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1           **MODELLING POLARITY-DRIVEN LAMINAR PATTERNS IN BILAYER**  
2   **TISSUES WITH MIXED SIGNALLING MECHANISMS**

3   JOSHUA W. MOORE<sup>1,2</sup>, TREVOR C. DALE<sup>3</sup>, AND THOMAS E. WOOLLEY<sup>2</sup>

ABSTRACT. Recent advances in high-resolution experimental methods have highlighted the significance of cell signal pathway crosstalk and localised signalling activity in the development and disease of numerous biological systems. The investigation of multiple signal pathways often introduces different methods of cell-cell communication, i.e. contact-based or diffusive signalling, which generates both a spatial and temporal dependence on cell behaviours. Motivated by cellular mechanisms that control cell-fate decisions in developing bilayer tissues, we use dynamical systems coupled with multilayer graphs to analyse the role of signalling polarity and pathway crosstalk in fine-grain pattern formation of protein activity. Specifically, we study how multilayer graph edge structures and weights influence the layer-wise (laminar) patterning of cells in bilayer structures, which are commonly found in glandular tissues. We present sufficient conditions for existence, uniqueness and instability of homogeneous cell states in the large-scale spatially discrete dynamical system. Using methods of pattern templating by graph partitioning to generate quotient systems, in combination with concepts from monotone dynamical systems, we exploit the extensive dimensionality reduction to provide existence conditions for the polarity required to induce fine-grain laminar patterns with multiple spatially dependent intracellular components. We then explore the spectral links between the quotient and large-scale dynamical systems to extend the laminar patterning criteria from existence to convergence for sufficiently large amounts of cellular polarity in the large-scale dynamical system, independent of spatial dimension and number of cells in the tissue.

**Keywords:** Pattern formation, Monotone systems, Pathway crosstalk, Spectral graph theory, Cell polarity.

4   1. INTRODUCTION

5           Cell-fate determination is the process of stem, or progenitor, cell commitment to transition to  
6 a differentiated state with adapted cellular functions [1]. This process enables the generation of  
7 specialised cell populations during organ development as cells propagate through lineage struc-  
8 tures with each cell-fate choice. Cell-fate decisions are typically regulated by tightly orchestrated  
9 intracellular protein cascades often termed genetic regulatory networks (GRNs) that describe  
10 complex intracellular protein interactions that depend on both the local cellular environment  
11 and intrinsic genetic properties of the cell [2]. Subsequently, the investigation of the autonomous  
12 spatial organisation of cell-fate biomarkers (active proteins for cell-fate regulation) in developing  
13 tissues has been of significant interest as a strategy to elucidate the intracellular mechanisms  
14 that govern such cellular behaviour bifurcations [3–5].

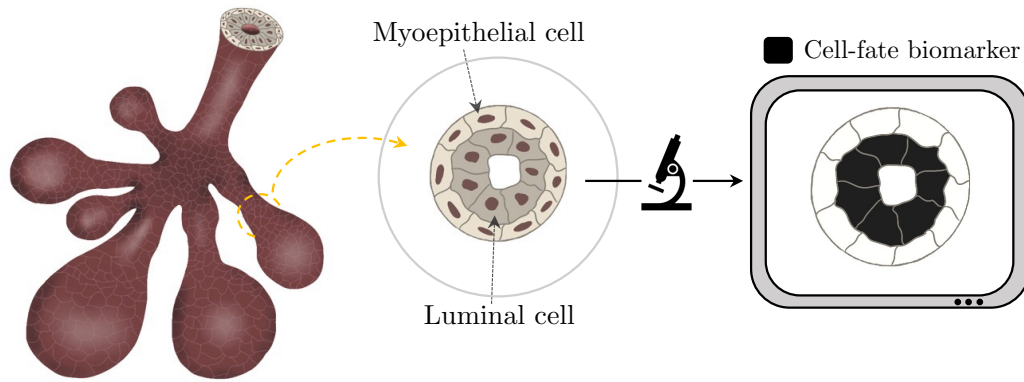
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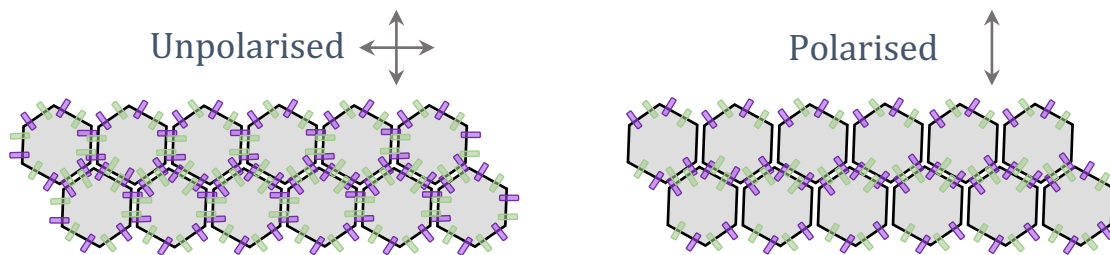
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(A) Bilayer structures in glandular tissues.



(B) Cell signalling polarity.

FIGURE 1. Schematic representation of the bilayer cellular architecture of ductal tissues and cellular polarity. (A) Branching tissue structures produce bilayer ducts of layer-wise contrasting epithelial cells with the outer and inner layers of myoepithelial and luminal cells, respectively, to facilitate the production and secretion for the transportation of substances such as sweat, saliva and breast milk [7–9]. Imaging the ducts for key cell-fate biomarkers highlight the presence of laminar patterns intracellular protein expression. Some well-established cell-fate biomarkers are p63 and Notch for myoepithelial and luminal cells in mammary and sweat glands [10, 11]. (B) An example contact-dependent signalling mechanism with activators and receptors are represented using purple and green rectangles depicting the effect of layer-wise polarity in a bilayer of cells.

15 Ductal structures commonly found in glandular tissues possess some of the most simple cell-  
 16 fate biomarker spatial patterning. Primarily comprised of just two cell types, these tissues  
 17 produce branching morphologies with a consistent bilayer ring of layer-wise contrasting cell  
 18 types (Figure 1A). To form the bilayer rings, undifferentiated cells self-organise to autonomously  
 19 produce distinct laminar patterns of opposing cell-fate biomarkers to promote the substance  
 20 transportation functions required of the organ [6]. Common examples of these laminar pattern  
 21 features can be found in glandular tissues, such as: the mammary, salivary and sweat glands  
 22 where the emergence of bilayer cell-type expression initiates the formation of ductal features  
 23 [7–9].

24 Though the spatial patterning of the cell-fate biomarkers can be simple in bilayer tissues, the  
 25 cellular regulatory mechanisms that generate these cell states are complex, often involving mul-  
 26 tiple inter-linked GRNs, which is referred to as pathway crosstalk [12]. Consequently, pathway  
 27 crosstalk may induce multiple modes of independent cell-cell communication channels, such as

28 contact-based (juxtacrine) or short-range diffusive (paracrine) protein interactions between local  
 29 cells [10, 13]. Thus, cells receive local tissue information from a range of sources to consistently  
 30 select the appropriate cell type for functional ductal formation.

31 In addition to the rich interactions of intracellular GRNs, a common control mechanism  
 32 present in developing tissues is cellular polarity. In a broad sense, cell polarity is the asymmetry  
 33 of the shape or molecular distribution of the cell [14]. In this study we focus on biochemical  
 34 asymmetries that are associated with localisation of signalling activators and receptors within,  
 35 or on, the surface of the cell and refer to this as polarity herein [15]. In general, polarity  
 36 in cell signalling proteins causes anisotropic communication flow between cells and therefore  
 37 specifies a niche of adjacent interaction cells, as shown in Figure 1B as an example of apical-basal  
 38 polarity in an epithelial bilayer. The role of polarity is well-established in mechanical processes  
 39 of mammalian development such as division and adhesion, to ensure consistent morphological  
 40 features of the tissue during growth [16].

41 There is growing evidence in a variety of biological systems in which polarity also acts as  
 42 a spatial coordinator of cell-fate specification and promotes mechanical feedback loops to pre-  
 43 serve local cell types for healthy tissue function [17]. However, the precise role polarity has in  
 44 influencing intracellular kinetics that govern cell-fate choices is widely unknown, since cell-cell  
 45 interactions compound with complex pathway crosstalk [16]. Therefore, in this study we explore  
 46 the interplay of polarity and multiple cell-cell signalling mechanisms associated with pathway  
 47 crosstalk in generating laminar patterns of biomarkers, conforming with the process of cell-fate  
 48 determination of developing bilayer tissues.

49 Following Turing's seminal paper in 1952 [18], the majority of theoretical results of pattern  
 50 formation in developmental biology focus on diffusion-driven instabilities of reaction-diffusion  
 51 (RD) systems [19, 20]. RD systems rely on the assumption that cells communicate using short-  
 52 range and/or long-range paracrine signalling mechanisms, namely the local diffusion of proteins  
 53 coupled with intracellular kinetics. However, there exists many pattern forming biological sys-  
 54 tems that rely on non-diffusive, juxtacrine communication, such as lateral-inhibition mechanisms,  
 55 where adjacent cells inhibit each other from converging to the same state, facilitating fine-grain  
 56 pattern formation [21, 22].

57 The fundamental differences in paracrine and juxtacrine signalling motivate contrasting mod-  
 58 elling approaches. The diffusion process in paracrine signalling extends to a spatial continuum  
 59 limit, generating small systems of partial differential equations (PDEs) allowing protein patterns  
 60 to form over multiple cell lengths to represent phenomena such as morphogen gradients over the  
 61 tissue [20]. In contrast, the discrete nature of juxtacrine signalling induced by membrane contact  
 62 necessitates the use of spatially discrete systems of ordinary differential equations (ODEs) [23].  
 63 Subsequently, these contrasting modelling paradigms restrict the specific continuum and discrete  
 64 approaches to pattern analysis in systems where both diffusive and non-diffusive mechanisms are  
 65 present.

66 Graphs representing spatially discrete analogues of diffusive mechanisms have previously been  
 67 employed to homogenise the analytical approaches to pattern formation and, further, investi-  
 68 gate cell structure on pattern emergence [24]. That is, graph vertices depict cells and edges  
 69 are drawn between cells if they are communicating via diffusive proteins. Critically, this ap-  
 70 proach preserves the concept of cell identity within diffusive models and transforms the systems  
 71 of PDEs into much larger systems of ODEs, consistent with the juxtacrine model formulation.

72 However, the central theme of pattern analysis is understanding the conditions that yield the  
73 degradation of stable homogeneity of the system and is typically conducted via linear stability  
74 analysis with coupled spatio-temporal components [25–27]. Consequently, the high-dimension of  
75 these ODE descriptions and required nonlinear kinetics of multicellular domains render analyt-  
76 ical approaches intractable, which lead to many studies focusing on spatially reduced systems  
77 accompanied by numerical simulations for the larger cellular domains [23, 25, 26]. Critically, the  
78 analysis conducted on such spatially reduced models have been shown to be insufficient for pre-  
79 dicting the types of patterning observed numerically [25], with similar results for cell-resolution  
80 discretised diffusive systems [28].

81 Adopting concepts from systems engineering, the application of interconnected dynamical  
82 systems theory was employed in [29] to derive analytic pattern formation conditions for jux-  
83 tacrine models, independent of the number of cells and therefore the size of the ODE system.  
84 Namely, cells were treated as input-output (IO) components within a circuit, i.e. cells receive  
85 signals and then produce response signal to other connected cells. This approach recasts the  
86 large-scale ODE system in a macroscopic perspective to analyse the behaviour of only the di-  
87 rectly spatially-dependent intracellular proteins using signal transfer functions. Furthermore,  
88 extending the low-dimensional quotient representations of graph coupled dynamical systems as  
89 formalised by Golubitsky, Stewart and co-authors [30–32] to IO systems, edge symmetries of  
90 the cell-cell connectivity graphs were exploited in [33] to develop methods of graph partitioning  
91 to form quotient graphs that represent a pattern template. Embedding the intracellular ODE  
92 systems defined by the GRNs within these quotient graphs produces a quotient interconnected  
93 dynamical system which is significantly smaller in dimension, specifically when analysing syn-  
94 chronised intercellular dynamics. Namely, these quotient systems were then used to provide  
95 pattern existence conditions for prescribed cellular patterns for interconnected juxtacrine mod-  
96 els. These methods of pattern predictions were later extended in [34] and [35] to simultaneously  
97 couple diffusive and non-diffusive signalling mechanisms within the interconnected dynamical  
98 systems framework using directed multilayer graphs, namely graphs with unidirectional edges  
99 connected to cells with multiple-input and output signals. However, the influence of edge weights  
100 on pattern existence and convergence in these multi-channel interconnected systems is yet to be  
101 investigated.

102 The spatial scalability of the interconnected methods of pattern analysis follows from the  
103 theory of monotone dynamical systems [36]. Provided the intracellular proteins regulated by the  
104 prescribed GRN react monotonically to intercellular stimuli, then global dynamics become pre-  
105 dictable in a closed-loop system of cells and facilitates the introduction of control theoretic tools  
106 for pattern stability [33]. Although, the restriction to bipartite connectivity graphs was imposed  
107 in [29] and [35] as a sufficient measure to preserve the monotonic behaviour of lateral-inhibition  
108 models in the large-scale forms, these restrictions limit the biological applications. Such condi-  
109 tions can be relaxed when seeking pattern existence in quotient systems as demonstrated in [33]  
110 but how such behaviour translates to the large-scale counterpart is not fully understood.

111 We have previously analysed the role of polarity in laminar pattern formation using intercon-  
112 nected methods for a single juxtacrine signalling mechanism [37]. In this study, we generalise  
113 and extend these results to include multiple signalling mechanisms of any type using a multilayer

114 graph approach as defined in [35]. Namely, we explore the interplay of multilayer network topol-  
 115 ogy and edge weights in laminar pattern formation in bilayer tissues using dynamical systems of  
 116 generic competitive kinetics.

117 Initially, we present conditions for the existence, uniqueness and instability of a homogeneous  
 118 steady state for large-scale multi-input-multi-output (MIMO) dynamical system, which extends  
 119 the conditions of [35] to yield analytically applicable statements for low-spatial order intracel-  
 120 lular GRNs. Thereafter we use methods of multilayer graph partitioning to derive polarity  
 121 conditions for the existence of laminar patterning in large-scale systems. Critically, we demon-  
 122 strate the graph commutativity requirements imposed in [35] for simultaneous diagonalisation  
 123 can be relaxed when seeking patterns of only two states, allowing a broader range of quotient  
 124 connectivities to be explored.

