\) and the resulting dispersion relation), these matrices will always be in terms of functions that can be expressed in terms of even powers of \(M_B\) and \(M_S\), and thus functions of \(M^2_B\) and \(M^2_S\). This dependence on the squares of these matrices follows as if \(f: \mathbb{C} \rightarrow \mathbb{C}\) is analytic, then a matrix-valued function can be defined via a power series in the matrix argument \([42]\).

The hyperbolic functions we will use are meromorphic with poles away from 0, and hence the ambiguity in defining the square root matrices, \(M_S\) and \(M_B\), does not play a role. Without loss of generality, we will consider the principal square roots of the matrices for definiteness, so that eigenvalues of \(M_B\) and \(M_S\) are the square roots with positive (or possibly zero) real parts of the eigenvalues of \(M^2_B\) and \(M^2_S\).

Proceeding, we then have the following solutions to equations \([19]\) and \([20]\) given by the hyperbolic matrix functions:
\[
s = \cosh((y - h - \epsilon)M_S)a, \quad b = \cosh(yM_B)\beta,
\]
for some nonzero constant vectors \(a, \beta\). We note these functions satisfy the no-flux conditions at the top and bottom boundaries by construction. We now use the coupling conditions \([15]\) to determine a condition for non-trivial \(a\) and \(\beta\). These read,
\[
-D_SM_S \sinh(\epsilon M_S)a = \eta (\cosh(\epsilon M_S)a - \cosh(hM_B)\beta),
\]
\[
D_B M_B \sinh(hM_B)\beta = \eta (\cosh(\epsilon M_S)a - \cosh(hM_B)\beta).
\]

We then have, writing equations \([24]\) and \([25]\) as a \(2n \times 2n\) block matrix, the following condition for non-trivial solutions to this system:
\[
\det \begin{pmatrix}
\eta \cosh(\epsilon M_S) + D_SM_S \sinh(\epsilon M_S) & -\eta \cosh(hM_B) \\
-\eta \cosh(hM_B) & -\eta \cosh(hM_B) - D_B M_B \sinh(hM_B)
\end{pmatrix} = 0.
\]

As this condition involves transcendental functions of \(\lambda\), we note that in general for a fixed spatial mode \(q\), there will be infinitely many values of \(\lambda\) for which equation \([26]\) is satisfied. Equivalently, \(q\) only differentiates between eigenmodes in the \(x\) direction, but cannot do so in \(y\), and so these eigenmodes must be captured via multiplicity in \(\lambda\).

While the condition given by equation \([26]\) is in principle computable, it is difficult to use to gain insight into Turing-like instabilities. Even simplifying the determinant condition is non-trivial, as the four blocks will not in general commute, so we now exploit the assumption of no reactions in the bulk to simplify this condition. We have that \(M^2_B\) is diagonal, and from our assumption that \(\Re(\lambda) > 0\), we have that its eigenvalues have positive real part. Therefore, the elements of \(\cosh(M_B)\) are given by the hyperbolic cosine of the diagonal elements of \(M_B\), and since these are all positive definite, \(\cosh(hM_B)\) is invertible.

Now we define the matrices \(A = \eta \cosh(\epsilon M_S) + D_SM_S \sinh(\epsilon M_S), B = -\eta \cosh(hM_B), C = \eta \cosh(\epsilon M_S), D = -\eta \cosh(hM_B) - D_B M_B \sinh(hM_B)\). By the above argument, we have that \(B\) is invertible. We then have that \([26]\) can be written (by exchanging rows and using the Schur complement) as,
\[
\det \begin{pmatrix}
A & B \\
C & D
\end{pmatrix} = (-1)^n \det(B) \det(C - DB^{-1}A) = 0.
\]

Noting that \(DB^{-1} = I_n + D_B M_B \tanh(hM_B)/\eta\), we have that
\[
C - DB^{-1}A = \eta \cosh(\epsilon M_S) - \left(I_n + \frac{1}{\eta} D_B M_B \tanh(hM_B)\right) \left(\eta \cosh(\epsilon M_S) + D_SM_S \sinh(\epsilon M_S)\right)
\]
\[
= - D_B M_B \tanh(hM_B) \left(\cosh(\epsilon M_S) + \frac{1}{\eta} D_SM_S \sinh(\epsilon M_S)\right) - D_SM_S \sinh(\epsilon M_S),
\]
so equation (27) is equivalent to,

\[
\det \left( D_B M_B \tanh(h M_B) \left( \cosh(\epsilon M_S) + \frac{1}{\eta} D_S M_S \sinh(\epsilon M_S) \right) + D_S M_S \sinh(\epsilon M_S) \right) = 0.
\] (28)

We note that the Turing instability conditions for the surface system in isolation – neglecting spatial structure in \( y \) – are precisely that the growth rates \( \lambda \) computed from \( \det(M_S) = 0 \) have negative real part for \( k_0 = 0 \), and positive real part for some \( k_0 > 0 \), and so this matrix encodes directly the classical case in this way. Furthermore, for a fixed \( \eta \), and with fixed model parameters, we expect that condition (28) admits infinitely many distinct values of \( \lambda \). The intuition for this is that in the uncoupled case (\( \eta = 0 \)), the surface domain is a rectangle and, hence, the surface eigenfunctions \( s(y) \) are also cosines of different spatial eigenvalues, which can vary independently from \( k_0 \). However, we know of no method to compute analytical expressions for such spatial eigenvalues in the coupled case, and so instead use condition (28) to compute \( \lambda \) directly, remaining aware of the inherent multiplicity. To further understand the dispersion relation given by (28), and how it relates to classical conditions for Turing instabilities, we now pursue several asymptotic reductions.

4 Instability Conditions in Thin Surface Regimes

In this section we compute instability conditions from equation (28) for a variety of distinguished limits modelling a thin surface region, as motivated by synthetic patterning in bacterial populations. First, we mention even simpler reductions of the system, as a consistency check of our dispersion relation. We show that patterning is equivalent in the limit of decoupling the interaction of the surface and bulk regions, that is for sufficiently small \( \eta \ll 1 \). This is pursued in Appendix A where the classical Turing conditions are recovered as the surface system becomes isolated, as required. In addition, in Appendix A we also demonstrate that no patterning can occur for classical Turing kinetics once all diffusion coefficients are equal in each of the regions, a direct analogue of the well known result that the classical Turing instability requires differential transport.

Noting that the full system is too rich to investigate in generality and that the non-dimensional surface depth parameters, \( \varepsilon \) and \( \epsilon_* \), are small in Table 2 for the motivating example of synthetic pattern formation in \( E. coli \) colonies, we proceed below to studying pattern formation instabilities with thin surface asymptotics. In the experimental setting of Grant et al. [34], the bacterial layer is always relatively thin, owing to transport constraints in the bacteria, though the agar layer can take different bulk heights. For this reason, after first introducing a thin surface limit of the dispersion relation (28) in Section 4.1 we consider subsequent limits of large or small bulk thickness, \( h \), in Section 4.2. We anticipate that the permeability of the interface, \( \eta \), is large in these experiments but do not have quantitative estimates, and so also consider our asymptotics across varying values of this parameter. In Section 4.3 we derive asymptotic results under a regular asymptotic assumption on \( k \) and \( \lambda \) (i.e. that they remain comparable with non-asymptotic terms in the dispersion relation), and collect these results in Table 3. Finally in Section 4.4 we give an example of distinguished limits where this asymptotic assumption breaks down. Throughout the following, we implicitly assume that the surface Jacobian, \( J_S \), has elements that are of the same order and thus of the order of \( \|J_S\|_\infty \), so that \( \|J_S A\|_\infty \) is of the same scale as \( \|J_S\|_\infty \|A\|_\infty \) for any matrix \( A \) considered.