125 Next, we investigate the spectral links between quotient and large-scale dynamical systems.  
 126 We demonstrate positional changes of the eigenvalues associated with laminar patterns in the  
 127 multigraphs are dependent on the amount of polarity for non-bipartite graphs. We then discuss  
 128 the implications of spectral rearrangements with respect to bipartite graphs and laminar pat-  
 129 terning. Finally, combining our insights from the spectral rearrangements and quotient system  
 130 analysis, we explore the convergence of laminar patterns in the associated large-scale dynamics  
 131 systems.

132 The structure of the study is as follows. In Section 2.1 we define the large-scale interconnected  
 133 dynamical system analysed in this study. In Section 2.2 we present conditions for the existence,  
 134 uniqueness and instability of a homogeneous steady state for large-scale MIMO dynamical sys-  
 135 tem. Our main results are presented in Section 3 where we introduce the necessary results  
 136 from monotone dynamical systems in Section 3.1 and then present conditions for the existence  
 137 of laminar patterning in Section 3.2. In Section 3.3, we demonstrate positional changes of the  
 138 eigenvalues associated with laminar patterns in the multigraphs are dependent on the amount of  
 139 polarity for non-bipartite graphs. Finally, in Section 3.4, we present sufficient polarity dependent  
 140 conditions for the convergence of laminar patterns in the large-scale systems.

## 141 2. EXISTENCE OF CELLULAR HETEROGENEITY

142 In this section, we are interested in deriving conditions for the existence and instability of a  
 143 homogeneous steady state (HSS) of a large-scale dynamical system that describes intracellular  
 144 kinetics within a tissue of cells. First, we define the types of interconnected dynamical sys-  
 145 tems considered in this study, namely, coupling the multiple input and output signal dynamics  
 146 of individual cells using weighted connectivity graphs associated with each respective signalling  
 147 mechanism. Thereafter, we exploit the repetitive structure of large-scale interconnected dynami-  
 148 cal systems to provide analytically tractable conditions for the existence, uniqueness and stability  
 149 of the HSS that is necessary for the investigation of polarity-driven cellular heterogeneity.

### 150 2.1. The signal polarity interconnected system for bilayer geometries with multiple 151 signal mechanisms.

152

Consider a large-scale interconnected dynamical systems representing  $N$  spatially discrete  
 cells, each containing  $n$  intracellular proteins. Namely, for each cell  $i \in \{1, \dots, N\}$ , let  $\mathbf{x}_i =$   
 $[x_{i,1}, \dots, x_{i,n}]^T \in X \subset \mathbb{R}_{\geq 0}^n$  be the concentration of the intracellular proteins. The cellular signal  
 inputs and outputs are defined by  $\mathbf{u}_i = [u_{i,1}, \dots, u_{i,r}]^T$ ,  $\mathbf{y}_i = [y_{i,1}, \dots, y_{i,r}]^T \in U, Y \subset \mathbb{R}_{\geq 0}^r$ ,

respectively, for  $1 \leq r \leq n$ . The interconnected ODE system has the form

$$\dot{\mathbf{x}}_i = \mathbf{f}(\mathbf{x}_i, \mathbf{u}_i), \quad (2.1)$$

$$\mathbf{y}_i = \mathbf{h}(\mathbf{x}_i), \quad (2.2)$$

153 where  $\dot{\mathbf{x}}_i$  represents the derivative with respect to time. The function  $\mathbf{f} : X \times U \rightarrow X$  defines  
 154 the intracellular protein dynamics which are dependent on external stimuli,  $\mathbf{u}_i$ , produced by  
 155 connected cells. We define cellular connectivity in terms of multiple signalling mechanisms later  
 156 in this section. Furthermore,  $\mathbf{h} : X \rightarrow Y$  describes the translation of intracellular dynamics  
 157 to signal outputs of the cell. We assume that both functions  $\mathbf{f}(\cdot)$  and  $\mathbf{h}(\cdot)$  are both  $\mathcal{C}^2$  over  
 158 their respective domains to ensure the continuity of the corresponding linearised system that is  
 159 required for the interconnected pattern analysis in Section 3. The structure of the IO system  
 160 (2.1) in context of the tissue is shown in Figure 2. For convenience when discussing tissue  
 161 behaviour, we define the large-scale vectorised counterparts of the intracellular state variables,  
 162 signal inputs and outputs by  $\mathbf{x} = [\mathbf{x}_1, \dots, \mathbf{x}_N]^T$ ,  $\mathbf{u} = [\mathbf{u}_1, \dots, \mathbf{u}_r]^T$  and  $\mathbf{y} = [\mathbf{y}_1, \dots, \mathbf{y}_r]^T$ .

163 For the transition of signal outputs to inputs, we assume that each output signal is independent  
 164 and defines a linear relationship between output and input signals. Let  $V := \{v_1, \dots, v_N\}$ , be  
 165 vertices representing the cells in the tissue, then for each output signal  $y_{i,j}$  there is an associated  
 166 connectivity graph  $\mathcal{G}_j = \mathcal{G}_j(V, E_j)$ , where  $E_j$  is the set of edges for each output signal mechanism  
 167  $1 \leq j \leq r$ . Note that the vertex set  $V$  is identical for each connectivity graph whereas edge  
 168 structure may differ between the respective graphs to allow for different signalling mechanisms  
 169 within the IO system (2.1). For example, the cellular connectivity graphs of contact-dependent  
 170 and long-diffusion mechanisms have potentially different edge structures as it is expected that  
 171 the average degree of the contact-based graph is less than that of a diffusive mechanism due to  
 172 the physical constraints of cellular junctions (Figure 2).

173 Algebraically, the cell-cell interaction graphs are represented using the weighted adjacency  
 174 matrix,  $\mathbf{W}_j \in \mathbb{R}_{\geq 0}^{N \times N}$ . Let  $\mathcal{W} := \{\mathbf{W}_j\}$  be the set of weighted and row-stochastic adjacency  
 175 matrices. That is, for any  $j \in \{1, \dots, r\}$  and any row  $i \in \{1, \dots, n\}$  then row-sum  $\sum_k (\mathbf{W}_j)_{ik} = 1$   
 176 which represents the weighted average of signal transfer between connected cells. In addition, we  
 177 assume that the connectivity graph  $\mathcal{G}_j$  associated with  $\mathbf{W}_j$  is undirected and connected, and thus  
 178  $\mathbf{W}_j$  is symmetric and irreducible, i.e., there exists no permutation matrix that transform  $\mathbf{W}_j$  to  
 179 upper triangular form [38]. We note that these assumptions are grounded in the underlying  
 180 biological interactions that we model. Specifically, the undirected property of the graph can be  
 181 justified by the symmetric signalling capacity that arises from the proximity-based juxtacrine  
 182 and paracrine signalling mechanisms. Moreover, the cells represented by vertices in  $\mathcal{G}_j$  belong  
 183 to a single tissue generating a connected structure.

184 To preserve the order of signal outputs,  $y_{i,j}$ , and therefore the cellular structure within the IO  
 185 system (2.1), we construct a global interconnection matrix,  $\mathbf{P}$ , by interweaving each  $\mathbf{W}_j \in \mathcal{W}$   
 186 in order of output signal defined by  $y_{i,j}$ . Namely,

$$\mathbf{P} = \sum_{j=1}^r \mathbf{W}_j \otimes \mathbf{D}_j, \quad (2.3)$$

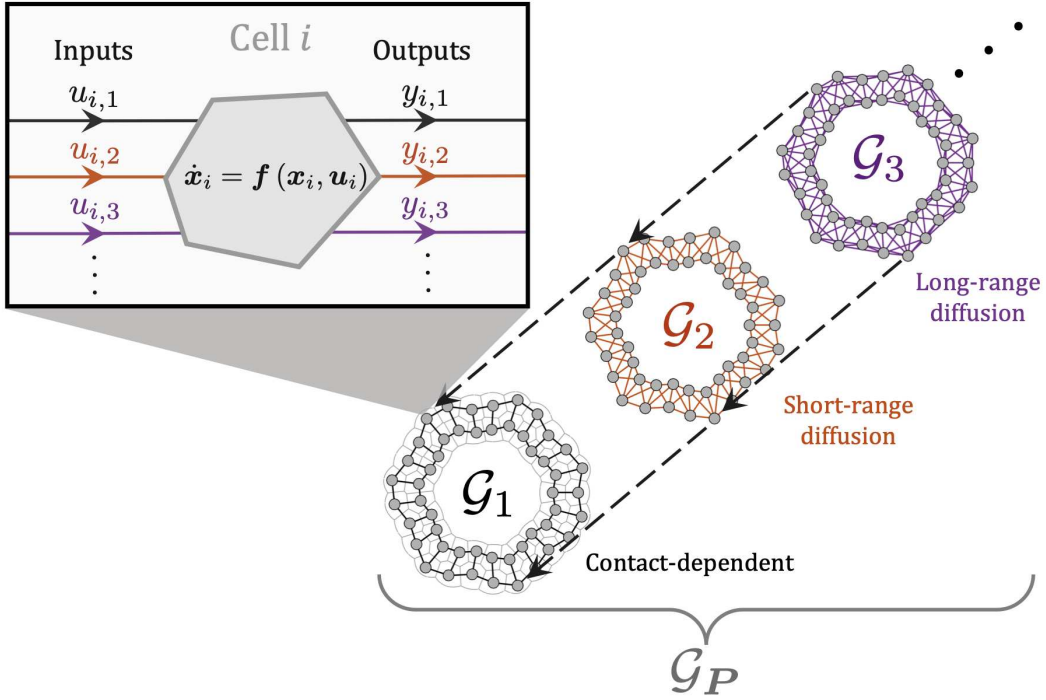


FIGURE 2. A graphical representation of the IO system (2.1) for bilayer geometries with multiple signalling mechanisms defined by the global adjacency matrix  $\mathbf{P}$  (2.3). Example 2D bilayer graphs are shown for contact-dependent short and long-range diffusion over the same vertex set representing the cells in the tissue, which are explicitly shown with membranes in  $\mathcal{G}_1$  to highlight the bilayer cellular structure. Each of the connectivity graphs is then embedded within the same vertex set as indicated by the dashed arrows. Therefore, each vertex contains the intracellular kinetics defined by the IO system (2.1) which responds to the signal outputs of adjacent cells for each signalling mechanism which are transformed to signal inputs by  $\mathbf{P}$  as defined in equation (2.5).

188 where  $\otimes$  is the Kronecker product and  $\mathbf{D}_j = \text{diag}(\delta_{j,1}, \dots, \delta_{j,r})$  for  $\delta_{i,j}$  the Kronecker delta  
 189 function

$$190 \quad \delta_{i,j} = \begin{cases} 1 & i = j, \\ 0 & i \neq j. \end{cases} \quad (2.4)$$

191 The global interconnection matrix  $\mathbf{P} \in \mathbb{R}^{rN \times rN}$  produces a multilayer graph  $\mathcal{G}_{\mathbf{P}}$  that is  
 192 layer-wise independent as shown in Figure 2. Critically, the construction of  $\mathbf{P}$  defines the linear  
 193 relationship between global signal outputs and inputs

$$194 \quad \mathbf{u} = \mathbf{P}\mathbf{y} \quad (2.5)$$

195 where cell-wise input-output structure is preserved. The fundamental cellular identity preserving  
 196 structure of  $\mathbf{P}$  is demonstrated in the following example.

197 **Example 2.1.** Consider the two general matrices

$$198 \quad \mathbf{W}_1 = \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix} \quad \text{and} \quad \mathbf{W}_2 = \begin{bmatrix} b_{11} & b_{12} \\ b_{21} & b_{22} \end{bmatrix} \quad (2.6)$$



199 for the IO system (2.1) with only two cells each with two signal inputs and outputs i.e.,  $r = 2$ .  
 200 Then the global interconnection matrix,  $\mathbf{P}$ , has the form

$$201 \quad \mathbf{P} = \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix} \otimes \begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix} + \begin{bmatrix} b_{11} & b_{12} \\ b_{21} & b_{22} \end{bmatrix} \otimes \begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix} = \begin{bmatrix} a_{11} & 0 & a_{12} & 0 \\ 0 & b_{11} & 0 & b_{12} \\ a_{21} & 0 & a_{22} & 0 \\ 0 & b_{21} & 0 & b_{22} \end{bmatrix}. \quad (2.7)$$

202 To study the role of layer-dependent signalling polarity for the generation of laminar pattern  
 203 in bilayer geometries, we consider each graph to consist of two layers  $\mathcal{L}_1 := \{v_1, \dots, v_{|\mathcal{L}_1|}\}$  and  
 204  $\mathcal{L}_2 := \{v_{|\mathcal{L}_1|+1}, \dots, v_N\}$  where  $|\mathcal{L}_1| = 1, \dots, N - 1$ , as shown in figures 2 and 3.

205 This layer-wise grouping of the vertices also provides consistent structure to the weighted  
 206 adjacency matrices  $\mathbf{W}_k \in \mathcal{W}$ . As a first-approach to the layer-dependent signal polarity, we  
 207 consider only two values of edge weights for connected cells in the same and different layers as  
 208 highlighted in Figure 3. Namely, consider the graph  $\mathcal{G}_k$  associated with  $\mathbf{W}_k$ , then if  $v_i, v_j \in$   
 209  $\mathcal{L}_1$  (or  $\mathcal{L}_2$ ) such that  $v_i$  and  $v_j$  are connected by an edge in  $\mathcal{G}_k$  and are in the same layer, then  
 210  $(\mathbf{W}_k)_{ij} = \hat{w}_1^{[k]}$ , for  $\hat{w}_1^{[k]}$  the row-normalised intralayer edge weight. Similarly, if  $v_i$  and  $v_j$  are in  
 211 different layers,  $v_i \in \mathcal{L}_1$  and  $v_j \in \mathcal{L}_2$ , and are connected in  $\mathcal{G}_k$  then,  $(\mathbf{W}_k)_{ij} = \hat{w}_2^{[k]}$ , for  $\hat{w}_2^{[k]}$  the  
 212 row-normalised interlayer edge weight. Consequently, when vertices are indexed consecutively  
 213 from  $\mathcal{L}_1$  then  $\mathcal{L}_2$ , each  $\mathbf{W}_k$  has block form

$$214 \quad \mathbf{W}_k = \begin{bmatrix} \widehat{\mathbf{W}}_{1, \mathcal{L}_1}^{[k]} & \widehat{\mathbf{W}}_{2, \mathcal{L}_1}^{[k]} \\ \widehat{\mathbf{W}}_{2, \mathcal{L}_1}^{[k]T} & \widehat{\mathbf{W}}_{1, \mathcal{L}_2}^{[k]} \end{bmatrix}, \quad (2.8)$$

215 where  $\widehat{\mathbf{W}}_{1, \mathcal{L}_1}^{[k]} \in \mathbb{R}_{\geq 0}^{|\mathcal{L}_1| \times |\mathcal{L}_1|}$  contains all intralayer connections scaled by  $\hat{w}_1^{[k]}$  for all the vertices in  
 216  $\mathcal{L}_1$ ,  $\widehat{\mathbf{W}}_{2, \mathcal{L}_1}^{[k]} \in \mathbb{R}_{\geq 0}^{|\mathcal{L}_1| \times |\mathcal{L}_2|}$  contains all interlayer connections scaled by  $\hat{w}_2^{[k]}$  for all the vertices in  
 217  $\mathcal{L}_1$ . Similarly  $\widehat{\mathbf{W}}_{1, \mathcal{L}_2}^{[k]} \in \mathbb{R}_{\geq 0}^{|\mathcal{L}_2| \times |\mathcal{L}_2|}$  accounts for the intralayer connections within  $\mathcal{L}_2$ . As each  $\mathcal{G}_k$   
 218 is undirected, the interlayer connections for all vertices in  $\mathcal{L}_2$  are represented by  $\widehat{\mathbf{W}}_{2, \mathcal{L}_1}^{[k]T}$ , that is,  
 219  $\mathbf{W}_k$  is symmetric.

220 **Example 2.2.** The weighted adjacency matrix  $\mathbf{W}_1$  associated with  $\mathcal{G}_1$  in Figure 3 has the block  
 221 matrices

$$222 \quad \widehat{\mathbf{W}}_{1, \mathcal{L}_1} = \begin{bmatrix} 0 & \hat{w}_1^{[1]} & 0 & \cdots & 0 & \hat{w}_1^{[1]} \\ \hat{w}_1^{[1]} & 0 & \hat{w}_1^{[1]} & & & \\ & & \ddots & & & \\ & & & \ddots & & \\ & & & & \hat{w}_1^{[1]} & 0 & \hat{w}_1^{[1]} \\ \hat{w}_1^{[1]} & 0 & \cdots & 0 & \hat{w}_1^{[1]} & 0 \end{bmatrix} \quad \text{and} \quad \widehat{\mathbf{W}}_{2, \mathcal{L}_1} = \begin{bmatrix} \hat{w}_2^{[1]} & 0 & 0 & \cdots & 0 & 0 \\ 0 & \hat{w}_2^{[1]} & 0 & & & \\ & & \ddots & & & \\ & & & \ddots & & \\ & & & & \ddots & \\ & & & & & 0 & \hat{w}_2^{[1]} & 0 \\ 0 & 0 & \cdots & 0 & 0 & 0 & \hat{w}_2^{[1]} \end{bmatrix}, \quad (2.9)$$

223 for  $\hat{w}_1^{[1]} = w_1^{[1]} / |w^{[1]}|$  and  $\hat{w}_2^{[1]} = w_2^{[1]} / |w^{[1]}|$  where  $|w^{[1]}| = 2w_1^{[1]} + w_2^{[1]}$  is the normalising factor  
 224 for all rows ensuring the row-stochastic property of  $\mathbf{W}_1$ . From the regularity of  $\mathcal{G}_1$  in Figure 3,  
 225 we have that  $\widehat{\mathbf{W}}_{1, \mathcal{L}_1} = \widehat{\mathbf{W}}_{1, \mathcal{L}_2}$  as the connections within layers are identical for  $\mathcal{L}_1$  and  $\mathcal{L}_2$ .

226 To summarise the internal cellular dynamics in terms of signal inputs and outputs, as pro-  
 227 posed in [29], we introduce the transfer function  $\mathbf{T} : U \rightarrow Y$  that describes cellular signal output  
 228 response with respect to changes to input signals determined by connected cells. It is assumed  
 229 that  $\mathbf{T}(\cdot)$  is bounded and  $\mathcal{C}^2$  which conforms with the biological context of the IO system (2.1),

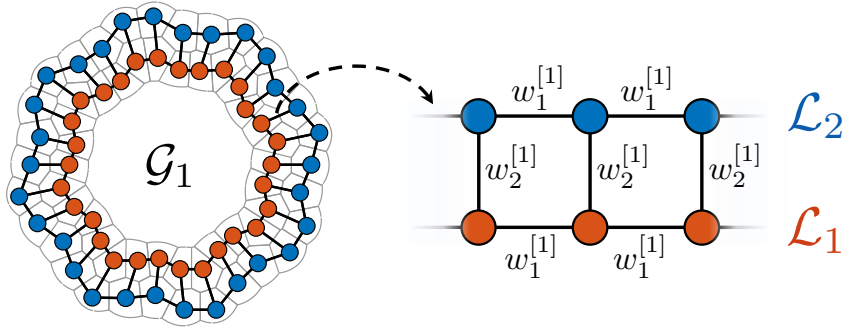


FIGURE 3. Layer-dependent edge weight structure in bilayer graphs. A 2D bilayer graph,  $\mathcal{G}_1$ , with contact-dependent edge connections highlight the layer vertex partitions with vertices in  $\mathcal{L}_1$  and  $\mathcal{L}_2$  coloured orange and blue, respectively. The edge weight structure within and between the layers is shown with edges between vertices in the same layer weighted by  $w_1^{[1]}$  and connected vertices in different layers weighted by  $w_2^{[1]}$ .

230 namely, intracellular expression must remain finite with continuous dependence on the cellular  
 231 microenvironment. The introduction of  $\mathbf{T}(\cdot)$  allows for the analysis of the IO system (2.1) from  
 232 an alternative macroscopic perspective, such that  $\mathbf{T}(\cdot)$  retains the underlying features of the  
 233 intracellular kinetics defined by  $\mathbf{f}(\cdot)$  and  $\mathbf{h}(\cdot)$  while not explicitly defining the intracellular in-  
 234 teractions. For instance, intercellular communication of lateral-inhibition and lateral-induction  
 235 pathways have a decreasing and increasing transfer function  $\mathbf{T}(\cdot)$ , respectively [29, 39]. Explic-  
 236 itly, the transfer function allows for the definition of the auxiliary input to output transition  
 237 relation

$$238 \quad \mathbf{u} = \mathbf{P}[\mathbf{T}(\mathbf{u}_1), \dots, \mathbf{T}(\mathbf{u}_N)]^T, \quad (2.10)$$

239 which reduces the analytic complexity of the macroscopic analysis of spatially driven pattern  
 240 formation in large-scale systems as the dependence of cellular coupling is more accessible in this  
 241 form [29, 33, 35]. However, these methods require that the characteristic behaviour of the GRNs  
 242 is known with respect to intercellular signals i.e., prescribing monotone properties for  $\mathbf{T}(\mathbf{u}_i)$ .

243 In the following section, we show that the zeros of the auxiliary input to output transition  
 244 equation (2.10) are the steady states of the IO system (2.1), thus enabling stability analysis  
 245 of the homogeneous steady state macroscopically. Subsequently, we derive conditions for the  
 246 existence and uniqueness of the HSS in the large-scale system. To induce polarity driven pattern  
 247 formation within the IO system (2.1), we seek sufficient conditions for the instability of HSS  
 248 dependent on the bilayer connectivity graphs  $\mathcal{G}_k$ , and in particular, the polarity weights,  $w_1^{[k]}$   
 249 and  $w_2^{[k]}$ .

## 250 2.2. Existence, uniqueness and stability of the homogeneous steady state in the 251 large-scale IO systems.

252 The majority of statements presented in this section were initially stated in [35] for MIMO IO  
 253 systems. Here, we have independently proven them and partially extended them to comment on  
 254 the uniqueness of the HSS. We include all results for completeness with a focus on the application  
 255 to mixed signal mechanisms in bilayer geometries. Consider the  $\mathcal{C}^2$  function  $\mathbf{S} : U \rightarrow X$  that  
 256 describes the changes to the intracellular kinetics  $\mathbf{x}_i$  by the input signals  $\mathbf{u}_i$  emanating from

257 connected cells. Therefore, the following statement demonstrates that the zeros of the auxiliary  
 258 transfer relation (2.10) are the steady states of the IO system (2.1).

259 **Lemma 2.1** ([34]). *Assume that for some  $\mathbf{u}_0 \in \mathbb{R}^r$  the function  $\mathbf{f}(\mathbf{x}, \mathbf{u}_0) = 0$  has a solution  
 260 denoted by  $\mathbf{x}_0 = \mathbf{S}(\mathbf{u}_0)$  and therefore  $\mathbf{T}(\mathbf{u}_0) = \mathbf{h}(\mathbf{S}(\mathbf{u}_0))$ . If  $\mathbf{u}_0$  satisfies*

$$261 \quad \begin{bmatrix} \mathbf{u}_0 \\ \vdots \\ \mathbf{u}_0 \end{bmatrix} = \mathbf{P} \begin{bmatrix} \mathbf{T}(\mathbf{u}_0) \\ \vdots \\ \mathbf{T}(\mathbf{u}_0) \end{bmatrix} \quad (2.11)$$

262 *then  $\mathbf{x}_0 = \mathbf{S}(\mathbf{u}_0)$  is a steady state of the IO system (2.1). Conversely, if  $\mathbf{S}(\cdot)$  is injective and  $\mathbf{x}_0$   
 263 is a fixed point of the IO system (2.1), then the corresponding  $\mathbf{u}_0$  satisfies the auxiliary system  
 264 (2.11).*

265 Following from Lemma 2.1, we now study the transfer dynamics defined by  $\mathbf{T}(\cdot)$  for the  
 266 existence of the steady states of the IO system (2.1). Critically, as  $\mathbf{T}(\cdot)$  represents changes in  
 267 intercellular signalling,  $\mathbf{T}(\cdot)$  is bounded. Therefore, the following statement ensures the existence  
 268 and uniqueness of a homogeneous steady states of the IO system (2.1) using the boundedness of  
 269 transfer dynamics.

270 **Lemma 2.2.** *Let  $\mathbf{u}_0 \in \mathbb{R}^r$  such that the conditions of Lemma 2.1 hold and  $\mathbf{u}^* = \mathbf{1}_N \otimes \mathbf{u}_0$ . Then,  
 271 there exists  $\mathbf{x}_0 \in \mathbb{R}^n$  such that  $\mathbf{x}^* = \mathbf{1}_N \otimes \mathbf{x}_0$  is a steady state of the IO system (2.1). Moreover,  
 272 if  $\partial \mathbf{f}(\mathbf{x}, \mathbf{u}_0) / \partial \mathbf{x}$  is invertible for all  $\mathbf{x} \in X$  then  $\mathbf{x}^*$  is unique.*

273 *Proof.* It is sufficient to show that there exists  $\mathbf{u}_0 \in \mathbb{R}^r$  such that  $\mathbf{u}^* = \mathbf{1}_N \otimes \mathbf{u}_0$  satisfies the  
 274 auxiliary system (2.11) as  $\mathbf{x}_0 = \mathbf{S}(\mathbf{u}_0)$ . As each  $\mathbf{W}_j \in \mathcal{W}$  is row-stochastic, then the global  
 275 interconnection matrix  $\mathbf{P}$  is also row-stochastic by construction. Consequently, there exists an  
 276 eigenvalue  $\lambda$  of  $\mathbf{P}$  such that  $\mathbf{P}\mathbf{1}_{rN} = \lambda\mathbf{1}_{rN}$  [40], and therefore the proof follows from verifying  
 277 the existence of  $\mathbf{u}_0$  that satisfies  $\mathbf{u}_0 = \lambda\mathbf{T}(\mathbf{u}_0)$ .

278 By the bounded property of  $\mathbf{T} : U \rightarrow Y$ , there exists some constant  $m > 0$  where  $\|\lambda\mathbf{T}(\cdot)\|_2 \leq$   
 279  $m$ . Consider the function  $\mathbf{F} : B_m \rightarrow B_m$  where  $\mathbf{F}(\cdot) = \lambda\mathbf{T}(\cdot)$  and  $B_m = \{\mathbf{v} \in \mathbb{R}^r : \|\mathbf{v}\|_2 \leq m\}$ ,  
 280 noting that  $B_m$  is convex and the continuity of  $\mathbf{F}(\cdot)$  is induced by the continuity of  $\mathbf{T}(\cdot)$ .  
 281 Therefore, by the Brouwer Fixed-Point Theorem [41], there exists some  $\mathbf{u}_0 \in B_m$  such that  
 282  $\mathbf{u}_0 = \mathbf{F}(\mathbf{u}_0) = \lambda\mathbf{T}(\mathbf{u}_0)$ .

283 The uniqueness of the HSS is guaranteed by the following. Assume that for any  $\mathbf{u}_0 \in \mathbb{R}^r$   
 284 there exists  $\bar{\mathbf{x}}_1, \bar{\mathbf{x}}_2 \in \mathbb{R}^n$  where both are solutions to  $\mathbf{f}(\mathbf{x}, \mathbf{u}_0) = \mathbf{0}$ . Specifically,  $f_j(\bar{\mathbf{x}}_1, \mathbf{u}_0) =$   
 285  $f_j(\bar{\mathbf{x}}_2, \mathbf{u}_0)$  for all  $j \in \{1, \dots, n\}$ . Therefore by the Mean Value Theorem [42], we construct the  
 286 linear system

$$287 \quad \begin{bmatrix} 0 \\ \vdots \\ 0 \end{bmatrix} = \begin{bmatrix} \frac{\partial f_1}{\partial x_1}(\mathbf{x}, \mathbf{u}_0) & \cdots & \frac{\partial f_1}{\partial x_n}(\mathbf{x}, \mathbf{u}_0) \\ \vdots \\ \frac{\partial f_n}{\partial x_1}(\mathbf{x}, \mathbf{u}_0) & \cdots & \frac{\partial f_n}{\partial x_n}(\mathbf{x}, \mathbf{u}_0) \end{bmatrix} \begin{bmatrix} \bar{\mathbf{x}}_{11} - \bar{\mathbf{x}}_{21} \\ \vdots \\ \bar{\mathbf{x}}_{1n} - \bar{\mathbf{x}}_{2n} \end{bmatrix} \quad (2.12)$$

288 and from the Invertible Matrix Theorem the kernel of  $\partial \mathbf{f} / \partial \mathbf{x}$  contains only the null vector [43],  
 289 *i.e.*  $\bar{\mathbf{x}}_1 = \bar{\mathbf{x}}_2$ . □

290 **Remark 2.1.** *If the transfer function  $\mathbf{T} : U \rightarrow Y$  is Lipschitz continuous with Lipschitz constant  
 291  $k \in (0, 1]$ , namely,*

$$292 \quad \|\mathbf{T}(\mathbf{u}_i) - \mathbf{T}(\mathbf{u}_j)\|_2 \leq k \|\mathbf{u}_i - \mathbf{u}_j\|_2 \quad (2.13)$$

293 for all  $\mathbf{u}_i, \mathbf{u}_j \in U$ . Then the HSS defined in Lemma 2.2 is unique by the Banach Fixed-Point  
 294 Theorem [44], independent of the invertibility of  $\mathbf{f}(\mathbf{x}, \mathbf{u})$ .