4.1 Thin Surface Limits (\( \varepsilon_*^2 / 3 \ll 1 \))

Here, we consider an asymptotically thin-surface, requiring \( \varepsilon \|M_S\|_\infty = H_\varepsilon \|M_S\|_\infty / \bar{L} \ll 1 \). First note that in the thin layer limit below, the surface Jacobian \( J_S \) only appears via

\[
D_S M_S^2 = \frac{k^2 Q}{\eta} D_S + \lambda I_n - J_S. \tag{29}
\]

In addition, given patterning (i.e. \( \Re(\lambda) > 0 \)), the matrix \( J_S \) cannot be dominated by the terms \( \lambda I_n \) or \( \frac{k^2 Q}{\eta} D_S \) within \( D_S M_S^2 \), since then the reaction kinetics are subleading in the requirements for patterning, which thus
contain only terms associated with pure diffusion at leading order. However, pure diffusion cannot induce patterning, as demonstrated in Appendix B. Thus we conclude that, given patterning

\[
\max(\|\lambda_k\|, k^2_{\beta} \|D_S\|_\infty) \sim O(\|J_S\|_\infty),
\]

(30)

and also that \(\|M_S\|_\infty\) has an upper bound (and in particular the \(k^2_{\beta}\) term is in fact bounded). Noting the boundedness of \(M_S\) we have that \(\cosh(\varepsilon M_S)\) is invertible, as for \(\varepsilon\) sufficiently small this matrix has a determinant which is asymptotically \(1 + \varepsilon^2 \text{trace}(M_S^2)/2 > 0\). In addition, for sufficiently small \(\varepsilon\), we have the Taylor expansion

\[
\tanh(\varepsilon M_S) = \varepsilon M_S \left(1 + O\left(\varepsilon^2 \|M_S\|_\infty^2 / 3\right)\right),
\]

(31)

where \(O(\varepsilon^2 \|M_S\|_\infty^2 / 3)\) means the same scale as \(\varepsilon^2 \|M_S\|_\infty^2 / 3, \text{ or smaller}\), as the surface thickness tends to zero. Thus by right multiplying \(\mathbf{25}\) by \(\cosh(\varepsilon M_S)^{-1}\) and Taylor expanding we obtain (to leading order) the relation

\[
\det\left(D_B M_B \tanh(h M_B) + \frac{1}{\eta} D_B M_B \tanh(h M_B) + I_n \right) D_S M_S^2 = 0,
\]

(32)

providing \(\varepsilon^2 \|M_S\|_\infty^2 / 3 \ll 1\) (ensuring the invertibility of \(\cosh(\varepsilon M_S)\) and the validity of the Taylor expansion above). Furthermore, noting that \(\varepsilon_s = \varepsilon \|D_S^{-1}\|_{\infty}^2\|J_S\|_{\infty}\) together with the relations \(\mathbf{30}\), which give the maximum scale of \(k_q\) and show that \(|\lambda||D_S^{-1}\| \sim O(\|D_S^{-1}\|_{\infty}||J_S||_{\infty}) \sim O(\|D_S^{-1}\|_{\infty}||J_S||_{\infty})\), we have

\[
\varepsilon^2 \|M_S\|_\infty^2 \sim \max\left(\varepsilon^2 k^2_q, \varepsilon^2 \|D_S^{-1}\|_\infty \varepsilon^2 \|D_S^{-1}\|_\infty \|J_S\|_\infty \right) \sim \varepsilon^2 \|D_S^{-1}\|_\infty = \varepsilon_s^2, \quad (33)
\]

using \(\|D_S^{-1}\|_{\infty} \geq 1/\|D_S\|_{\infty}\). The latter inequality is immediate in the two species case on writing \(D_S = \text{diag}(a,a\xi)\) with \(\xi \leq 1\), as then \(\|D_S^{-1}\|_{\infty} = 1/(a\xi) \geq 1/a = 1/\|D_S\|_{\infty}\) with a trivial generalisation to higher number of species. Hence, for conditions associated with patterning, the relative error in the leading order thin surface approximation arising from equation \(\mathbf{31}\) is \(\varepsilon_s^2 / 3\) and thus we require \(\varepsilon_s^2 / 3 \ll 1\). Despite the very large range of potential parameters in Table \(\mathbf{2}\) the scales for synthetic patterning in bacterial colonies are consistent with this bound.

4.2 Consideration of bulk depth \(h\)

Noting \(D_S \approx D_B\) at least for the parameter estimates of Tables \(\mathbf{1}\) and also relations \(\mathbf{30}, \mathbf{33}\) we also have

\[
\|M_B^2\|_{\infty} = \|k^2_{\beta} I_n + \lambda D_B^{-1}\|_{\infty} \sim O(\|D_S^{-1}\|_\infty), \quad \|D_B M_B^2\|_{\infty} = \|k^2_{\beta} D_B + \lambda I_n\|_{\infty} \sim O(\|J_S\|_{\infty}).
\]

(34)

Hence an appropriate scale for the largest component of \(h M_B\) is \(h_s = h \|D_S^{-1}\|_{\infty}^{1/2}\), which ranges from small to large in Table \(\mathbf{2}\) and thus we proceed to consider simplifications of the expression \(M_B \tanh(h M_B)\) within the instability condition \(\mathbf{28}\) for small and large values of \(h_s\). For the small \(h_s\) limit a Taylor series expansion immediately gives \(M_B \tanh(h M_B) \sim h M_B^2\), with relative corrections on the scale of \(h^2_s / 3\) and we also have

\[
\|M_B \tanh(h M_B)\|_{\infty} \sim h \|M_B^2\|_{\infty} \sim h_s \|M_B\|_{\infty} \quad \text{for } h_s \ll 1.
\]

(35)

For large \(h_s\) simplifications, first note that \(M_B^2\) is diagonal, with diagonal components that have positive real parts since \(\Re(\lambda) > 0\) as we require instability. Furthermore, similar to the synthetic patterning explored in experimental studies \(\mathbf{23, 24, 27}\), we are interested in lateral patterning (in the \(x\)-direction of Fig. \(\mathbf{1}\)) thus, we take \(k^2_q > 0\) and enforce \(k^2_q \geq \pi / L\) by wavemode selection, which bounds the real part of \(M_B^2\) away from zero.

For \(z \in \mathbb{C}\) with \(\Re(z) \neq 0\), we have the limit

\[
z \tanh(z) \rightarrow \text{Sign}(\Re(z)) z \quad \text{as } |z| \rightarrow \infty,
\]
as may be deduced by writing $z$ in terms of its real and imaginary parts, with subsequent use of the properties of trigonometric and hyperbolic functions. In addition we have, without loss of generality, defined $M_B$ by the diagonal matrix with positive semi-definite real part for the square root of the diagonals of $M_B^*$, and in fact no such square root has zero real part since the diagonals of $M_B^*$ have positive real part. Consequently, at leading order we have in the large $h_*$ limit that $h M_B \tanh (h M_B) \to h M_B$ and thus $M_B \tanh (h M_B) \to M_B$, with

$$
\|M_B \tanh (h M_B)\|_\infty \sim \|M_B\|_\infty \quad \text{for } h_* \gg 1.
$$

Finally, with this definition of $M_B$, which is diagonal with terms whose real parts are bound away from zero, we also have that the diagonal elements, and hence the matrix norm, do not blow up on taking the hyperbolic tangent (all of its singularities lie on the imaginary axis) and thus $\| \tanh (h M_B) \|_\infty \sim O(1)$ for $h_* \sim O(1)$. This may be summarised together with equations (35) and (36) via

$$
\|M_B \tanh (h M_B)\|_\infty \sim \min (h_* \|M_B\|_\infty, \|M_B\|_\infty) = \min (h_* 1) \|M_B\|_\infty.
$$

We are now in a position to consider the small $\varepsilon_*$ thin surface, limit of the instability condition given by equation (28), considering the full range of values of $h_*$, which is a measure of the non-dimensional depth of the bulk relative to the patterning lengthscale. We also consider the case $h_* \sim O(\varepsilon_*)$ for relative completeness, even though Tables 1/2 highlight that $h_* \gg \varepsilon_*$ is anticipated for experiments with synthetic pattern formation within bacterial populations.

4.3 Thin surface asymptotic regimes with $k_\eta^2 \|D_S\|_\infty, |\lambda| \sim \text{ord} (\|J_S\|_\infty)$

An example of patterning when $k_\eta^2 \|D_S\|_\infty, |\lambda| \sim \text{ord} (\|J_S\|_\infty)$ is given in the next subsection, but here we consider thin surface asymptotics with $\epsilon^2 / 3 \ll 1$ on fixing $k_\eta^2 \|D_S\|_\infty, |\lambda| \sim \text{ord} (\|J_S\|_\infty)$, where ord $\|J_S\|_\infty$ is defined to mean both $O(\|J_S\|_\infty)$ and not $o(\|J_S\|_\infty)$. Hence we are considering pattern formation that occurs on the timescales of the kinetics with a lengthscale associated with the timescale of the kinetics and the (largest) diffusion scale and, as previously noted, this simplifies the instability condition (28) at leading order to

$$
\det \left( D_B M_B \tanh (h M_B) + \epsilon \left( \frac{1}{\eta} D_B M_B \tanh (h M_B) + I_n \right) D_S M_2^2 \right) = 0,
$$

where the scale of the non-dimensional permeability $\eta$ is unknown, and the possible values of $h_* = h \|D_S^{-1}\|_\infty^{1/2} \sim h \|M_B\|_\infty$ are wide-ranging. Hence, there are several nontrivial distinguished limits, which we proceed to document. Where possible, we will also relate these limits to the isolated surface case, where $\lambda$ is determined by the dispersion relation $\det (M_2^2) = \det (\lambda I_n + k_\eta^2 D_S - J_S) = 0$, in order to understand the impact of the bulk on the classical single-domain situation.