295 As we seek spatially driven instabilities of the HSS, we assume the asymptotic stability of  
 296  $\mathbf{x}^*$  in the absence of cellular connections. We say a fixed-point of a system is stable if the  
 297 associated Jacobian has all eigenvalues with negative real-part. Therefore, we are assuming that  
 298  $\mathbf{A} := \partial \mathbf{f} / \partial \mathbf{x}_i$  evaluated at  $\mathbf{x}_0$  is stable i.e., the intracellular kinetics are not self-exciting in the  
 299 absence of interconnections.

300 A necessary feature for polarity-driven pattern formation in spatially discrete interconnected  
 301 systems is the connectivity-induced instability of the HSS,  $\mathbf{x}^*$ , associated with the IO system  
 302 (2.1), which can be approached by linearisation. The following results provided a convenient  
 303 method of analysing the linear stability of homogeneous large-scale IO systems by assuming each  
 304 cellular connectivity graph  $\mathcal{G}_j$  commutes, thus enabling the parallel computation of eigenvalues  
 305 for each adjacency matrix  $\mathbf{W}_j \in \mathcal{W}$ , reducing the dimensionality of the linearisation.

306 **Lemma 2.3.** Let  $\mathbf{A} := \partial \mathbf{f} / \partial \mathbf{x}_i$ ,  $\mathbf{B} := \partial \mathbf{f} / \partial \mathbf{u}_i$  and  $\mathbf{C} := \partial \mathbf{h} / \partial \mathbf{x}_i$ , be each evaluated at the steady  
 307 state  $\mathbf{x}_0$  for fixed  $\mathbf{u}_0$ . Let the steady state of the global IO system be  $\mathbf{x}^* = \mathbf{1}_N \otimes \mathbf{x}_0$ . Assume  
 308 that all  $\mathbf{W}_j \in \mathcal{W}$  commute and denote  $\mathbf{\Lambda}_j = \text{diag}(\lambda_{1,j}, \dots, \lambda_{r,j})$  where  $\lambda_{i,j}$  is the  $j$ th eigenvalue  
 309 of  $\mathbf{W}_i$  w.r.t. the common eigenbasis of all matrices in  $\mathcal{W}$ . Then  $\mathbf{x}^*$  is asymptotically stable if  
 310  $\mathbf{A} + \mathbf{B}\mathbf{\Lambda}_j\mathbf{C}$  is stable for all  $j$  and unstable otherwise.

*Proof.* Linearisation of the global IO system (2.1) about the fixed point  $\mathbf{x}^* = \mathbf{I}_N \otimes \mathbf{x}_0$  yields the  
 Jacobian

$$\begin{aligned} \mathbf{J} &= \mathbf{I}_N \otimes \mathbf{A} + (\mathbf{I}_N \otimes \mathbf{B}) \mathbf{P} (\mathbf{I}_N \otimes \mathbf{C}), \\ &= \mathbf{I}_N \otimes \mathbf{A} + (\mathbf{I}_N \otimes \mathbf{B}) \left( \sum_{i=1}^r \mathbf{W}_i \otimes \mathbf{D}_i \right) (\mathbf{I}_N \otimes \mathbf{C}), \\ &= \mathbf{I}_N \otimes \mathbf{A} + \sum_{i=1}^r \mathbf{W}_i \otimes \mathbf{B} \mathbf{D}_i \mathbf{C}, \end{aligned} \quad (2.14)$$

311 by direct substitution of the definition of  $\mathbf{P}$  in terms of the independent signalling mechanisms  
 312 and the mixed products property of Kronecker products [45]. As  $\mathbf{W}_i \mathbf{W}_j = \mathbf{W}_j \mathbf{W}_i$  for all  
 313  $\mathbf{W}_i, \mathbf{W}_j \in \mathcal{W}$  and all matrices  $\mathbf{W}_i$  are real and symmetric, then there exists a matrix  $\mathbf{R}$  that  
 314 simultaneously diagonalises all adjacency matrices  $\mathbf{W}_i \in \mathcal{W}$  [43]. Moreover, the eigenbasis  
 315 defined by  $\mathbf{R}$  fixes the order of the diagonal entries in each  $\mathbf{Z}_i = \mathbf{R}^{-1} \mathbf{W}_i \mathbf{R} = \text{diag}(\lambda_{i,1}, \dots, \lambda_{i,N})$   
 316 such that the sum of the diagonalised matrices  $\mathbf{Z}_i$  are unique. Specifically, reordering the  
 317 eigenvectors that form the eigenbasis  $\mathbf{R}$  would only permute the sum of the diagonal values of  
 318  $\mathbf{Z}_i$ .

Consider the transformed Jacobian  $\mathbf{H} = (\mathbf{R}^{-1} \otimes \mathbf{I}_n) \mathbf{J} (\mathbf{R} \otimes \mathbf{I}_n)$  then by the mixed products  
 property of Kronecker products

$$\begin{aligned} \mathbf{H} &= (\mathbf{R}^{-1} \otimes \mathbf{I}_n) (\mathbf{I}_N \otimes \mathbf{A}) (\mathbf{R} \otimes \mathbf{I}_n) + (\mathbf{R}^{-1} \otimes \mathbf{I}_n) \left( \sum_{i=1}^r \mathbf{W}_i \otimes \mathbf{B} \mathbf{D}_i \mathbf{C} \right) (\mathbf{R} \otimes \mathbf{I}_n), \\ &= \mathbf{R}^{-1} \mathbf{I}_N \mathbf{R} \otimes \mathbf{I}_n \mathbf{A} \mathbf{I}_n + \sum_{i=1}^r \mathbf{R}^{-1} \mathbf{W}_i \mathbf{R} \otimes \mathbf{I}_n \mathbf{B} \mathbf{D}_i \mathbf{C} \mathbf{I}_n, \\ &= \mathbf{I}_N \otimes \mathbf{A} + \sum_{i=1}^r \mathbf{Z}_i \otimes \mathbf{B} \mathbf{D}_i \mathbf{C}. \end{aligned} \quad (2.15)$$

319 By the diagonal structure of  $\mathbf{Z}_i$  the matrix  $\mathbf{H}$  has the block diagonal form

$$320 \quad \mathbf{H} = \begin{bmatrix} \mathbf{A} + \sum_{i=1}^r \lambda_{i,1} \mathbf{B} \mathbf{D}_i \mathbf{C} & & \\ & \ddots & \\ & & \mathbf{A} + \sum_{i=1}^r \lambda_{i,n} \mathbf{B} \mathbf{D}_i \mathbf{C} \end{bmatrix}, \quad (2.16)$$

321 and therefore, as  $\sum_{i=1}^r \lambda_{i,j} \mathbf{B} \mathbf{D}_i \mathbf{C} = \mathbf{B} \mathbf{\Lambda}_j \mathbf{C}$  then the eigenvalues of  $\mathbf{H}$  are those of  $\mathbf{A} + \mathbf{B} \mathbf{\Lambda}_j \mathbf{C}$   
 322 for all  $1 \leq j \leq N$ . Consequently, if  $\mathbf{A} + \mathbf{B} \mathbf{\Lambda}_j \mathbf{C}$  has eigenvalues with all negative real-part, for  
 323 all  $1 \leq j \leq N$ , then  $\mathbf{H}$  is stable and therefore the stability of  $\mathbf{J}$  follows by the bijection between  
 324 the linearised systems  $\mathbf{H}$  and  $\mathbf{J}$ .  $\square$

325 Before discussing the behaviour of flows of the IO system (2.1) near the HSS, we first introduce  
 326 a convenient condition for the instability of a matrix.

327 **Lemma 2.4** ([33]). *If  $\mathbf{M} \in \mathbb{R}^{n \times n}$  is stable then  $(-1)^n \det(\mathbf{M}) > 0$ . Conversely, if  $(-1)^n \det(\mathbf{M}) <$   
 328  $0$  then  $\mathbf{M}$  has an eigenvalue with positive real-part.*

329 Invoking lemmas 2.3 and 2.4 leads to the following sufficient condition for the instability of  
 330 the HSS associated with an IO system (2.1) with commuting connectivity graphs  $\mathcal{G}_j$ .

331 **Theorem 2.1.** *Consider the large-scale IO system (2.1) that is spatially coupled via the global  
 332 interconnection matrix  $\mathbf{P}$  (2.3) such that each  $\mathbf{W}_j \in \mathcal{W}$  commute. Denote  $\mathcal{DT} := \partial \mathbf{T} / \partial \mathbf{u}_i$  and  
 333 let  $\mathbf{\Lambda}_j = \text{diag}(\lambda_{1,j}, \dots, \lambda_{r,j})$  where  $\lambda_{i,j}$  is the  $j$ th eigenvalue of  $\mathbf{W}_i$  w.r.t. the common eigenbasis  
 334 of all matrices in  $\mathcal{W}$ . Then the HSS  $\mathbf{x}^* = \mathbf{1}_N \otimes \mathbf{x}_0$  is unstable if there exists a  $\mathbf{\Lambda}_j$  such that*

$$335 \quad \prod_{i=1}^r (1 - \mu_{i,j}) < 0, \quad (2.17)$$

336 where  $\mu_{i,j}$  are the eigenvalues of  $\mathbf{\Lambda}_j \mathcal{DT}(\mathbf{u}_0)$  and  $\mathbf{u}_0$  is the steady state input vector associated  
 337 with  $\mathbf{x}_0$ .

*Proof.* By Lemma 2.3 we only need to show that there exists a positive eigenvalue of  $\mathbf{A} + \mathbf{B} \mathbf{\Lambda}_j \mathbf{C}$   
 for  $\mathbf{\Lambda}_j$  some diagonal matrix of eigenvalues of all matrices  $\mathbf{W}_i \in \mathcal{W}$  to demonstrate the instability  
 of the HSS. Consider  $(-1)^n \det(\mathbf{A} + \mathbf{B} \mathbf{\Lambda}_j \mathbf{C})$ , then by Sylvester's Determinant Theorem [46] we  
 have that,

$$\begin{aligned} (-1)^n \det(\mathbf{A} + \mathbf{B} \mathbf{\Lambda}_j \mathbf{C}) &= (-1)^n \det(\mathbf{A}) \det(\mathbf{I}_r + \mathbf{\Lambda}_j \mathbf{C} \mathbf{A}^{-1} \mathbf{B}), \\ &= (-1)^n \det(\mathbf{A}) \det(\mathbf{I}_r - \mathbf{\Lambda}_j \mathcal{DT}(\mathbf{u}_0)), \end{aligned} \quad (2.18)$$

338 where the final equality holds from  $\mathcal{DT}(\mathbf{u}_0) = -\mathbf{C} \mathbf{A}^{-1} \mathbf{B}$  as derived in [29]. As  $\mathbf{A}$  is stable  
 339 by assumption we have that  $\mathbf{A}^{-1}$  exists and  $(-1)^n \det(\mathbf{A}) > 0$  by Lemma 2.4. Therefore if  
 340  $\det(\mathbf{I}_r - \mathbf{\Lambda}_j \mathcal{DT}(\mathbf{u}_0)) < 0$  then  $\mathbf{x}^* = \mathbf{1}_N \otimes \mathbf{x}_0$  is unstable, by the converse statement of Lemma  
 341 2.4. Hence as the determinant of a matrix is the product of the eigenvalues [43], we have that

$$342 \quad \det(\mathbf{I}_r - \mathbf{\Lambda}_j \mathcal{DT}(\mathbf{u}_0)) = \prod_{i=1}^r (1 - \mu_{i,j}) \quad (2.19)$$

343 for all matrices  $\mathbf{\Lambda}_j$  ( $1 \leq j \leq N$ ).  $\square$

344 Applying the HSS instability condition derived in Theorem 2.1 IO systems with one, or two,  
 345 spatially dependent components, known as single-input-single-output (SISO) and double-input-  
 346 double-output (DIDO) interconnected systems produces simple forms of the instability condition

347 (2.17). Explicitly, the IO system (2.1) is SISO when  $r = 1$  and DIDO when  $r = 2$ . Let  $\text{Spec}(\mathbf{M})$   
 348 denote the set of eigenvalues of  $\mathbf{M}$  then, critically, we recover the SISO instability condition  
 349 initially derived in [29] where we allow for generic intracellular kinetics here.

350 **Corollary 2.1.** *Consider the large-scale IO system (2.1) and denote  $\mathcal{DT} := \partial\mathbf{T}/\partial\mathbf{u}_i$ . We have:*

351 (i) *If the IO system (2.1) is SISO with connectivity matrix  $\mathbf{W}_1$  then the HSS  $\mathbf{x}^* = \mathbf{1}_N \otimes \mathbf{x}$*   
 352 *is unstable if*

$$353 \quad 1 < \lambda_{1,j} T'(u_0) \quad (2.20)$$

354 *for some  $\lambda_{1,j} \in \text{Spec}(\mathbf{W}_1)$ .*

355 (ii) *If the IO system (2.1) is DIDO with global interconnection matrix  $\mathbf{P}$  constructed by the*  
 356 *commutative adjacency matrices  $\mathbf{W}_1$  and  $\mathbf{W}_2$  then the HSS  $\mathbf{x}^* = \mathbf{1}_N \otimes \mathbf{x}$  is unstable if*

$$357 \quad 1 < \text{tr}(\mathbf{\Lambda}_j \mathcal{DT}(\mathbf{u}_0)) - \det(\mathbf{\Lambda}_j \mathcal{DT}(\mathbf{u}_0)) \quad (2.21)$$

358 *for some  $\mathbf{\Lambda}_j = \text{diag}(\lambda_{1,j}, \lambda_{2,j})$ , where  $\lambda_{1,j} \in \text{Spec}(\mathbf{W}_1)$  and  $\lambda_{2,j} \in \text{Spec}(\mathbf{W}_2)$  both*  
 359 *associated with the same eigenvector.*

*Proof.* In the case of a SISO system when  $r = 1$ , the  $T : U \rightarrow V$  is a scalar function and we have that inequality (2.17) simply becomes  $1 - \lambda_{1,j} T'(u_0) < 0$  yielding the SISO condition (2.20). For a DIDO system where  $r = 2$ , there are two potentially different adjacency matrices  $\mathbf{W}_1$  and  $\mathbf{W}_2$  that form  $\mathbf{P}$ . Therefore from inequality (2.17) we have that

$$\begin{aligned} 0 &> (1 - \mu_1)(1 - \mu_2) = 1 + \text{tr}(-\mathbf{\Lambda}_j \mathcal{DT}(\mathbf{u}_0)) + \det(-\mathbf{\Lambda}_j \mathcal{DT}(\mathbf{u}_0)) \\ &= 1 - \text{tr}(\mathbf{\Lambda}_j \mathcal{DT}(\mathbf{u}_0)) + \det(\mathbf{\Lambda}_j \mathcal{DT}(\mathbf{u}_0)) \end{aligned} \quad (2.22)$$

360 using the relations between determinant, trace and the eigenvalues of a matrix [43]. Rearrange-  
 361 ment of inequality (2.22) yields the DIDO HSS instability condition (2.21).  $\square$

362 The HSS instability conditions outlined in Theorem 2.1 allow the study of polarity regimes  
 363 via graph edge weights to induce heterogeneity of cellular states within the bilayer tissues using  
 364 analytic methods. Critically, the sufficient patterning conditions of Theorem 2.1 are independent  
 365 of the precise intracellular kinetics and we do not impose any specific feature on the transfer  
 366 function,  $\mathbf{T}(\cdot)$ , other than the mild requirement of boundedness that follows immediately when  
 367 modelling protein dynamics.

368 In the following section, we introduce methods of graph partitioning for templating laminar  
 369 patterns in bilayer geometries that produce analytic conditions for the existence of the laminar  
 370 patterns with multiple signalling mechanisms. Formally, we define laminar patterns in bilayer  
 371 graphs in the following way.

372 **Definition 2.1** (Laminar patterns in bilayer IO systems). *Let  $\mathbf{x}_+$  and  $\mathbf{x}_-$  be heterogeneous*  
 373 *steady states of the IO system (2.1), i.e.,  $\mathbf{x}_+, \mathbf{x}_- \in X$  and  $\mathbf{x}_- \neq \mathbf{x}_+$ . The IO system (2.1)*  
 374 *is said to have converged to a laminar pattern state if for some  $t_0 > 0$  we have  $\mathbf{x}_i = \mathbf{x}_-$  and*  
 375  *$\mathbf{x}_j = \mathbf{x}_+$  for  $i = 1, \dots, |\mathcal{L}_1|$  and  $j = |\mathcal{L}_1| + 1, \dots, N$  and for all  $t > t_0$ .*

376 When seeking laminar pattern formation the bilayer IO systems 2.1, we show that the commu-  
 377 tative properties of the adjacency matrices  $\mathbf{W}_j \in \mathcal{W}$  required for the HSS instability condition in  
 378 Theorem 2.1 can be relaxed when seeking dichotomous cell states in bilayer structures with same  
 379 layer connectivity symmetries, namely semi-regular bilayer graphs. In addition, by restricting  
 380 the characteristic behaviour of intracellular kinetics to competitive interactions, we ensure that

381 the HSS instability converges to laminar patterns by applying results from monotone dynamical  
 382 system theory.

### 383 3. LAMINAR PATTERN CONVERGENCE WITH MONOTONE KINETICS IN SEMI-REGULAR BILAYER 384 GRAPHS

385 The instability of the HSS of the IO system (2.1) does not imply the existence of stable het-  
 386 erogeneous cell states, even in systems with a unique HSS and bounded dynamics as there may  
 387 exist oscillatory or chaotic solution trajectories. We leverage results from monotone dynamics  
 388 systems and techniques of graph symmetry reduction to ensure the convergence to dichotomous  
 389 cell states at the instance of HSS instability in the bilayer geometries. These methods of discrete  
 390 pattern analysis were first introduced for SISO systems in [33] and later briefly extended to  
 391 MIMO systems in [35]. Here we demonstrate the applicability of these methods to two-state  
 392 pattern formation with pathway crosstalk kinetics in bilayer geometries. In addition, we empha-  
 393 sise the link to the corresponding large-scale IO system (2.1), namely, when are the predicted  
 394 patterns in the symmetry reduced system preserved in the large-scale system.

#### 395 3.1. Monotone kinetics for pattern convergence.

396 Let  $\phi_t(\mathbf{x}_1)$  and  $\phi_t(\mathbf{x}_2)$  be two solutions to the IO system (2.1) where  $\mathbf{x}_1 \leq \mathbf{x}_2$  are initial  
 397 conditions. It is said that the dynamical system (2.1) is monotone if  $\phi_t(\mathbf{x}_1) \leq \phi_t(\mathbf{x}_2)$  for all  
 398  $t \in [0, \infty)$  [47]. Furthermore, the IO system (2.1) is said to be strongly monotone if  $\phi_t(\mathbf{x}_1) <$   
 399  $\phi_t(\mathbf{x}_2)$  for all  $t \in [0, \infty)$  [47]. Critically, the property of strong monotonicity is crucial for the  
 400 asymptotic convergence of solutions  $\phi_t(\mathbf{x})$  on bounded domains  $X \subset \mathbb{R}_{\geq 0}^n$ , analogous to the  
 401 Monotone Convergence Theorem for bounded sequences [48].

402 A dynamical system can be shown to be monotone by studying the sign structure of the  
 403 associated Jacobian matrix on convex domains. The trajectory domain  $X$  is convex if for any  
 404  $\mathbf{a}, \mathbf{b} \in X$  then  $t\mathbf{a} + (1-t)\mathbf{b} \in X$  for all  $t \in [0, 1]$ , i.e., there exists a line segment between any two  
 405 points in the domain that lies in the interior of  $X$ . The monotone identification via the Jacobian  
 406 matrix relies on the inter-component monotonicity of vector-valued functions and the convexity  
 407 of their respective domains, as initially studied by Kamke [49], leading to the classification of  
 408 type K functions. Namely, a function  $\mathbf{g}(\cdot)$  is said to be type K if for each  $i$ ,  $\mathbf{g}_i(\mathbf{a}) \leq \mathbf{g}_i(\mathbf{b})$   
 409 for any two points  $\mathbf{a}, \mathbf{b} \in X$  satisfying  $\mathbf{a} \leq \mathbf{b}$  and  $\mathbf{a}_i = \mathbf{b}_i$  where  $X$  is a convex domain [47].  
 410 The identification of type K functions in dynamical systems leads to the sufficient condition for  
 411 monotone trajectories.

412 **Lemma 3.1** (Type K monotone systems [47]). *Consider the general autonomous dynamical*  
 413 *system*

$$414 \quad \dot{\mathbf{z}} = \mathbf{g}(\mathbf{z}), \quad (3.1)$$

415 *where  $\mathbf{z} \in Z$  and  $Z \subset \mathbb{R}^n$  is convex. Then the dynamical system (3.1) is monotone if it is type*  
 416 *K. Furthermore, by the Fundamental Theorem of Calculus, the general autonomous dynamical*  
 417 *system (3.1) is guaranteed to be type K when the row-sums of the associated Jacobian satisfy*

$$418 \quad \sum_{j \neq i} \frac{\partial \mathbf{g}_i}{\partial \mathbf{z}_j} \geq 0 \quad (3.2)$$

419 *for all  $1 \leq i \leq n$ .*

420 A direct consequence of Lemma 3.1 is that the IO system (2.1) is monotone provided that all  
 421 off-diagonal components of the associated Jacobian are non-negative for all  $\mathbf{x} \in X$  as previously  
 422 applied in large-scale IO pattern formation studies [29, 34]. In addition, Hirsch provided a  
 423 sufficient condition for strong monotonicity that is dependent on the irreducibility of the Jacobian  
 424 of the dynamical system [50]. Specifically, a matrix  $\mathbf{M}$  is said to be irreducible if there exists  
 425 no permutation matrix  $\mathbf{U}$  such that  $\mathbf{U}^T \mathbf{M} \mathbf{U}$  is in upper block triangular form [46].

426 **Lemma 3.2** ([47]). *Consider the dynamical system (3.1) as in Lemma 3.1. If the Jacobian,  $\frac{\partial \mathbf{g}}{\partial \mathbf{z}}$ ,*  
 427 *is irreducible and type K for all  $z \in Z$  then system (3.1) is strongly monotone.*

428 The combination of lemmas 3.1 and 3.2 yield sufficient conditions for the identification of  
 429 strongly monotone dynamical systems using standard linearisation methods, which are particu-  
 430 larly applicable to interconnected dynamical systems. Namely, connected graphs have irreducible  
 431 adjacency matrices [38].

432 Time-dependent monotone systems are often characterised into two distinctive classes: coop-  
 433 erative dynamics where all solutions are monotone in forward-time ( $t \rightarrow \infty$ ), and competitive  
 434 dynamics where all solutions are monotone in backward-time ( $t \rightarrow -\infty$ ) [47]. It has previously  
 435 been demonstrated that competitive dynamics lead to pattern generation in large-scale IO sys-  
 436 tems, specifically, when studying processes of mutual cellular inhibition which are a common  
 437 feature of cell-fate dynamics in developing tissues [29]. For example, the lateral-inhibition inter-  
 438 actions of Notch1 and Delta1 are often found in tissues with a dichotomy of spatially organised  
 439 cell-types and conform to the monotone competitive description [10, 26]. Subsequently, we focus  
 440 our attention on competitive intracellular kinetics which leads to the following assumption on  
 441 the behaviour of the transfer function  $\mathbf{T}(\cdot)$  to ensure the asymptotic convergence of solutions  
 442 with tissue heterogeneity.

(A1) The derivative of the transfer function  $\mathcal{DT}(\mathbf{u})$  of the IO system (2.1) has one of the  
 following sign structures

$$\mathcal{S}_1 = \begin{bmatrix} - & + & \cdots & - & + \\ + & - & \cdots & + & - \\ & & \vdots & & \\ - & + & \cdots & - & + \\ + & - & \cdots & + & - \end{bmatrix} \quad \mathcal{S}_2 = \begin{bmatrix} - & - & \cdots & - & - \\ - & - & \cdots & - & - \\ & & \vdots & & \\ - & - & \cdots & - & - \\ - & - & \cdots & - & - \end{bmatrix}$$

443 for all  $\mathbf{u} \in U$  where any sign can be replaced by zero provided  $\mathcal{DT}(\mathbf{u})$  is irreducible.

444 Critically, the conditions imposed on the intracellular kinetics by (A1) are not restrictive in  
 445 the context of cellular pattern formation as activation and repression of intracellular signals are  
 446 typically modelled using monotonic functions, such as Hill or logistic functions that relate to  
 447 Michaelis-Menten kinetics for enzyme-catalyst reactions [51]. Furthermore, the irreducibility of  
 448  $\mathcal{DT}(\mathbf{u})$  follows immediately if there exist no zero entries. That is, each spatially dependent  
 449 component is continuously dependent on all other spatially dependent components.

450 In the following section, we will use the competitive properties of the transfer function to  
 451 predict the existence of laminar pattern formation in bilayer geometries graph partitioning. In  
 452 particular, we focus on the analysis of the transfer function, as this considers only the spatially



453 dependent components of the IO system (2.1), which potentially reduces the dimensionality of  
 454 the analysis while preserving the underlying behaviour of the system.

### 455 3.2. Dimension reduction by graph partition for polarity laminar pattern existence.

456

457 Methods of graph partitioning have previously been employed in large-scale IO systems to  
 458 predict the existence of patterns with a predefined pattern structure [33, 34]. These predefined  
 459 pattern structures allow for the construction of bespoke systems by exploiting the symmetries  
 460 of the cellular connectivity graphs,  $\mathcal{G}_k$ , thereby analysing only representative vertices from each  
 461 pattern partition of the large-scale graphs, vastly reducing the dimensionality associated IO sys-  
 462 tems. Under the assumption of monotone transfer kinetics (A1), we provide sufficient conditions  
 463 for the existence of polarity-driven laminar patterns in bilayer geometries with multiple spatially  
 464 dependent components using graph partitioning. Critically, we demonstrate the prior require-  
 465 ment of commutative connectivity graphs  $\mathcal{G}_j$  can be relaxed when seeking patterns with only  
 466 two contrasting states.

467 The method of pattern templating via graph partitions seeks to group cells that are assumed  
 468 to have the same steady-state solutions and therefore impose that cells within the same group  
 469 behave identically, or more formally described as synchronised vertex dynamics [30, 52, 53]. This  
 470 assumption allows for the study of two representative cells from each layer in the bilayer large-  
 471 scale graphs,  $\mathcal{G}_k$ , to predict the existence of laminar patterns as shown in Figure 4. Formally,  
 472 we are assuming the existence of an equitable partition,  $\pi_2$ , of the vertices  $v_i \in V$  into the  
 473 pattern groups  $\mathcal{L}_1$  and  $\mathcal{L}_2$  of each layer for all connectivity graphs  $\mathcal{G}_k$ . This means that  $v \in \mathcal{L}_i$   
 474 has the same number of adjacent vertices in both  $\mathcal{L}_1$  and  $\mathcal{L}_2$ , independent of the vertex,  $v$  [38].  
 475 We are imposing that cells within the same layer have the same edge connectivity structure,  
 476 and therefore the connectivity graphs  $\mathcal{G}_k$  must be layer-wise regular as highlighted in Figure 4.  
 477 However, we note that these connectivity symmetries are not necessary for the construction of  
 478 an equitable partition with weighted edges [53]. Algebraically, the partition  $\pi_2$  is equitable if  
 479 there exists some  $\bar{w}_{ij}^{[k]} > 0$  such that

$$480 \quad \sum_{v \in \mathcal{L}_j} \hat{w}_{uv}^{[k]} = \bar{w}_{ij}^{[k]} \quad \forall u \in \mathcal{L}_i, \quad (3.3)$$

481 where  $\hat{w}_{ij}^{[k]}$  are the  $ij$ -th elements of the row-stochastic adjacency matrix  $\mathbf{W}_k \in \mathcal{W}$  [38]. In  
 482 addition, we say that the laminar pattern partition,  $\pi_2$ , is simultaneously equitable if  $\pi_2$  is  
 483 equitable for all graphs  $\mathcal{G}_k$ .