**Case 1** $h_* \ll \varepsilon_* \ll 1$: Noting that $h_* \ll \varepsilon_*$ is equivalent to $h \ll \varepsilon$ by definition, in this limit equation (38) reduces to,

$$
\det \left( \frac{1}{\eta} D_B M_B \tanh (h M_B) + I_n \right) D_S M_2^2 = \det \left( \frac{1}{\eta} D_B M_B \tanh (h M_B) + I_n \right) \det (D_S) \det (M_2^2) = 0.
$$

However the determinant with the hyperbolic tangent term cannot generate a root with $\Re(\lambda) > 0$ and thus patterning. In particular, in Appendix A.2 following equation (53), it is shown that when $\Re(z^2) > 0$ one also has $\Re(z \tanh(z)) > 0$. With $\Re(\lambda) > 0$ for patterning, let $z^2 = h^2 (k_\eta^2 + \lambda/d_B)$, where $d_B$ is a bulk diffusion coefficient. Thus $z^2$ is an eigenvalue of $h^2 M_2^2$, and all eigenvalues of this matrix are of this form. Furthermore, we have $\Re(z^2) > 0$ where $z$ is an eigenvalue of $h M_B$ and satisfies $\Re(z \tanh(z)) > 0$. However for the hyperbolic tangent term in equation (39) to generate a root, at least one eigenvalue of $h M_B$, that is one such $z$, must satisfy $\Re(z \tanh(z)) < 0$, a contradiction, thus showing there are no roots from the determinant involving the hyperbolic tangent. Hence, noting $D_S$ is positive definite, the only roots are those of the isolated Turing modes, independent of $\eta$, and determined purely from $\det (M_2^2) = 0$. 


Case II $\varepsilon_\ast \ll 1$, $h_\ast /\varepsilon_\ast = h/\varepsilon = \hat{h} \sim \mathrm{ord}(1)$: This limit corresponds to the entire domain being thin with respect to the lengthscale in the $x$ direction ($\hat{L}$). In this case we have,

$$\det \left( \hat{h} D_B M_B^2 + \left( \frac{\hat{h}^2}{\eta} D_B M_B^2 + I_n \right) D_S M_S^2 \right) = 0. \quad (40)$$

Equation (40) is a slight modification of the isolated surface Turing conditions in 1-D given by $\det(M_S^2) = 0$, and can similarly be written as an $n$th order polynomial in $\lambda$. Further, if $\eta \ll \varepsilon \|D_B M_B^2\|_\infty \sim \mathrm{ord}(\varepsilon \|J_S\|_\infty)$, then the conditions for instability are precisely those for an isolated surface. Similarly, if $\eta = \mathrm{ord}(\varepsilon \|D_B M_B^2\|_\infty) \sim \mathrm{ord}(\varepsilon \|J_S\|_\infty)$, then we are left with a ‘quadratic’ dispersion relation, which does not simplify from the form given in (40) (‘quadratic’ meaning this dispersion relation will give a polynomial of order $2n$ for $\lambda$, compared to the standard $n$th order polynomial). In general such a relation could lead to quite different values of $\lambda$ from the isolated case, though we will not analyse it further here. If $\eta \gg \varepsilon \|D_B M_B^2\|_\infty \sim \mathrm{ord}(\varepsilon \|J_S\|_\infty)$, then we have the instability condition,

$$\det \left( \lambda (1 + \hat{h}) I_n + k_q^2 (\hat{h} D_B + D_S) - J_S \right) = 0, \quad (41)$$

which can be seen as a homogenisation, or averaging, of the bulk and surface layers. Such an averaged dispersion relation has the potential to increase the ability of the system to pattern compared to the isolated case by, e.g., introducing, or increasing, the differential diffusion between species.

In some other (experimentally relevant) cases this averaged system will decrease the ability of the system to pattern compared to the isolated case. For instance, the necessary differential diffusion for Turing patterning may be due to, for example, substrate binding that is only present in the surface system. In an inert bulk region, there are fewer physical scenarios where differential diffusion is likely as most biological proteins are roughly the same size. In such a case, we have that $D_B = c_B I$, so that (41) can be rearranged to give,

$$\det \left( \left( \lambda (1 + \hat{h}) + k_q^2 c_B \right) I_n + k_q^2 D_S - J_S \right) = 0, \quad (42)$$

which we can see as a shrinking and shifting to the left a root $\lambda$ coming from the isolated case. Effectively then, such a scenario leads to a smaller instability region in parameter space, subject to the wavemode selection constraint that $k_q = q\pi/\hat{L}$, for a natural number $q$.

Another plausibly relevant case of equation (41) is if $D_S = D_B$, i.e. the surface and bulk diffusivities are the same. Here, the dispersion relation is that of the classic case except $\lambda$ and $k_q^2$ are both scaled by $(1 + \hat{h})$. Hence the allowed values of $(\lambda, k_q^2)$ are those of the classic case divided by $(1 + \hat{h})$, which shrinks the range of the allowed patterning wavenumbers relative to the classic case and thus leads to a smaller Turing space compared to the isolated surface system, though again subject to the wavemode selection constraint.

Case III $\varepsilon_\ast \ll h_\ast$: This case proceeds similarly regardless of whether $h_\ast \ll 1$, $h_\ast \sim \mathrm{ord}(1)$ or $h_\ast \gg 1$. Noting $D_S \approx D_B$, $\|D_S M_S^2\|_\infty \sim \|J_S\|_\infty$, from equations (29) and (30), $\|M_B\|_\infty \sim O(\|D_S^{-1} J_S\|_\infty^{1/2})$ by square rooting the first of relations (34), and equation (37), that is $\|M_B \tanh(h M_B)\|_\infty \sim \min(h_\ast, 1) \|M_B\|_\infty \sim \min(h_\ast, 1) \|D_S^{-1} J_S\|_\infty^{1/2}$, we have

$$\frac{\varepsilon \|D_S M_S^2\|_\infty}{\|D_B M_B \tanh(h M_B)\|_\infty} \sim \|D_S\|_\infty \|D_S^{-1} J_S\|_\infty^{1/2} \min(h_\ast, 1) \sim \frac{\varepsilon \|D_S^{-1} J_S\|_\infty}{\|D_S\|_\infty \|D_S^{-1} J_S\|_\infty \|D_S^{-1} J_S\|_\infty^{1/2} \min(h_\ast, 1)}.$$  

Noting $\|D_S^{-1}\|_\infty \|D_S\|_\infty \geq 1$, as deduced just below equation (33), and $\varepsilon_\ast = \varepsilon \|D_S^{-1} J_S\|_\infty^{1/2}$ we thus have

$$\frac{\varepsilon \|D_S M_S^2\|_\infty}{\|D_B M_B \tanh(h M_B)\|_\infty} \lesssim \frac{\varepsilon_\ast}{\min(h_\ast, 1)} \sim \max \left( \varepsilon_\ast, \frac{\varepsilon_\ast}{h_\ast} \right) \ll 1.$$  

Hence the final term from relation (32), that is $\varepsilon D_S M_S^2$, may always be dropped relative to the first term, that is $D_B M_B \tanh(h M_B)$. This reveals that the instability condition simplifies to

$$\det \left( D_B M_B \tanh(h M_B) \left( \varepsilon \frac{D_S M_S^2 + I_n}{\eta} \right) \right) = \det(D_B M_B \tanh(h M_B)) \det \left( \frac{\varepsilon}{\eta} D_S M_S^2 + I_n \right) = 0. \quad (43)$$
4.4 Further thin surface asymptotic regimes with $|\lambda|, k_\eta^2 \|D_S\|_\infty \sim \text{ord}(\epsilon^{1/2} \|J_S\|_\infty)$

There exist nontrivial asymptotic limits which are not described by $|\lambda|, k_\eta^2 \|D_S\|_\infty \sim \text{ord}(\|J_S\|_\infty)$, which can lead to instabilities not captured in Table 3, as we now show. In particular, with $|\lambda|, k_\eta^2 \|D_S\|_\infty \sim \text{ord}(\epsilon^{1/2} \|J_S\|_\infty)$ and $D_B \approx D_S$ we then have $\epsilon D_S M_\eta^2 = -\epsilon J_S + \text{ord}(\epsilon^{3/2} \|J_S\|_\infty)$, $\|D_B M_B^2\|_\infty \sim \text{ord}(|\lambda|, k_\eta^2 \|D_B\|_\infty) \sim \text{ord}(\epsilon^{1/2} \|J_S\|_\infty)$ and finally $h M_B^\infty \sim \text{ord}(\epsilon^{3/4} \|D_S^{-1} J_S\|_\infty^{1/2}) \sim \text{ord}(\epsilon^{3/4} h \|S\|_\infty^{1/2})$. Hence from equation (32), after expanding $\tanh(z) \sim z (1 + \text{ord}(z^2/3))$ for small $z$, we have at leading order

$$\det \left( h D_B M_B^2 + \frac{\epsilon h}{\eta} D_B M_B^2 D_S M_\eta^2 + \epsilon D_S M_\eta^2 \right) = 0,$$

(44)

with relative corrections of $h^2 \epsilon^2 / (3e^{3/2})$ which is required to be much less than unity. Further, noting we have already assumed $h \sim \text{ord}(\epsilon^{1/2})$, we thus additionally require $\epsilon^2 / (3\epsilon^{1/2}) \ll 1$ for equation (44) to hold. For the range of parameters detailed in Table 2 we have $\epsilon^2 / (3\epsilon^{1/2}) \in [1.8 \times 10^{-5}, 2.3]$, and thus we have equation (44) is typically valid for parameters associated with synthetic patterning in bacterial colonies, but not always.

We proceed by noting that the first and third terms of equation (44) are $\text{ord}(\epsilon \|J_S\|_\infty)$ and the second is $\text{ord}(\epsilon^2 / \|J_S\|_\infty^2 / \eta)$. Writing

$$\lambda = \epsilon^{1/2} \mu, \quad h = \epsilon^{1/2} \hat{h}, \quad k_\eta^2 D_B = \epsilon^{1/2} K_\eta^2 D_B \quad \text{with} \quad |\mu|, K_\eta^2 \|D_B\|_\infty \sim \text{ord}(\|J_S\|_\infty), \quad \hat{h} \sim \text{ord}(1).$$

We then have $h D_B M_B^2 = \epsilon \hat{h}(K_\eta^2 D_B + \mu I_n)$, and can factor an $\epsilon$ from equation (44) to obtain,

$$\det \left( \epsilon \hat{h}(K_\eta^2 D_B + \mu I_n) - \frac{\epsilon \hat{h}}{\eta} (K_\eta^2 D_B + \mu I_n) J_S - J_S \right) = 0,$$

(45)
which, in general, can admit nontrivial instabilities due to the coupling of the surface and the bulk. In particular, when $\eta \gg \varepsilon ||J_S||_{\infty}$, so that the second term is no longer retained in the leading order, we find that the growth rates $\mu$ are given as the eigenvalues of $J_S/\hat{h} - \hat{K}^2 DB$.

This matrix resembles the classical isolated-surface case except with a scaling of the kinetics by $\hat{h}$ and the appearance of the bulk diffusion parameters, rather than those in the surface. Hence, we can use usual methods (e.g. the Routh-Hurwitz criterion) to determine parameters that lead to instability in this case, noting that any values of $\lambda$ associated with instability will be of modulus $\mathcal{O}(\varepsilon^{1/2})$, and hence will be associated with slow growing modes. Additionally, we anticipate that such modes will also exhibit small amplitude patterns, as is typical due to center-manifold reduction near Turing-type bifurcations [19], and hence may not be visible against experimental noise. While other distinguished limits may exist which do not fall into the classifications given in Table 3, for brevity we do not pursue a systematic classification of these here. In the next section we will show that almost all numerically computed dispersion relations given by condition (28) fall within the asymptotics given in Table 3, with the exception of the case given in equation (45) which was found numerically first, and subsequently motivated the above scaling.

5 Numerical Exploration of Example Systems

As an example of these dynamics we consider the Schnakenberg kinetics for surface reactants $u_S = (u_S, v_S)$

given by

$$f_S(u_S, v_S) = \left( a - u_S + u_S^2 v_S, b - u_S^2 v_S \right)$$

with $a \geq 0, b > 0$. The spatially homogeneous steady state is given by $u_S^* = u_B^* = (a + b, b/(a + b)^2)$. Unless otherwise stated, we will assume equal diffusion coefficients between the surface and the bulk given by the diagonal matrices $D_S = D_B = \text{diag}(d_u, d_v)$. Without bulk reactions, and given linear interfacial conditions as summarised by equation (12) with the relations (5) and (7), we can immediately apply condition (28) to determine whether, or not, we expect a solution to pattern, and then compare these predictions with numerical simulations of the full nonlinear system.

Numerically computing $\lambda$ from condition (28) is substantially more involved than typical Turing-type analyses (e.g. for polynomial dispersion relations [63]) due to the transcendental nature of this determinant condition. In particular, we expect that for any given wave mode in the $x$ direction given by $k_q = q\pi/\hat{L}$, for a natural number $q$, we have infinitely many distinct values of $\lambda$. These essentially correspond to the wavemodes in the $y$ direction which we have found only implicitly in our construction of the dispersion relation. So to determine if, for a given set of parameters, condition (28) admits a value of $\lambda$ with $\Re(\lambda) > 0$ we resort to numerical heuristics. While fast general-purpose methods exist for rootfinding of polynomials over the complex numbers [62], we are unaware of similar methods for more complicated functions. In lieu of this, we developed a set of numerical heuristics to accurately determine whether or not a value of $\lambda$ with $\Re(\lambda) > 0$ exists, and tested this against full numerical simulations. We make use of the Matlab function PatternSearch as well as a deflation algorithm based on Muller’s method to find many candidate roots with positive real part [62] and then discard any which are spurious. Throughout this section, we denote the largest such root by $\max(\Re(\lambda))$, noting that even in the classical case this maximum is needed as there are generically $n$ distinct values of $\lambda$.

We first consider numerical constructions of dispersion relations for the small-asymptotic limits described in the previous section. Here we consider $\Re(\lambda)$ as a continuous function of the spectral parameter in the $x$ direction, $k_q$, as is commonly done [63]. For $\varepsilon, \varepsilon_s \ll 1$, we have that the isolated reaction-diffusion system can admit growth rates $\lambda$ comparable to a classical one dimensional reaction-diffusion system, which we will denote by $\lambda_C$ (which can be computed in the standard way [63]). We can then consider the maximum value of $\Re(\lambda)$ (across all values of $\lambda$ found from condition (28)), and compare this to the isolated case. We have confirmed these dispersion relations against full numerical simulations by simulating on a domain of lateral size $\hat{L}$ such that a particular mode $k_q = q\pi/\hat{L}$ is admissible, and observing a patterned solution.

We plot these dispersion curves in Figure 2 for a variety of the geometric and coupling parameters. As anticipated, the coupling strength $\eta$ and geometric parameters $h, h_s$ and $\varepsilon, \varepsilon_s$ each influence the shape of these dispersion curves greatly. We now compare these curves to the predictions in Table 3.

For Case
I ($h \ll \epsilon$), we see that $\max(\Re(\lambda))$ is almost unchanged to the standard case up to small corrections not captured by the asymptotics. In Case II ($h \sim \epsilon$), we observe approximate equivalence of the dispersion curve to the isolated case for small $\eta$, and an apparent change in the dispersion relation for increasing $\eta$.

The Case III behaviour ($h \gg \epsilon$) is consistent with the asymptotics of Table 3 whenever $\Re(\lambda) > 0$ except for $\eta \sim \epsilon \|S\|_{\infty}$ and $\eta \gg \epsilon \|S\|_{\infty}$ at relatively small values of $k_q/\pi$. Given these constraints, this mismatch is anticipated to be due to the interaction between the surface kinetics and the bulk diffusion, as described in Section 4.4 given the thin surface approximation $\epsilon_2^2/(3\epsilon^{1/2}) \ll 1$ with $k_q^2\|D_S\|_{\infty} |\lambda| \sim \text{ord}(\epsilon^{1/2} \|S\|_{\infty})$. As a consistency test of this suggested mechanism, in Figure 2(d) we replace $D_B$ by a scaled identity matrix so that differential diffusion in the bulk is no longer present, and we see that all of the dispersion curves, for smaller values of $k_q/\pi$ and $\eta$ sufficiently large, fall below the axis as expected. This is true for different scalar multiples of the identity, such as $D_B = d_v I_2$ where the dispersion curves were even more stable.

We remark that considering other parameters demonstrates that this nontrivial bulk-surface interaction can lead to a non-monotonic behaviour of the dispersion relation with respect to $\eta$.
As in the classical case, we expect that for sufficiently large domains, any region where $R(\lambda) > 0$ should admit a patterned state. We confirmed this using $L = 100$ for each of the dispersion curves, finding that they admitted patterned solutions for long time simulations if and only if $R(\lambda) > 0$ for some region in $k_q$-space. Similar to the classical case, the layered model is always observed to stable at $k_q = 0$ though with a local maximum at this point, in contrast to the behaviour of the classical Turing instability dispersion relation.