484 Let  $\bar{\mathbf{W}}_k \in \mathbb{R}_{\geq 0}^{2 \times 2}$  be the reduced adjacency matrix for the quotient graph  $\mathcal{G}_{k, \pi_2} = \mathcal{G}_k / \pi_2$  as  
 485 depicted in Figure 4, that are element-wise composed with the constants defined by equation  
 486 (3.3). Applying the IO preserving interconnection matrix definition (2.3) to the set of reduced  
 487 adjacency matrices, we have the reduced interconnection matrix of the form,

$$488 \quad \bar{\mathbf{P}} = \sum_{i=1}^r \bar{\mathbf{W}}_i \otimes \mathbf{D}_i, \quad (3.4)$$

489 noting that the row-stochastic property of each  $\mathbf{W}_i \in \mathcal{W}$  is preserved in the quotient mapping  
 490 such that each  $\bar{\mathbf{W}}_i$  is row-stochastic. In particular, as the partition  $\pi_2$  allocates the vertices

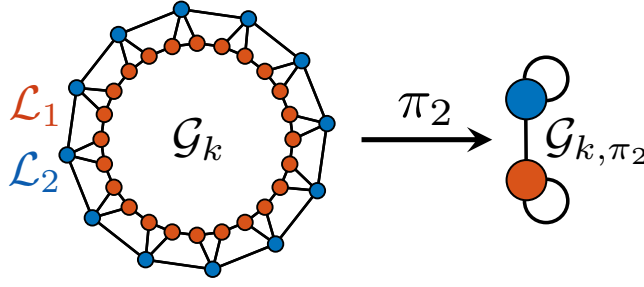


FIGURE 4. Templating for laminar patterns in bilayer geometries using the equitable partition  $\pi_2$ . The cellular connectivity graph,  $\mathcal{G}_k$ , is semi-regular such vertices within the same layer,  $\mathcal{L}_1$  or  $\mathcal{L}_2$ , have the same number of adjacent vertices in each of the layers which induce an edge symmetry with respect to vertices in the same layer. The equitable partition,  $\pi_2$ , leverages the edge symmetries of  $\mathcal{G}_k$  to generate a quotient graph  $\mathcal{G}_{k, \pi_2}$  consisting of two representative cells, one from each layer  $\mathcal{L}_1$  and  $\mathcal{L}_2$ .

491  $v \in V$  into either of the sets,  $\mathcal{L}_1$  or  $\mathcal{L}_2$ , each reduced adjacency matrix is of the form,

$$492 \quad \overline{\mathbf{W}}_i = \begin{bmatrix} a_i & 1 - a_i \\ 1 - b_i & b_i \end{bmatrix} \quad (3.5)$$

493 for all  $1 \leq i \leq r$ , where  $a_i, b_i \in (0, 1)$  are composed of the polarity weights  $w_1^{[i]}$  and  $w_2^{[i]}$ .  
494 Explicitly,  $a_i$  and  $b_i$  have the layer-dependent form

$$495 \quad a_i = \frac{n_{1, \mathcal{L}_1}^{[i]} w_1^{[i]}}{n_{1, \mathcal{L}_1}^{[i]} w_1^{[i]} + n_{2, \mathcal{L}_1}^{[i]} w_2^{[i]}} \quad \text{and} \quad b_i = \frac{n_{1, \mathcal{L}_2}^{[i]} w_1^{[i]}}{n_{1, \mathcal{L}_2}^{[i]} w_1^{[i]} + n_{2, \mathcal{L}_2}^{[i]} w_2^{[i]}}, \quad (3.6)$$

496 where the superscripts correspond to the spatial connectivity mechanism,  $i$ , and  $n_{1, \mathcal{L}_j}^{[i]} \geq 1$  and  
497  $n_{2, \mathcal{L}_j}^{[i]} \geq 1$  are the number of connected vertices in the same and opposing layer, respectively,  
498 from the perspective of each layer,  $j = 1, 2$ . For example,  $n_{1, \mathcal{L}_1}^{[k]} = n_{1, \mathcal{L}_2}^{[k]} = 2$ ,  $n_{2, \mathcal{L}_1}^{[k]} = 1$  and  
499  $n_{2, \mathcal{L}_2}^{[k]} = 2$  for  $\mathcal{G}_k$  in Figure 4.

500 A key property of the equitable partition,  $\pi_2$ , is the preservation of eigenvalues when mapping  
501 between the large-scale and quotient graphs, that is,  $\text{Spec}(\overline{\mathbf{W}}_i) \subset \text{Spec}(\mathbf{W}_i)$  [38]. Using this  
502 property, any spatially driven instability of the HSS observed in the quotient system also exists  
503 in the associated large-scale system. However, to apply the HSS instability conditions derived  
504 in Theorem 2.1 to large-scale connectivity graphs, we require that all  $\overline{\mathbf{W}}_i$  must commute to  
505 generate a common eigenbasis for simultaneous diagonalisation. Commutativity is not preserved  
506 in the quotient transformation in general due to the reduced form of equation (3.5). Although,  
507 the following statement enables the use of the HSS instability conditions independent of the  
508 commutative properties of  $\overline{\mathbf{W}}_i$  by demonstrating the existence of a common eigenbasis for all  
509 reduced adjacency matrices partitioned by  $\pi_2$ , independent of commutativity.

510 **Lemma 3.3.** Let  $\overline{\mathbf{P}} \in \mathbb{R}_{\geq 0}^{2r \times 2r}$  be the reduced mixed interconnection matrix (3.4) associated with  
511 the equitable partition  $\pi_2$ . Given any matrix  $\widetilde{\mathbf{M}} \in \mathbb{R}^{r \times r}$  where  $\mathbf{M} = \mathbf{I}_2 \otimes \widetilde{\mathbf{M}}$  the eigenvalues of  
512  $\overline{\mathbf{P}}\mathbf{M}$  are those of  $\widetilde{\mathbf{M}}$  and  $\overline{\Lambda}_2 \widetilde{\mathbf{M}}$  where  $\overline{\Lambda}_2 = \text{diag}(a_1 + b_1 - 1, \dots, a_r + b_r - 1)$ .

513 *Proof.* By definition of the family of the reduced adjacency matrices (3.5),  $\text{Spec}(\overline{\mathbf{W}}_i) = \{1, a_i +$   
514  $b_i - 1\}$ , where all reduced adjacency matrices share the common eigenvector  $\mathbf{v}_1 = \mathbf{1}_2$ , associated

515 with the common eigenvalue  $\bar{\lambda}_{i,1} = 1$ . Without loss of generality, let  $\tilde{\mathbf{R}}$  be the transformation  
 516 matrix for  $\bar{\mathbf{W}}_1$  such that  $\tilde{\mathbf{R}}^{-1}\bar{\mathbf{W}}_1\tilde{\mathbf{R}}$  is in Jordan normal form [43]. Specifically, as  $\mathbf{v}_1$  must  
 517 represent a column of  $\tilde{\mathbf{R}}$  as it is an eigenvector for all  $\bar{\mathbf{W}}_i$ , then let  $\mathbf{v}_1$  form the first column of  
 518  $\tilde{\mathbf{R}}$  such that  $\tilde{\mathbf{R}}^{-1}\bar{\mathbf{W}}_1\tilde{\mathbf{R}}$  has diagonal entries 1 and  $a_1 + b_1 - 1$ , respectively. Moreover, as each  
 519  $\bar{\mathbf{W}}_i \in \mathbb{R}_{\geq 0}^{2 \times 2}$ , then  $\tilde{\mathbf{R}}^{-1}\bar{\mathbf{W}}_i\tilde{\mathbf{R}}$  must be upper triangular form as 1 is a common eigenvalue for  
 520 all  $1 \leq i \leq r$ , that is,  $\tilde{\mathbf{R}}$  simultaneously upper triangularises the family of reduced adjacency  
 521 matrices such that each  $\tilde{\mathbf{R}}^{-1}\bar{\mathbf{W}}_i\tilde{\mathbf{R}}$  has diagonal entries 1 and  $a_i + b_i - 1$ .

Consider the invertible transformation  $\mathbf{R} = \tilde{\mathbf{R}} \otimes \mathbf{I}_r$ . Denote the adjacency triangulation  
 transformation of  $\bar{\mathbf{P}}\mathbf{M}$  by  $\mathbf{H} = \mathbf{R}^{-1}\bar{\mathbf{P}}\mathbf{M}\mathbf{R}$ . Therefore, we have that

$$\begin{aligned} \mathbf{H} &= \left( \tilde{\mathbf{R}}^{-1} \otimes \mathbf{I}_r \right) \bar{\mathbf{P}}\mathbf{M} \left( \tilde{\mathbf{R}} \otimes \mathbf{I}_r \right), \\ &= \left( \tilde{\mathbf{R}}^{-1} \otimes \mathbf{I}_r \right) \left( \sum_{i=1}^r \bar{\mathbf{W}}_i \otimes \mathbf{D}_i \right) \left( \mathbf{I}_2 \otimes \tilde{\mathbf{M}} \right) \left( \tilde{\mathbf{R}} \otimes \mathbf{I}_r \right), \\ &= \sum_{i=1}^r \tilde{\mathbf{R}}^{-1} \bar{\mathbf{W}}_i \tilde{\mathbf{R}} \otimes \mathbf{D}_i \tilde{\mathbf{M}}. \end{aligned} \quad (3.7)$$

522 Specifically,  $\mathbf{H}$  is of block upper triangle form such that

$$523 \quad \mathbf{H} = \begin{bmatrix} \mathbf{I}_r \tilde{\mathbf{M}} & \mathbf{Z} \tilde{\mathbf{M}} \\ \mathbf{0} & \bar{\Lambda}_2 \tilde{\mathbf{M}} \end{bmatrix}, \quad (3.8)$$

524 where  $\mathbf{Z}$  is some real  $r \times r$  matrix constructed by interweaving the upper right entries of the  
 525 transformed reduced adjacency matrices. Thus the eigenvalues of  $\mathbf{H}$  are those of  $\tilde{\mathbf{M}}$  and  $\bar{\Lambda}_2 \tilde{\mathbf{M}}$ ,  
 526 and therefore are the eigenvalues of  $\bar{\mathbf{P}}\mathbf{M}$  via bijective transformation defined by  $\mathbf{R}$ .  $\square$

527 Subsequently, by seeking the existence of laminar patterns using the partition  $\pi_2$ , Lemma 3.3  
 528 enables an analytic approach to determine the spatially driven instability of the HSS with any  
 529 combination of layer-wise semi-regular bilayer graphs. Specifically, we need only determine the  
 530 eigenvalues of  $\mathcal{DT}(\mathbf{u}^*)$  to ensure the HSS instability condition (2.17) is satisfied.

531 By applying the strongly monotone properties of the transfer kinetics outlined in Section 3.1,  
 532 we seek to ensure the asymptotic convergence of heterogeneous solutions in the instance of HSS  
 533 instability. However, it can be shown (see Lemma A.1 in Appendix A) that the interconnection  
 534 matrix,  $\mathbf{P}$ , and consequently the reduced interconnection matrix  $\bar{\mathbf{P}}$  is reducible, and therefore  
 535 unable to conform to the strongly monotone criteria in Lemma 3.2. However, we recover the  
 536 irreducibility of  $\mathbf{P}$  and  $\bar{\mathbf{P}}$  by multiplication with a suitable class of matrices.

537 **Lemma 3.4.** *Let  $\mathbf{P}$  be the mixed interconnection matrix (2.3) and  $\mathbf{Q} = \text{diag}(\mathbf{Q}_1, \dots, \mathbf{Q}_N)$  such*  
 538 *that  $\mathbf{Q}_k \in \mathbb{R}^{r \times r}$  is irreducible for each  $k \in \{1, \dots, N\}$ . Then  $\mathbf{P}\mathbf{Q}$  is irreducible.*

539 *Proof.* A graph is said to be strongly connected if there exists a path between any two vertices.  
 540 We aim to show that the graph defined by the weighted adjacency matrix  $\mathbf{P}\mathbf{Q}$  is strongly  
 541 connected and therefore use the property that a graph is strongly connected if and only if the  
 542 associated adjacency matrix is irreducible [54].

543 For an unweighted, nonnegative adjacency matrix  $\mathbf{M}$ , it can be shown that the  $(i, j)$ th element  
 544 of  $\mathbf{M}^k$  represents the number of ways to travel from vertex  $v_i$  to vertex  $v_j$  along exactly  $k$  edges.  
 545 Therefore if  $\mathbf{M}$  defines a connected graph of  $l$  vertices, then  $\mathbf{M}^l$  contains no zero entries for all  
 546  $(i, j)$ , that is, there exists a path between any two vertices in less than, or equal, to  $l$  steps [54].  
 547 The converse statement is also true. In the case of weighted, nonnegative adjacency matrices,

548 the elements  $(i, j)$  of  $\mathbf{M}^k$  no longer represent the number of ways to get from vertex  $i$  to vertex  
 549  $j$  along exactly  $k$  edges, but nevertheless are non-zero if there exists a path between  $v_i$  to vertex  
 550  $v_j$  along  $k$ , or less, edges.

551 The set of vertices has cardinality  $|V_{\mathbf{PQ}}| = rN$  owing to the total number of interconnections  
 552 within the large-scale IO system (2.1). Hence consider the adjacency matrix  $(\mathbf{PQ})^{rN}$ . From  
 553 Lemma A.3 it can be shown that

$$554 \quad \mathbf{P}^{rN} = \left( \sum_{i=1}^r \mathbf{W}_i \otimes \mathbf{D}_i \right)^{rN} = \sum_{i=1}^r \mathbf{W}_i^{rN} \otimes \mathbf{D}_i, \quad (3.9)$$

555 and by the above argument  $\mathbf{W}_i^{rN}$  has no zero elements as each  $\mathbf{W}_i$  represents a connected graph of  
 556  $N$  vertices. Therefore,  $\mathbf{P}^{rN}$  is the interweave of  $r$  completely non-zero matrices and thus w.l.o.g.  
 557 for any non-zero elements  $p_{i,j}$  of  $\mathbf{P}^{rN}$  then  $p_{i\pm r,j}$  and  $p_{i,j\pm r}$  are also non-zero. Specifically there  
 558 exist no two non-zero elements in  $\mathbf{P}^{rN}$  that are more than  $r$  elements apart in each row and  
 559 column, as in Example 2.1 where  $r = 2$ ,  $N = 2$ . In addition, define  $\mathbf{Q}_k^{rN} := \tilde{\mathbf{Q}}_k$ . By assumption,  
 560  $\tilde{\mathbf{Q}}_k$  has no zero entries for all  $i, j \in \{1, \dots, r\}$  by irreducibility and so  $\mathbf{Q}^{rN} = \text{diag}(\tilde{\mathbf{Q}}_1, \dots, \tilde{\mathbf{Q}}_N)$ .  
 561 Applying the definition of the matrix product, the elements of  $(\mathbf{PQ})^{rN}$  are given by

$$562 \quad (\mathbf{P}^{rN} \mathbf{Q}^{rN})_{ij} = \sum_{k=1}^{rN} p_{i,k} \tilde{q}_{k,j} \neq 0 \quad (3.10)$$

563 for all  $i, j \in \{1, \dots, rN\}$ , as every column of  $\mathbf{Q}^{rN}$  contains  $r$  consecutive non-zero elements. There-  
 564 fore  $(\mathbf{PQ})^{rN}$  is a non-zero matrix which implies that the graph of  $\mathbf{PQ}$  is strongly connected,  
 565 thus  $\mathbf{PQ}$  is irreducible.  $\square$

566 The statement of Lemma 3.4 applies also to the reduced interconnection matrix  $\bar{\mathbf{P}}$  as it has  
 567 identical structure to the corresponding large-scale interconnection matrix  $\mathbf{P}$  and therefore the  
 568 irreducibility of the product is preserved under the quotient mapping by  $\pi_2$ . Hence by ensuring  
 569 the irreducibility of the Jacobian of the reduced IO system (2.1) spatially coupled by  $\bar{\mathbf{P}}$ , then  
 570 by Lemma 3.4 and (A1), the following statement provides polarity-dependent conditions that  
 571 guarantee the existence of laminar patterns in semi-regular bilayer graphs by using the strongly  
 572 monotone dynamics of solution trajectories.