To compare these dispersion relations against numerical simulations of the full nonlinear system, we compute a heterogeneity functional determining how far a solution is from a homogeneous state \[6\]. For simplicity, and because the surface layer is of primary interest in synthetic pattern formation within bacterial colonies, we only consider the heterogeneity of the activator in the surface. We define the heterogeneity functional as

$$F_h(u_S) = c \int_0^1 \int_h^{h+\epsilon} \left( \frac{\partial u_S}{\partial x} \right)^2 + \left( \frac{\partial u_S}{\partial y} \right)^2 \, dy \, dx,$$

where $c \in \mathbb{R}^+$ is simply a positive definite scaling parameter. Note that $F_h(u_S) \geq 0$ and for $u_S \in C^1$, $F(u_S) = 0$ iff $u_S$ is spatially homogeneous. While we do not anticipate this metric to necessarily be comparable to max($R(\lambda)$), we note that near the boundary of a Turing instability, the amplitude of patterns and their growth rates in time both scale with the distance from the bifurcation point, typically as a square root of the growth rate \[19\]. Hence this functional should at least qualitatively scale with the growth of max($R(\lambda)$) near the onset of instability.

To use this heterogeneity functional, the full system \([8]–[12]\) was solved until a final time of $t = 10^5$ to ensure a good representation of the steady state pattern. The initial data were taken to be $u_0 = u^*(1 + \xi_u(x,y))$ and $v_0 = v^*(1 + \xi_v(x,y))$ with $\xi_u$ and $\xi_v$ random fields such that at each value of $(x,y)$, they are independently and identically distributed normal random variables with zero mean and variance $10^{-4}$. The equations were simulated using the COMSOL Multiphysics® software \[16\] with at least $2 \times 10^4$ second-order triangular finite elements. A non-uniform mesh was constructed such that the surface region $\Omega_S$ was resolved with at least 10 distinct triangular elements in any vertical cross-section. Convergence was checked in spatial and temporal discretisations, and a relative tolerance of $10^{-5}$ was given to the adaptive timestepping algorithm.

In Figure 5 we give examples of this heterogeneity functional across the ranges of the geometric parameters $\epsilon$, $h$, and $\eta$, alongside predictions from the instability condition \([28]\). As anticipated by the asymptotics, for very small $\epsilon$ (Figure 5a), we see the system fails to support spatial patterns for $\eta \geq 3.9 \times 10^{-4}$. Additionally, we see a jump in the value of the heterogeneity between $\eta = 8 \times 10^{-5}$ and $\eta = 10^{-4}$. We plot values of $u_B$ in Figure 4 across this jump to demonstrate that this discontinuity in the value of the spatial heterogeneity $F_h(u_S)$ for these parameters is due to different nonlinear modes emerging as parameters are varied, and so it is sensible that it is not captured in the linear analysis. Other discontinuities in the plots of the heterogeneity functional in Figure 5 are similarly due to different final modes being selected.

In Figures 3(b) and (d) we see a region of intermediate values of $\eta$ for which no patterning occurs, and more broadly across all of Figure 5 we see that a minimal value of max($R(\lambda)$) occurs approximately for $\eta$ within the range ($10^{-3}, 1$). We show examples of the mode selection process from Figure 3(d) in Figure 5. For small $\eta = 10^{-4}$, we see stable multiple-spike solutions that are essentially confined to the surface. As $\eta$ increases further to $10^{-1}$, a single-spike solution is observed, at a smaller amplitude as the dispersion relation has just crossed the instability threshold given in Figure 3(d). Further increases to large $\eta$ lead to stable spike solutions that remain essentially vertically homogeneous in the surface, but have small transverse variations in the bulk due to the change in reaction kinetics across the interface, as illustrated for $\eta = 10^5$. Further increasing $\eta$ sharpens these spike solutions across the domain, but does not impact the number of modes. Besides the discontinuities in the heterogeneity due to nonlinear mode selection, there is often a good match between the linear analysis (e.g. value of max($R(\lambda)$)) and the heterogeneity, which can be expected near to the Turing bifurcation points in simpler settings due to the existence of normal forms of the pattern amplitude \[19\].

In all of Figure 3 we observe that max($R(\lambda)$) appears to asymptotically approach a fixed value for either $\eta \rightarrow 0$ (which corresponds to the static Turing conditions) or $\eta \rightarrow \infty$, with the latter always being smaller than the former, though this may just be a feature of the parameters explored here. However, in (3c) (and the other cases noted in the caption), we observe that an instability occurs for all values of $\eta$, which is confirmed by numerical simulations of the full system.
As a further example which helps visualise the impact of varying the geometric parameters and coupling constant \( \eta \), we observe patterns primarily confined to the surface but with some interaction with the bulk in Figure 6. Again some mode selection effects are present (two vs three spot solutions for small and larger values of \( \eta \) respectively), though due to generic aspects of multistability in two spatial dimensional systems [25], we suspect these depend somewhat on initial data, rather than just parameter values. Finally in Figure 7 we give an example where no change in the number of unstable modes was apparent for variation in \( \eta \), though the structure of the solution does change.

Within Turing-unstable regimes, the surface largely drives the structure of the modes and hence the patterns can be thought of as quasi-one-dimensional (Figures 6-7). The permeability \( \eta \) does control how much structure there is, both in the bulk in general and in the surface modes’ variation in the \( y \) direction, though in all cases the largest spatial variation is along the lateral coordinate \( x \). For the largest permeability we explored (\( \eta = 10^5 \)), we see that the sizes of the surface and bulk can have a significant impact on the relative shape of the solutions in the bulk region (cf Figures 5(c), 6(c), and 7(c)). In particular we see that the deepest part of the bulk (\( y = 0 \)) in Figure 5(c) and 7(c) maintain a fairly distinct periodic pattern between high and low activator concentrations, whereas the larger bulk in Figure 6(c) is substantially more homogeneous at \( y = 0 \). We also note that for intermediate values of \( \eta \), Figures 6(b) and 7(b) have the largest visual gradients in the activator in the surface layer, consistent with the intermediate-\( \eta \) values having significant impacts on the predicted values of \( \text{max}(\Re(\lambda)) \) in Figure 2.

This further demonstrates nontrivial...
Fig. 4 One-dimensional plots of $u_S$ corresponding to parameters in Figure 3(a) for two values of $\eta$ in the top two panels, and plots of the corresponding $u_B$ below (with $L = 1$ in all cases). The surface concentration $u_S$ is effectively homogeneous in the $y$ direction, and so is essentially a one-dimensional pattern, shown above. Note that the bulk concentrations are almost homogeneous, whereas the surface concentrations are not (compare the scales of $u_S$ and $u_B$).

Fig. 5 Plots of $u_S$ and $u_B$ corresponding to parameters in Figure 3(d) for three values of $\eta$, and $L = 1$. Here, $\varepsilon = 10^{-2}$ and $h = 10^{-1}$.

impacts of the bulk geometry on the structure of emergent patterns, and such leeching into the bulk may be useful to help quantify its impact in synthetic systems.

6 Discussion

Motivated by recent interest in a range of biological contexts, we have developed and analysed a general class of reaction-diffusion models of pattern formation in stratified media, though with an absence of reactions in the bulk and a linear coupling between the layers. We have derived a criterion for pattern-forming
Turing Patterning in Stratified Domains

Fig. 6 Plots of $u_S$ and $u_B$ corresponding to parameters in Figure 3 with $h = 0.5$ and $\varepsilon = 10^{-1}$ for three values of $\eta$, and $\tilde{L} = 1$.

Fig. 7 Plots of $u_S$ and $u_B$ corresponding to parameters in Figure 3 except that $h = 10^{-1}$ and $\varepsilon = 10^{-1}$ for three values of $\eta$, and $\tilde{L} = 1$.

instability in such media, given by equation (26). In Appendix A we showed that the absence of differential transport within each layer entails no patterning for these systems, in direct analogy to the classical Turing instability. We have also demonstrated a range of interesting behaviours via asymptotic reductions in thin domains, and numerical simulations. In particular, this setting of a linearly coupled system with no reactions in the bulk with a thin surface layer is also of significant biological interest, as several groups are using bacterial colonies on inert substrates as a medium for engineered pattern formation via synthetic biology [34, 7, 44]. However, as far as we are aware, there is little theoretical understanding of how the inert substrate impacts the surface reaction-diffusion systems in these kinds of geometries. Additionally, to accurately model the real complexity of these experimental systems we would need to account for intracellular (i.e. non-diffusible) proteins which play a role in the reactions, as our reaction-diffusion framework only captures the dynamics of diffusible signalling molecules.

Nevertheless, even in the simplified setting of an inert bulk and a thin surface, the computed instability criteria are much richer than in the classical case. For instance, the nine distinguished limits for $|\lambda|, k^2_D S_\infty \sim \text{ord}(||J_S||_\infty)$ given in Table 3 demonstrate a variety of behaviours not predicted by analysing the surface reaction-diffusion system alone, as is typical in applications. In addition, these distinguished
limits, though emergent from a complex multi-parameter system, depend on only three non-dimensional parameter groupings, $\epsilon_s, h_s, \text{and } \eta/\epsilon || J_S ||_\infty)$. The first two of these respectively are the surface and bulk depth relative to the lateral lengthscale, i.e. the basic geometry. The final grouping is $\eta/\epsilon || J_S ||_\infty = \tau/|H_e|| J_S ||_\infty$). Noting that $\tau$ is chosen such that $|| J_S ||_\infty \sim \text{ord}(1)$, one can deduce more generally that $\tau/|| J_S ||_\infty$ is the dimensional timescale of surface reaction. Hence the final parameter grouping is the ratio of the interface permeability to the surface velocity scale, $|| J_S ||_\infty H_e/\tau$, with the latter in turn given by the ratio of the surface depth and reaction timescale.

We further note that our instability condition [28], recovers the usual features of Turing instabilities, such as requiring differential diffusion for their onset, and reducing to the polynomial dispersion relation when the bulk becomes uncoupled from the surface. The explicit coupling between bulk diffusion and surface reactions given by [45] when $|\lambda|, k^2_0 || D_S ||_\infty \sim \eta \epsilon^{1/2}[J_S][|| J_S ||_\infty]$ suggests additional distinguished limits from those in Table 3, the associated instabilities possess slower growth rates, but nonetheless highlight substantial and non-trivial impacts of the bulk on the system’s ability to pattern. We anticipate that there are other examples of nontrivial surface-bulk coupling driven instabilities, as suggested in the discussion of the Averaged and Quadratic cases in Table 3 but leave investigation of these to further work.

Broadly, our asymptotic and numerical results on thin surfaces suggest that the presence of the inert bulk generally decreases the ability of the surface system to undergo a Turing instability compared to an isolated system. The exceptional cases, such as the homogenised limit [41] and the explicit coupling in equation (45), can in principle lead to larger Turing spaces, though we have shown in some realistic cases such as equal bulk diffusions ($D_B = I_n$) that these do not enlarge the Turing space. Note that in systems where diffusion varies significantly between domains (e.g. non-diffusible proteins in the surface) the parameter space that admits pattern formation can increase with increasing bulk size (see, for instance [40, 8]). Exploring such interplay will be the focus of future work.

Our results suggest that experiments should aim to design large and robust parameter regimes using classical criteria for pattern-formation (e.g. using design approaches such as in [22]), as diffusion into the bulk region will likely decrease the size of such Turing spaces. We have shown that even in cases where the broad influence of the bulk is to decrease the ability of the system to pattern, such a decrease will be non-monotonic in the geometric and transport parameters of the bulk region in general, as illustrated with the non-dimensional bulk depth, $h$ and permeability, $\eta$. Finally, we have instances of instability such that the bulk domain is a necessary component to drive an otherwise stable surface system to a patterned state (e.g. equation (45)), though we leave systematic analysis of such instabilities for future work.

We remark that the mode selection phenomena we have illustrated can be understood in the context of finite-size effects, which are well-studied in the classical case [63]. Namely given a dispersion relation for $\mathcal{R}((\lambda_C(k_0)))$, where $\lambda_C$ is the growth rate of a classical Turing mode, one can tune the geometry to select different spatial eigenvalues $k_0$ to give rise to non-monotonic effects as, for instance, the domain size is increased. However, here the effects are more subtle as we cannot explicitly compute the relationship between $\lambda$ and eigenvalues of the full spatial operator, and so can only implicitly observe these effects. Nevertheless, these mode selection effects appear to be more prevalent compared to classical cases requiring very small domains and other fine-tuning [63]. Additionally, they appear to be more prevalent across a wide range of geometric parameters, whereas the classical cases have been studied almost entirely in terms of a scalar length, and are generally restricted in parameter regimes where they occur.

There are numerous extensions of these results that are worth pursuing. In the example setting of bacterial colony formation on an agar substrate, one might need to augment the bulk evolution with a degradation reaction. We remark that such a simple addition leads to substantial complexity as, if the surface equilibrium is nonzero, then there does not exist a homogeneous equilibrium across the whole coupled system (a degradation reaction in the bulk by itself will always lead to a homogeneous zero equilibrium concentration). We anticipate that the mathematical structure in this case will be even more intricate. A simpler addition, also of relevance to bacterial patterning on agar, would be the inclusion of non-diffusible reactants in the surface region. This approach would also pave the way to account for all gene regulatory dynamics in a quantitative model based on mass action kinetics. In such a case, we can apply techniques to incorporate the impact of such reactants on the surface reaction kinetics directly (in the linearised system) [45]. Along similar lines, more complicated transport functions $g$ across the membrane can be studied, again leading to new possibilities of differential transport, which can easily be added to the analysis implemented here.
There are many biological examples of physical layered media with reactions in multiple different spatial domains, such as in the epithelial-mesenchymal coupling during the development of the skin in mammals [83]. For example, in the study of hair follicle morphogenesis, a substantial amount of biochemical research has implicated Turing-type instabilities in the formation of follicle primordia [51]. More recently, it has been suggested that a simple activator-inhibitor system is insufficient to capture the dynamical complexity in hair follicle patterning, and so suggestions have been made that such patterns arise due to many coupled processes, which will undoubtedly occur across the different domains of the epithelium and the developing mesenchyme [32]. Similar remarks can be made about many kinds of skin and other organ patterning events across a range of species, suggesting that general methodologies for stratified reaction-diffusion systems would be useful to elucidate underlying physico-chemical mechanisms. Mathematically, the limit of $\eta \to \infty$ can be thought of as a step function heterogeneity, as explored in [51], so that the systems studied here are also in some sense a generalisation of piecewise-constant reaction-diffusion problems.

While we have explored exemplar reaction-diffusion systems in such coupled domains, there are more general transport mechanisms that could be studied. Both chemotaxis and a range of mechanical taxis, as well as mechanical forces, could be included in such a model. We note that a numerical study [32] has made some progress towards such a model. The linear stability analysis for such problems is involved, but the approach presented here generalises to these settings. Of course, in the absence of a homogeneous steady state, one must develop new methods for the analysis of pattern-forming instabilities. This has been done recently for heterogeneous steady states [53], but extending such an analysis to these coupled geometries is nontrivial.

Finally we mention that one could generalise from our setting of two planar domains to many more coupled domains, or to more complicated geometric settings, including those relevant for more realistic models of development, such as in the blastula stage or later stages of epithelial-mesenchymal development on complicated morphologies. While our approach may be generalisable to very different geometric settings, the dispersion relation we have found in this simple case is already somewhat difficult to analyse, and full numerical simulations may be more expeditious. Nevertheless, analytically tractable results for this family of problems are valuable in understanding the role of coupled domain structures in pattern formation, as such scenarios are ubiquitous in biological settings.

Acknowledgements A.L.K. and E.A.G. are grateful for support from BBSRC grant BB/N006097/1; V.K. is grateful for support from the European Regional Development Fund-Project ‘Center for Advanced Applied Science’ (no. CZ.02.1.01/0.0/0.0/16_019/0000778) and the Mathematical Institute at the University of Oxford. In compliance with BBSRC’s open access initiative, the data in this paper is available from [http://dx.doi.org/xx.xxxx/xxxxxxxxxxxxxxxxxx](http://dx.doi.org/xx.xxxx/xxxxxxxxxxxxxxxxxx).

A Further analysis of possible patterning instabilities: surface isolation and equal diffusion coefficients

Here, we compute instability conditions from equation (28) for further distinguished limits, in particular (i) the limiting of decoupling the interaction of the surface and bulk regions, that is for sufficiently small $\eta \ll 1$ and (ii) the limit of equal diffusion coefficients in each region for classical Turing patterning systems. In particular, (i) provides a useful consistency check of the modelling framework, while (ii) confirms that in the absence of differential transport within at least one layer or between them, patterning cannot occur for classical Turing systems, in direct analogy to the behaviour of the single layer classical Turing instability.

A.1 Patterning in the limit of an isolated surface system, via sufficiently small non-dimensionalised permeability

We consider the patterning conditions for the isolated surface system in the case that $\eta \to 0$. Here, we denote the eigenvalues of $M_B$ as $\mu_B$, the eigenvalues of $M_S$ as $\mu_S$, and the eigenvalues of $I_n$ as $\nu$, where $p = 1, 2, \ldots, n$ in all cases, where $n$ is again the number of reactants. By continuity of the determinant, equation (28) reduces for sufficiently small $\eta$ to the condition

$$\det(M_B \tanh(hM_B)) \det(M_S \sinh(\varepsilon M_S)) = 0,$$

so that the bulk and surface components decouple. As these hyperbolic trigonometric functions are at worst meromorphic, the zero of the determinants occur for eigenvalues of the matrices $M_B$, $M_S$ that, respectively, are roots of $z \tanh(hz) = 0$, $z \sinh(\varepsilon z) = 0$ for $z \in \mathbb{C}$. Hence, equation (47) is satisfied whenever $\mu_B = j\pi/h$, or $\mu_S = j\pi/\varepsilon$, with $j \in \{0, 1, 2, \ldots\}$, natural.