573 **Theorem 3.1** (Existence of laminar patterns with semi-regular graphs). *Consider the IO system*  
 574 *(2.1) with interconnection matrix  $\mathbf{P}$  (2.3). Let  $\pi_2$  be the layer-wise simultaneously equitable*  
 575 *partition for all bilayer connectivity graphs,  $\mathcal{G}_k$ , defined by  $\mathbf{P}$  such that the associated reduced*  
 576 *interconnection matrix  $\bar{\mathbf{P}}$  (3.4) defines the reduced IO system of representative cells from each*  
 577 *layer. Assuming that (A1) is satisfied and there exists  $\bar{\mathbf{A}}_2$  such that the HSS instability condition*  
 578 *(2.17) holds for all  $n_{1,\mathcal{L}_i}^{[k]} w_1^{[k]} \leq n_{2,\mathcal{L}_i}^{[k]} w_2^{[k]}$  ( $i \in \{1, 2\}, k \in \{1, \dots, r\}$ ), then any solutions in the*  
 579 *neighbourhood of the HSS,  $\mathbf{x}^*$ , converge to laminar patterns in the reduced system.*

580 *Proof.* Following from Lemma 2.1 we consider the auxiliary dynamic system defined by the  
 581 transfer kinetics for the reduced IO system

$$582 \quad \begin{bmatrix} \dot{\mathbf{z}}_1 \\ \dot{\mathbf{z}}_2 \end{bmatrix} = - \begin{bmatrix} \mathbf{z}_1 \\ \mathbf{z}_2 \end{bmatrix} + \bar{\mathbf{P}} \begin{bmatrix} \mathbf{T}(\mathbf{z}_1) \\ \mathbf{T}(\mathbf{z}_2) \end{bmatrix} := \mathbf{F}(\mathbf{z}), \quad (3.11)$$

583 as this represents the behaviour of reduced IO system using only the spatially dependent com-  
 584 ponents. Note that the fixed points of the auxiliary system (3.11) are those of the IO system  
 585 (2.1). Namely, the auxiliary system (3.11) has HSS  $\mathbf{z}^* = \mathbf{1}_2 \otimes \mathbf{u}_0$  for the cell-wise input steady

586 state  $\mathbf{u}_0$  associated with  $\mathbf{x}^*$ . Linearising the auxiliary system about the HSS yields the following  
 587 Jacobian

$$588 \quad \frac{\partial \mathbf{F}}{\partial \mathbf{z}}(\mathbf{z}^*) = -\mathbf{I}_{2r} + \bar{\mathbf{P}}(\mathbf{I}_2 \otimes \mathcal{DT}(\mathbf{z}^*)). \quad (3.12)$$

589 First, we show that sign structures,  $\mathcal{S}_1$  and  $\mathcal{S}_2$  of (A1), are equivalent up to linear transforma-  
 590 tion on the Jacobian (3.12), thereby ensuring the competitive solution dynamics of the auxiliary  
 591 system (3.11). Following that, we then use a competitive to cooperative bijective transforma-  
 592 tion to show that the auxiliary system is strongly monotone. Critically, the boundedness in  
 593 combination with strongly monotone kinetics of the transfer function ensures the convergence  
 594 of heterogeneous solutions in the auxiliary system (3.11) and thus the reduced IO system by  
 595 Lemma 2.1. A sketch of the following proof is given in Figure 5.

596 Denote the reflection transformation  $\mathbf{M} = \mathbf{I}_2 \otimes \tilde{\mathbf{M}}$  where

$$597 \quad \tilde{\mathbf{M}} = \text{diag}((-1), (-1)^2, \dots, (-1)^{r-1}, (-1)^r). \quad (3.13)$$

Note that  $\tilde{\mathbf{M}}^{-1} = \tilde{\mathbf{M}}$  and therefore  $\mathbf{M}^{-1} = \mathbf{M}$ . Introducing the coordinate transformation  
 $\mathbf{w} = \mathbf{M}\mathbf{z}$  which converts between Jacobians with sign structures  $\mathcal{S}_1$  and  $\mathcal{S}_2$ . Explicitly, consider  
 the auxiliary system (3.11) with  $\text{Sgn}(\mathcal{DT}(\cdot)) = \mathcal{S}_2$ , then the Jacobian (3.12) with respect to  $\mathbf{w}$   
 yields

$$\begin{aligned} \mathbf{M} \left( \frac{\partial \mathbf{F}}{\partial \mathbf{z}}(\mathbf{M}\mathbf{w}) \right) \mathbf{M} &= \left( \mathbf{I}_2 \otimes \tilde{\mathbf{M}} \right) \left( -\mathbf{I}_{2r} + \bar{\mathbf{P}}(\mathbf{I}_2 \otimes \mathcal{DT}(\mathbf{M}\mathbf{w})) \right) \left( \mathbf{I}_2 \otimes \tilde{\mathbf{M}} \right), \\ &= -\mathbf{I}_{2r} + \left( \mathbf{I}_2 \otimes \tilde{\mathbf{M}} \right) \left( \sum_{i=1}^r \bar{\mathbf{W}}_i \otimes \mathbf{D}_i \right) \left( \mathbf{I}_2 \otimes \mathcal{DT}(\mathbf{M}\mathbf{w}) \right) \left( \mathbf{I}_2 \otimes \tilde{\mathbf{M}} \right), \\ &= -\mathbf{I}_{2r} + \left( \sum_{i=1}^r \bar{\mathbf{W}}_i \otimes \mathbf{D}_i \right) \left( \mathbf{I}_2 \otimes \tilde{\mathbf{M}} \right) \left( \mathbf{I}_2 \otimes \mathcal{DT}(\mathbf{M}\mathbf{w}) \right) \left( \mathbf{I}_2 \otimes \tilde{\mathbf{M}} \right), \\ &= -\mathbf{I}_{2r} + \left( \sum_{i=1}^r \bar{\mathbf{W}}_i \otimes \mathbf{D}_i \right) \left( \mathbf{I}_2 \otimes \tilde{\mathbf{M}} \mathcal{DT}(\mathbf{M}\mathbf{w}) \tilde{\mathbf{M}} \right), \end{aligned} \quad (3.14)$$

598 where the third and fourth equality follow from the commutativity of diagonal matrices and the  
 599 mixed multiplication property of the Kronecker product. The transformed Jacobian (3.14) is a  
 600 non-positive matrix as

$$601 \quad \left( \tilde{\mathbf{M}} \mathcal{DT}(\mathbf{M}\mathbf{w}) \tilde{\mathbf{M}} \right)_{ij} = (-1)^{i+j} (\mathcal{DT}(\mathbf{M}\mathbf{w}))_{ij} \quad (3.15)$$

602 by direct computation. For  $i+j$  odd,  $(\mathcal{DT}(\mathbf{M}\mathbf{w}))_{ij}$  switches sign, i.e.  $\tilde{\mathbf{M}} \mathcal{DT}(\mathbf{M}\mathbf{w}) \tilde{\mathbf{M}}$  has sign  
 603 structure  $\mathcal{S}_2$ . Therefore, we continue by considering the transfer function with  $\text{Sgn}(\mathcal{DT}(\cdot)) = \mathcal{S}_2$ .

604 The Jacobian (3.12) with  $\text{Sgn}(\mathcal{DT}(\cdot)) = \mathcal{S}_2$  is a non-positive matrix as all element of  $\bar{\mathbf{P}}$  are  
 605 non-negative. From Lemma 3.3 the polarity dependent eigenvalues  $\lambda_{i,2}$  of  $\bar{\mathbf{W}}_i$  have eigenvectors,  
 606  $\mathbf{v}_{i,2}$ , with sign structure  $\text{Sgn}(\mathbf{v}_{i,2}) = [-, +]^T$ . Therefore, motivated by polarity-driven patterning  
 607 and the requirement of the positivity of the dominant instability mode for monotone kinetics  
 608 [47], we construct a transformation,  $\mathbf{R}$ , to ensure that any polarity driven instability satisfies the  
 609 monotonicity criteria, that is, monotone with respect to alternating domains. Then consider the  
 610 transformation  $\mathbf{R} = \tilde{\mathbf{R}} \otimes \mathbf{I}_r$  where  $\tilde{\mathbf{R}} = \text{diag}(-1, 1)$ . Noting again that  $\mathbf{R}^{-1} = \mathbf{R}$  as  $\tilde{\mathbf{R}}^{-1} = \tilde{\mathbf{R}}$ .  
 611 By similar calculations as above, it can be shown that by the coordinate transformation  $\mathbf{w} = \mathbf{R}\mathbf{z}$

612 the Jacobian (3.12) has the form

$$613 \quad \mathbf{R} \left( \frac{\partial \mathbf{F}}{\partial \mathbf{z}} (\mathbf{R}\mathbf{w}) \right) \mathbf{R} = -\mathbf{I}_{2r} + \sum_{i=1}^r \tilde{\mathbf{R}}\tilde{\mathbf{W}}_i\tilde{\mathbf{R}} \otimes \mathbf{D}_i\mathcal{DT}(\mathbf{R}\mathbf{w}), \quad (3.16)$$

614 where the quotient adjacency matrix is transformed to the following form

$$615 \quad \tilde{\mathbf{R}}\tilde{\mathbf{W}}_i\tilde{\mathbf{R}} = \begin{bmatrix} a_i & -(1-a_i) \\ -(1-b_i) & b_i \end{bmatrix}. \quad (3.17)$$

616 Therefore, let  $\tau(i) = (i-1) \bmod r + 1$  then the row-sum of the transformed auxiliary Jacobian  
617 (3.16) can be expressed as

$$\sum_{j \neq i} \left( \mathbf{R} \left( \frac{\partial \mathbf{F}}{\partial \mathbf{z}} (\mathbf{R}\mathbf{w}) \mathbf{R} \right) \right)_{ij} = \begin{cases} (2a_{\tau(i)} - 1) \sum_{j=1, i \neq j}^r (\mathbf{D}_{\tau(i)}\mathcal{DT}(\mathbf{R}\mathbf{w}))_{ij} & 1 \leq i \leq r, \\ (2b_{\tau(i)} - 1) \sum_{j=1, i \neq j}^r (\mathbf{D}_{\tau(i)}\mathcal{DT}(\mathbf{R}\mathbf{w}))_{ij} & r+1 \leq i \leq 2r. \end{cases} \quad (3.18)$$

618 Hence by the assumption  $n_{1, \mathcal{L}_i}^{[k]} w_1^{[k]} \leq n_{2, \mathcal{L}_i}^{[k]} w_2^{[k]}$  ( $i \in \{1, 2\}, k \in \{1, \dots, r\}$ ), we have that  $2a_k - 1 \leq 0$   
619 and  $2b_k - 1 \leq 0$  by direct substitution into equation (3.6). Critically, as  $\mathcal{DT}(\mathbf{R}\mathbf{w})$  is a negative  
620 matrix, we have that

$$622 \quad \sum_{j \neq i} \left( \mathbf{R} \left( \frac{\partial \mathbf{F}}{\partial \mathbf{z}} (\mathbf{R}\mathbf{w}) \mathbf{R} \right) \right)_{ij} \geq 0 \quad (3.19)$$

623 for all  $i \in \{1, \dots, 2r\}$ , thus satisfying the type K condition in Lemma 3.1. Furthermore, by  
624 Lemma 3.4, the transformed auxiliary Jacobian (3.16) is irreducible and therefore the auxiliary  
625 dynamical system (3.11) is strongly monotone (cooperative) with respect to the laminar pattern  
626 transformation  $\mathbf{R}$ .

627 The cooperative auxiliary dynamical system (3.11) is monotone with respect to the standard  
628 domain  $\mathbb{R}_{\geq 0}^{2r}$  and has a positive eigenvector  $\mathbf{v} > 0$  associated with the polarity driven instability  
629  $\bar{\mathbf{A}}_2$  of the transformed HSS  $\mathbf{R}\mathbf{z}^*$  by the Perron-Frobenius Theorem [55]. Consequently, for  
630 small  $\epsilon$ , any solution starting at  $\mathbf{R}\mathbf{z} = \mathbf{R}\mathbf{z}^* + \epsilon\mathbf{v}$  must have positive derivative and increase  
631 in the transformed trajectory domain  $\mathbb{R}_{\geq 0}^{2r}$  [47]. Critically, if the solutions of the cooperative  
632 auxiliary dynamical system (3.11) are bounded, then the strongly monotone property ensures  
633 the convergence to another steady state,  $\mathbf{R}\mathbf{z}^{**} \neq \mathbf{R}\mathbf{z}^*$ .