For the bulk component, this implies that $\det(M_B^2 + (j\pi/h)^2 I_n) = 0$ which, as this is a diagonal matrix, implies that the allowed growth rates are given by

$$\lambda_p = -D_B(k_q^2 + (j\pi/h)^2) = -D_B\pi^2(q^2 + (j/h)^2) \leq 0, \text{ for } q, j \in \{0, 1, \ldots\}, p \in \{1, 2, \ldots, n\},$$

with \( D_{B,p} \) denoting the \( p \)th component of the diffusion matrix \( D_B \), and recalling that \( k_q = q \pi / L \). Hence these solutions do not drive an instability, noting that although these eigenvalues formally break the assumption we made that \( \Re(\lambda) > 0 \) used to deduce equation (28), we have equation (47) is precisely (29) in the limit \( \eta \to 0 \), and, hence, we have not used this assumption to determine these eigenvalues.

Similarly, by the preceding discussion of the eigenvalues \( \mu_{i,p} \) of \( M_\delta \), we have a condition for the existence of nontrivial eigenvectors for these eigenvalues given by

\[
\det(M_\delta^2 - \mu_{i,p}^2 I_a) = \det((q^2 + j/\epsilon) \pi^2 I_a + D_\delta^{-1}(\lambda I_a - J_\delta)) = 0,
\]

which we recognise as exactly the condition one would find to compute \( \lambda \) in a traditional \( n \) species reaction-diffusion system posed on a rectangle with Neumann boundary data [25]. In practice finding all such solutions for a given eigenpair \( q, j \) is a straightforward numerical problem. While these computations are more easily implemented by noting that the \( \eta = 0 \) limit does separate into two uncoupled regions that can be analysed via standard methods, these results serve as a useful consistency check of equation (28).

### A.2 Identical Diffusion Coefficients Within Regions

Below we assume that the surface Jacobian, \( J_s \), can be diagonalised, noting that diagonalisable matrices are a dense subset of complex-valued matrices and thus the results derived below therefore hold in general due to continuity. In particular, we now show that if there is identical diffusion for different species within each region, then the spatially homogeneous steady state is linearly stable to the perturbations given by equation (16) for kinetics in the surface layer that allow the classical Turing instability.

To proceed, we let \( D_s = c_s J_s \) and \( D_B = c_B I_a \), so that \( M_\delta^2 = (k_q^2 + \lambda/c_B) I_a \). As \( J_s \) can be diagonalised, we can also diagonalise \( M_\delta^2 = (k_q^2 + \lambda/c_B) I_a = (1/c_s) J_s \). Making this additional assumption, we rewrite these matrices using the \( n + 1 \) scalar values \( m_p^2 \) and \( \eta \) where \( m_p \) are the eigenvalues of \( J_s \) and \( \eta = 1, 2, \ldots, n \). We note that these scalars will never be zero as they are real and non-negative, we require \( \Re(\lambda) > 0 \) and, restricting ourselves to kinetics that exhibit the classical Turing instability, we have stable kinetics in the absence of diffusion, and thus \( \Re(\eta) < 0 \).

With the assumption of identical diffusion coefficients, all of the matrices in equation (28) are diagonal and proportional to the identity except for \( M_\delta^2 \), which can be diagonalised using the eigenvectors of \( J_s \). Noting that all functions involving \( M_\delta \) in (28) are both even functions and analytic, we can simultaneously diagonalise the entire condition using these eigenvectors. Doing so, we arrive at the following \( n \) scalar conditions from equation (28),

\[
c_{g}m_{B} \tanh(h_{B}) \left( \cosh(\eta m_{p}) + \frac{c_{\mu}}{\eta} m_{p} \sinh(\eta m_{p}) \right) + c_{\mu} m_{p} \sinh(\eta m_{p}) = 0,
\]

where we note that only one of these conditions must be satisfied, and hence they lead to independent roots for \( \lambda \). We observe that \( \cosh(\eta m_{p}) \neq 0 \) cannot occur once \( m_{p} \) does not have zero real part, which is enforced by assumptions. Hence we can divide equation (50) by this factor to yield,

\[
\frac{c_{\mu}c_{g}m_{B}m_{p}}{\eta} \tanh(h_{B}) \cosh(\eta m_{p}) + c_{\mu} m_{p} \tanh(h_{B}) + c_{g}m_{B} \tanh(h_{B}) = 0.
\]

We now argue that there are no possible instabilities given condition (51). We note that \( \Re(\lambda) > 0 \), both \( m_{p}^2 \) and \( m_{g}^2 \) must have strictly positive real part. Given \( k_q^2 \leq 0 \), \( \Re(\eta) > 0 \), \( \Re(\eta m_{p}) < 0 \), we have \( \Re(m_{p}^2) > 0 \), \( \Re(\eta m_{p}) > 0 \) and hence \( \arg(m_{p}^2) \in (-\pi/4, \pi/4) \cup (-\pi, -3\pi/4) \cup (3\pi/4, \pi) \), with \( m_{p} = 0 \) excluded (and identical bounds for \( m_{g} \)). Thus all roots of \( m_{p} \cosh(\eta m_{p}) \) and \( m_{g} \tanh(h_{B}) \) are excluded and one may divide (51) by \( c_{\mu}c_{g}m_{B}m_{p} \cosh(\eta m_{p}) \tanh(h_{B}) \) to obtain,

\[
\frac{1}{\eta} + \frac{1}{c_{g}m_{B} \tanh(h_{B})} + \frac{1}{c_{\mu} m_{p} \tanh(h_{B})} = 0.
\]

Letting \( z = x + iy \in \mathbb{C} \), \( x, y \in \mathbb{R} \), with \( \Re(z^2) > 0 \), we will show that \( \Re(z \tanh(z)) > 0 \), and subsequently apply this for \( z = m_{B} \) and \( z = m_{B} \). Letting \( Q = (\cosh(x) \cos(y))^2 + (\sinh(x) \sin(y))^2 \), we can compute

\[
\Re(z \tanh(z)) = \frac{x \cosh(x) \sinh(x) - y \cos(y) \sin(y)}{Q} = \frac{x \sinh(2x) - y \sin(2y)}{2Q}.
\]

Additionally, \( \Re(z^2) > 0 \) implies \( x^2 > y^2 \) which then forces \( \Re(z \tanh(z)) > 0 \) since \( x \sinh(2x) - y \sin(2y) > 0 \) for \( x^2 > y^2 \). The latter holds given \( x > 0, y \in (-x, x) \) since then

\[
x \sinh(x) > y \sin(y) \geq y \sin(y).
\]

The first inequality holds as the real function \( x \sinh(x) \) is even and, for \( x > 0 \), monotonic increasing, while the second inequality follows using the fact \( \sinh(y) \geq |y| \geq \sin(y) \) for all real \( y \) and the odd parity of \( y \sin(y) \). The case for \( x < 0 \) is then inherited from the case \( x > 0 \) by parity. Hence, the left hand side of equation (52) must have a strictly positive real part, and so this equation can never be satisfied. Therefore, in the case of identical diffusion coefficients within each region, there are no values of \( \lambda \) with positive real part.
B Pure diffusion does not induce patterning

We now show that diffusion alone, in the absence of reaction terms, cannot induce patterning in the modelling framework. In particular in the absence of reaction kinetics, and given our linear interfacial conditions, each species decouples and can be considered in isolation. Without loss of generality we consider surface and bulk concentrations of the first species, denoted $c_B$ and $c_S$ below with respective diffusion coefficients $d_B$, $d_S$ in each region. Then, with $\Omega_B$ and $\Omega_S$ denoting the bulk and surface regions, as in Figure 1 and the subscript $t$ denoting a time derivative, we have

$$\frac{1}{2} \frac{\partial}{\partial t} \left[ \int_{\Omega_B} dV_B \nabla c_B \cdot \nabla c_B + \int_{\Omega_S} dV_S \nabla c_S \cdot \nabla c_S \right]$$

$$= \int_{\Omega_B} dV_B \nabla c_B \cdot \nabla c_B + \int_{\Omega_S} dV_S \nabla c_S \cdot \nabla c_S$$

$$= \int_{\Omega_B} dV \nabla \cdot (c_B d_B \nabla c_B) - c_B d_B \nabla^2 c_B + \int_{\Omega_S} dV \nabla \cdot (c_S d_S \nabla c_S) - c_S d_S \nabla^2 c_S$$

$$= \int_{\partial \Omega_B} dS_B d_B \frac{\partial c_B}{\partial n} + \int_{\partial \Omega_S} dS_S d_S \frac{\partial c_S}{\partial n} - \int_{\Omega_B} dV \nabla^2 c_B + \int_{\Omega_S} dV \nabla^2 c_S$$

$$= - \int_{\Omega_B} dV \nabla^2 c_B + \int_{\Omega_S} dV \nabla^2 c_S \leq 0,$$

where the surface integrals in the fourth line vanish, courtesy of the zero flux boundary conditions [10] and [11], and the interfacial conditions (12), on noting relations (6) and (7). This can also be recognised as a free energy inequality, or equivalently an entropy inequality, corresponding to the second law of thermodynamics given a Fickian diffusive flux as a constitutive relation [39]. Hence a standard measure of heterogeneity cannot increase and thus initial conditions that are close to homogeneous (in the sense of a suitable Sobolov norm) cannot induce patterns.

References

1. K. Anguige and M. Röger. Global existence for a bulk/surface model for active-transport-induced polarisation in biological cells. Journal of Mathematical Analysis and Applications, 448(1):213–244, 2017.

2. M. Asllani, J. D. Challenger, F. S. Pavone, L. Sacconi, and D. Fanelli. The theory of pattern formation on directed networks. Nature communications, 5:4517, 2014.

3. F. K. Balagaddé, H. Song, J. Ozaki, C. H. Collins, M. Barnet, F. H. Arnold, S. R. Quake, and L. You. A synthetic escherichia coli predator–prey ecosystem. Molecular Systems Biology, 4(1), 2008.

4. S. Basu, Y. Gerchman, C. H. Collins, F. H. Arnold, and R. Weiss. A synthetic multicellular system for programmed pattern formation. Nature, 434(7037):1130–1134, 2005.

5. D. L. Benson, P. K. Maini, and J. A. Sherratt. Unravelling the turing bifurcation using spatially varying diffusion coefficients. Journal of Mathematical Biology, 37(5):381–417, 1998.

6. C. Berding. On the heterogeneity of reaction-diffusion generated pattern. Bulletin of mathematical biology, 49(2):233–252, 1987.

7. C. R. Boehm, P. K. Grant, and J. Haseloff. Programmed hierarchical patterning of bacterial populations. Bulletin of mathematical biology, 59(2):233–252, 1987.

8. F. Brauns, G. Pawlik, J. Halatek, J. Kerssemakers, E. Frey, and C. Dekker. Bulk-surface coupling reconciles Min-protein pattern formation in vitro and in vivo. bioRxiv, page 2020.03.01.971952, 2020.

9. E. O. Budrene and H. C. Berg. Complex patterns formed by motile cells of escherichia coli. Nature, 349(6310):630, 1991.

10. E. O. Budrene and H. C. Berg. Dynamics of formation of symmetrical patterns by chemotactic bacteria. Nature, 376(6535):49, 1995.

11. Y. Cao, Y. Feng, M. D. Ryser, K. Zhu, G. Herschlag, C. Cao, K. Marusak, S. Zauscher, and L. You. Programmable assembly of pressure sensors using pattern-forming bacteria. Nature Biotechnology, 35(11):1087–1093, 2017.

12. Y. Cao, M. D. Ryser, S. Payne, B. Li, C. V. Rao, and L. You. Collective Space-Sensing Coordinates Pattern Scaling in Engineered Bacteria. Cell, 165(3):620–630, 2016.

13. A. J. Catllá, A. McNamara, and C. M. Topaz. Instabilities and patterns in coupled reaction-diffusion layers. Physical Review E, 85(2):026215, 2012.

14. M. A. J. Chaplain, M. Ganesh, and I. G. Graham. Spatio-temporal pattern formation on spherical surfaces: numerical simulation and application to solid tumour growth. Journal of mathematical biology, 42(5):387–423, 2001.

15. S. J. Chapman, R. Erban, and S. A. Isaacson. Reactive boundary conditions as limits of interaction potentials for brownian and langevin dynamics. SIAM Journal on Applied Mathematics, 76(1):368–390, 2016.

16. COMSOL Multiphysics® v. 5.4. http://www.comsol.com. COMSOL AB, Stockholm, Sweden.

17. S. D. Conte and C. De Boor. Elementary numerical analysis: an algorithmic approach, volume 78. SIAM, 2017.

18. E. J. Crampin, E. A. Gaffney, and P. K. Maini. Reaction and diffusion on growing domains: scenarios for robust pattern formation. Bulletin of mathematical biology, 61(6):1093–1120, 1999.

19. M. C. Cross and P. C. Hohenberg. Pattern formation outside of equilibrium. Reviews of modern physics, 65(3):851, 1993.

20. G. C. Cruywagen and J. D. Murray. On a tissue interaction model for skin pattern formation. Journal of Nonlinear Science, 2(2):217–240, 1992.

21. D. Cusseledu, L. Edelstein-Keshet, J. A. Mackenzie, S. Portet, and A. Madzumase. A coupled bulk-surface model for cell polarisation. Journal of theoretical biology, 2018.

22. N. Dalchau, M. J. Smith, S. Martin, J. R. Brown, S. Emmott, and A. Phillips. Towards the rational design of synthetic cells with prescribed population dynamics. Journal of the Royal Society Interface, 9(76):2883–2898, 2012.
59. A. Madzvamuse, E. A. Gaffney, and P. K. Maini. Stability analysis of non-autonomous reaction-diffusion systems: the effects of growing domains. *Journal of mathematical biology*, 61(1):133–164, 2010.

60. P. K. Maini, T. E. Woolley, R. E. Baker, E. A. Gaffney, and S. S. Lee. Turing’s model for biological pattern formation and the robustness problem. *Interface focus*, 2(4):487–496, 2012.

61. C. Mou, B. Jackson, P. Schneider, P. A. Overbeek, and D. J. Headon. Generation of the primary hair follicle pattern. *Proceedings of the National Academy of Sciences*, 103(24):9075–9080, 2006.

62. D. E. Muller. A method for solving algebraic equations using an automatic computer. *Mathematical tables and other aids to computation*, 10(56):208–215, 1956.

63. J. D. Murray. *Mathematical Biology II: Spatial Models and Biomedical Applications*. Springer-Verlag, Berlin Heidelberg, 3rd edition, 2003.

64. H. Nakao and A. S. Mikhailov. Turing patterns in network-organized activator–inhibitor systems. *Nature Physics*, 6(7):544, 2010.

65. J. Nauman, P. Campbell, F. Lanni, and J. Anderson. Diffusion of insulin-like growth factor-i and ribonuclease through fibrin gels. *Biophys J.*, 92(12):4444–50, 2007.

66. K. Page, P. K. Maini, and N. A. Monk. Pattern formation in spatially heterogeneous turing reaction–diffusion models. *Physica D: Nonlinear Phenomena*, 181(1-2):80–101, 2003.

67. K. M. Page, P. K. Maini, and N. A. M. Monk. Complex pattern formation in reaction–diffusion systems with spatially varying parameters. *Physica D: Nonlinear Phenomena*, 202(1-2):95–115, 2005.

68. F. Paquin-Lefebvre, W. Nagata, and M. J. Ward. Pattern formation and oscillatory dynamics in a 2-d coupled bulk-surface reaction-diffusion system. *arXiv preprint arXiv:1810.00251*, 2018.

69. S. Payne, B. Li, Y. Cao, D. Schaeffer, M. D. Ryser, and L. You. Temporal control of self-organized pattern formation without morphogen gradients in bacteria. *Molecular systems biology*, 9(1):697, 2013.

70. R. G. Plaza, F. Sanchez-Garduno, P. Padilla, R. A. Barrio, and P. K. Maini. The effect of growth and curvature on pattern formation. *Journal of Dynamics and Differential Equations*, 16(4):1093–1121, 2004.

71. A. Rätz. Turing-type instabilities in bulk–surface reaction–diffusion systems. *Journal of Computational and Applied Mathematics*, 289:142–152, 2015.

72. A. Rätz and M. Röger. Symmetry breaking in a bulk–surface reaction–diffusion model for signalling networks. *Nonlinearity*, 27(8):1805, 2014.

73. F. Sánchez-Garduño, A. L. Krause, J. A. Castillo, and P. Padilla. Turing–Hopf patterns on growing domains: the torus and the sphere. *Nonlinearity*, 481:136–150, 2019.

74. J. J. Tabor, H. M. Salis, Z. B. Simpson, A. A. Chevalier, A. Levskaya, E. M. Marcotte, C. A. Voigt, and A. D. Ellington. A Synthetic Genetic Edge Detection Program. *Cell*, 137(7):1272–1281, 2009.

75. L. M. Vilaca, M. C. Milinkovitch, and R. Ruiz-Baier. Numerical approximation of a 3d mechanochemical interface model for skin patterning. *Journal of Computational Physics*, 384:363–404, 2019.

76. T. Woolley. Visions of mathematics, chapter 48: Mighty morphogenesis, 2014.

77. L. Yang, M. Dolnik, A. M. Zhabotinsky, and I. R. Epstein. Spatial resonances and superposition patterns in a reaction-diffusion model with interacting Turing modes. *Physical review letters*, 88(20):208303, 2002.

78. L. Yang and I. R. Epstein. Oscillatory Turing patterns in reaction-diffusion systems with two coupled layers. *Physical review letters*, 90(17):178303, 2003.