634 The transfer function  $\mathbf{T}(\cdot)$  is bounded and so there exists  $b > 0$  such that  $\|\bar{\mathbf{P}}[\mathbf{T}(\mathbf{z}_1), \mathbf{T}(\mathbf{z}_2)]^T\|_2 <$   
635  $b$  for all  $\mathbf{z}_i$ . Thus, as the cooperative auxiliary dynamical system (3.11) is monotone with respect  
636 to  $\mathbb{R}_{\geq 0}^{2r}$ , we have that the sets centered about the HSS  $\mathcal{V}_{\pm} = \mathbf{R}\mathbf{z}^* \pm (\mathbb{R}_{\geq 0}^{2r} \cap [0, b]^{2r})$  are forward  
637 invariant, i.e.  $\phi_t(\mathbf{R}\mathbf{z}) \in \mathcal{V}_{\pm}$  for all  $t \in [0, \infty)$ . Therefore all solutions are bounded within a  
638 compact domain and thus converge to  $\mathbf{R}\mathbf{z}^{**} \neq \mathbf{R}\mathbf{z}^*$  by the Cooperative Irreducible Convergence  
639 Theorem (Theorem 4.3.3 in [47]). Subsequently, the corresponding non-transformed system  
640 (3.11) must have each vertices with solutions in  $\mathcal{V}_+$  and  $\mathcal{V}_-$ , respectively, ensuring contrasting  
641 cell-wise solutions. Finally, as any steady state solution to the auxiliary dynamical system (3.11)  
642 is a steady state of the associated reduced IO system (2.1), by Lemma 2.1 the reduced IO system  
643 (2.1) converges to laminar patterns.  $\square$

644 From Theorem 3.1 we can conclude that the existence of a polarity-driven instability of the  
645 HSS implies the existence of heterogeneous steady states within the quotient system. This follows  
646 as solution trajectories diverge when transforming between competitive to cooperative systems

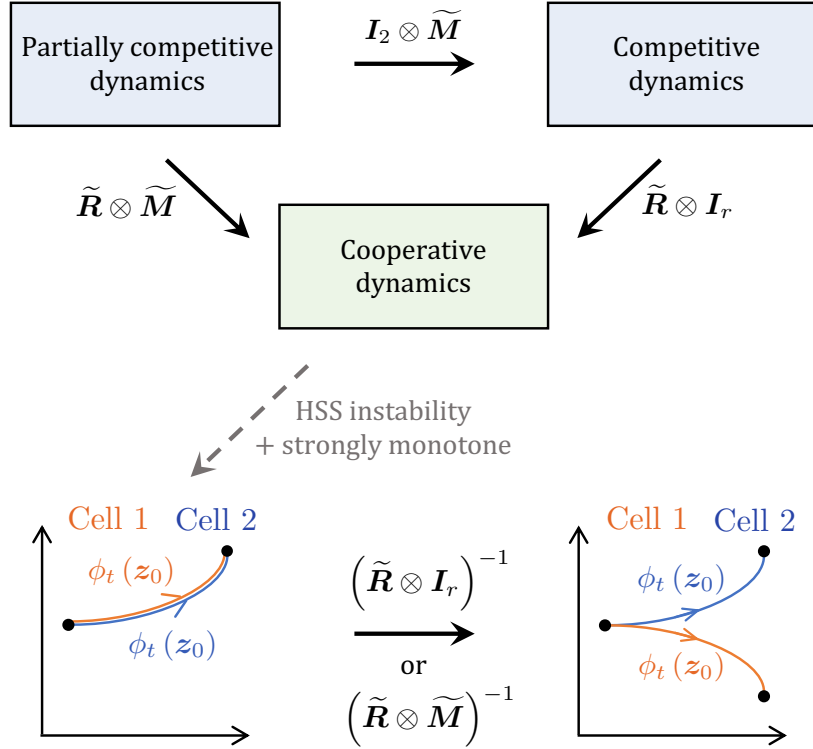


FIGURE 5. A sketch of the proof of Theorem 3.1 for system transformations where  $I_r$  is the identity matrix,  $\tilde{M}$  is the competitive sign structure transformation,  $\tilde{R}$  is the competitive to cooperative transformation and  $\phi_t(z_0)$  is a solution trajectory with initial condition  $z_0$ .

647 as highlighted in Figure 5. Moreover, as the competitive dynamics of the reduced IO system  
 648 (2.1) are isomorphic to cooperative dynamics, all periodic solutions are unstable [56], implying  
 649 the convergence to contrasting cell states. The following example demonstrates how Theorem  
 650 3.1 can be applied to prove the existence of laminar patterns in large-scale IO systems.

651 **Example 3.1.** Consider the DIDO system with two spatially-dependent components describing  
 652 lateral-inhibition with a diffusive crosstalk as represented in Figure 6,

$$\dot{x}_{i,1} = g_1(u_{i,1}) \cdot g_2(x_{i,2}) \cdot f_1(u_{i,2}) - x_{i,1}, \quad (3.20)$$

$$\dot{x}_{i,2} = f_2(x_{i,1}) - x_{i,2}, \quad (3.21)$$

$$\dot{x}_{i,3} = g_3(x_{i,1}) - x_{i,3}, \quad (3.22)$$

$$y_{i,1} = x_{i,2}, \quad (3.23)$$

$$y_{i,2} = x_{i,3}, \quad (3.24)$$

653 for each cell  $1 \leq i \leq 60$ . The functions  $f_j$  and  $g_j$ ,  $j = 1, 2, 3$ , are positive, bounded, and  
 654 increasing and decreasing functions, respectively, of the form,

$$655 \quad f_j(x) = \frac{x^{k_j}}{\alpha_j + x^{k_j}} \quad \text{and} \quad g_j(x) = \frac{1}{1 + \beta_j x^{h_j}} \quad (3.25)$$

656 where  $\alpha_j, \beta_j, k_j, h_j > 0$ . Let  $u_{i,1}$  and  $u_{i,2}$  be defined by short-range diffusion and contact-based  
 657 bilayer connectivity graphs  $\mathcal{G}_1$  and  $\mathcal{G}_2$ , respectively as in Figure 6. Explicitly, we have that outputs

658 are converted to inputs via the global interconnection matrix such that  $\mathbf{u} = (\mathbf{W}_1 \otimes \mathbf{D}_1 + \mathbf{W}_2 \otimes \mathbf{D}_2) \mathbf{y}$   
 659 for  $\mathbf{W}_1, \mathbf{W}_2 \in \mathcal{W}$ . Here, we focus on the associated reduced IO system (3.20-3.24) which is de-  
 660 fined by the simultaneously equitable partition  $\pi_2$ . Namely, in the reduced IO system, outputs  
 661 are converted to inputs by  $\mathbf{u} = (\overline{\mathbf{W}}_1 \otimes \mathbf{D}_1 + \overline{\mathbf{W}}_2 \otimes \mathbf{D}_2) \mathbf{y}$  where

$$\overline{\mathbf{W}}_1 = \begin{bmatrix} \frac{2w_1^{[1]}}{2w_1^{[1]}+4w_2^{[1]}} & \frac{4w_2^{[1]}}{2w_1^{[1]}+4w_2^{[1]}} \\ \frac{4w_2^{[1]}}{2w_1^{[1]}+4w_2^{[1]}} & \frac{2w_1^{[1]}}{2w_1^{[1]}+4w_2^{[1]}} \end{bmatrix} \quad \text{and} \quad \overline{\mathbf{W}}_2 = \begin{bmatrix} \frac{2w_1^{[2]}}{2w_1^{[2]}+2w_2^{[2]}} & \frac{2w_2^{[2]}}{2w_1^{[2]}+2w_2^{[2]}} \\ \frac{2w_2^{[2]}}{2w_1^{[2]}+2w_2^{[2]}} & \frac{2w_1^{[2]}}{2w_1^{[2]}+2w_2^{[2]}} \end{bmatrix} \quad (3.26)$$

663 such that  $n_{1,\mathcal{L}_1}^{[1]} = n_{1,\mathcal{L}_2}^{[1]} = n_{1,\mathcal{L}_1}^{[2]} = n_{1,\mathcal{L}_2}^{[2]} = 2$ ,  $n_{2,\mathcal{L}_1}^{[1]} = n_{2,\mathcal{L}_2}^{[1]} = 4$  and  $n_{2,\mathcal{L}_1}^{[2]} = n_{2,\mathcal{L}_2}^{[2]} = 2$ . We seek  
 664 to show the existence of polarity driven laminar patterns using the quotient graphs and so we  
 665 first require the HSS of the IO system (3.20-3.24), then we derive the derivative of the transfer  
 666 function  $DT(\mathbf{u}_i)$ , highlighting that (A1) is satisfied. Applying Theorem 3.1, we generate polarity  
 667 regimes for the existence of patterning.

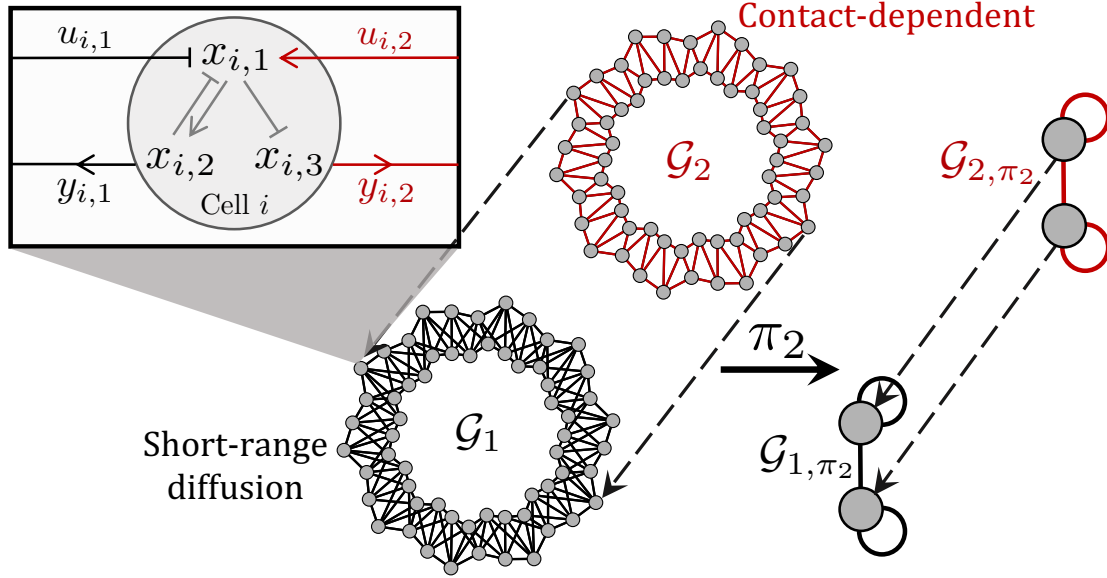


FIGURE 6. A schematic of the IO system considered in Example 3.1.

668 The HSS of the IO system (3.20 - 3.24) can be determined by solving

$$669 \quad g_1(f_2(x_1^*)) \cdot g_2(f_2(x_1^*)) \cdot f_1(g_3(x_1^*)) - x_1^* = 0 \quad (3.27)$$

670 for  $x_1^*$  by setting  $u_{i,1} = x_{i,2}$  and  $u_{i,2} = x_{i,3}$ , conforming to homogeneous input and outputs of the  
 671 tissue. Furthermore, the HSS defined by solving equation (3.27) is always stable in the absence of  
 672 interconnections. This can be shown by considering the linearisation of the intracellular kinetics

$$673 \quad \mathbf{A} := \frac{\partial \mathbf{f}}{\partial \mathbf{x}_i} = \begin{bmatrix} -1 & f_1 g_1 g_2' & 0 \\ f_2' & -1 & 0 \\ g_3' & 0 & -1 \end{bmatrix}. \quad (3.28)$$

674 As  $\det(\mathbf{A}) = f_1 g_1 f_2' g_2' - 1 < 0$  always holds by the monotonicity of the functions  $f_j$  and  $g_j$ ,  
 675 then the HSS defined by solving equation (3.27) is unique by Lemma 2.2. In addition,  $\mathbf{A}$  has  
 676 eigenvalues

$$677 \quad \mu_1 = -1, \quad \mu_2 = -1 + \sqrt{f_1 g_1 f_2' g_2'} \quad \text{and} \quad \mu_3 = -1 - \sqrt{f_1 g_1 f_2' g_2'} \quad (3.29)$$



678 and so as  $\mu_1, \Re(\mu_2), \Re(\mu_3) < 0$  we have that  $\mathbf{A}$  is stable. Thus any instability of the HSS will  
679 be induced by the interconnection of cells in the tissue.

680 The derivative of the transfer function can be determined by linearisation of the IO kinetics  
681 (3.20 - 3.24) as demonstrated in [29] such that  $\mathcal{DT}(\mathbf{u}_i) = -\mathbf{CA}^{-1}\mathbf{B}$  where  $\mathbf{B}$  and  $\mathbf{C}$  are the  
682 linearised inputs and outputs respectively as in Lemma 2.3. For the IO system (3.20 - 3.24), the  
683 derivative of the transfer function has the form

$$\begin{aligned} \mathcal{DT}(\mathbf{u}_i) &= -\det(\mathbf{A})^{-1} \begin{bmatrix} 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & f_1 g_1 g'_2 & 0 \\ f'_2 & 1 & 0 \\ g'_3 & f_1 g_1 g'_2 g'_3 & 1 - f_1 g_1 g'_2 \end{bmatrix} \begin{bmatrix} f_1 g_2 g'_1 & g_1 g_2 f'_1 \\ 0 & 0 \\ 0 & 0 \end{bmatrix} \\ &= -\det(\mathbf{A})^{-1} \begin{bmatrix} f_1 g_2 f'_2 g'_1 & g_1 g_2 f'_1 f'_2 \\ f_1 g_2 g'_1 g'_3 & g_1 g_2 f'_1 g'_3 \end{bmatrix}, \end{aligned} \quad (3.30)$$

684 where each of the functions  $f_j$  and  $g_j$  are evaluated using the corresponding arguments for the  
685 given input state  $\mathbf{u}_i$ . The multiplication of bounded functions are bounded [43] and subsequently  
686  $\mathcal{DT}(\mathbf{u}_i)$  is element-wise bounded as  $f_j, g_j, f'_j$  and  $g'_j$  are bounded. In addition, from the mono-  
687 tonicity of  $f_j$  and  $g_j$  we have that

$$688 \quad \text{Sgn}(\mathcal{DT}(\mathbf{u}_i)) = \begin{bmatrix} - & + \\ + & - \end{bmatrix} = \mathcal{S}_1 \quad (3.31)$$

689 and so the IO system (3.20 - 3.24) satisfies (A1). Therefore by Theorem 3.1 we have that the IO  
690 system (3.20 - 3.24) spatially coupled using the quotient graphs  $\mathcal{G}_{1,\pi_2}$  and  $\mathcal{G}_{2,\pi_2}$ , the instability  
691 of the HSS in addition to the monotone polarity conditions  $w_1^{[1]} \leq 2w_2^{[1]}$  and  $w_1^{[2]} \leq w_2^{[2]}$ , produce  
692 contrasting cell-wise states.

693 By Corollary 2.1 we apply the DIDO instability inequality (2.21) to the IO system (3.20 -  
694 3.24). As  $\det(\mathcal{DT}(\mathbf{u}_i)) = 0$ , the DIDO instability inequality (2.21) reduces to  $1 < \text{tr}(\overline{\mathbf{A}}_2 \mathcal{DT}(\mathbf{u}_i))$ ,  
695 namely the HSS is unstable only if

$$696 \quad 1 < -\det(\mathbf{A})^{-1} \left( \left( \frac{w_1^{[1]} - 2w_2^{[1]}}{w_1^{[1]} + 2w_2^{[1]}} \right) f_1 g_2 g'_1 f'_2 + \left( \frac{w_1^{[2]} - w_2^{[2]}}{w_1^{[2]} + w_2^{[2]}} \right) g_1 g_2 f'_1 g'_3 \right) \quad (3.32)$$

697 for the reduced IO system (3.20 - 3.24). The monotone polarity conditions  $w_1^{[1]} \leq 2w_2^{[1]}$  and  
698  $w_1^{[2]} \leq w_2^{[2]}$  of Theorem 2.1 confirm that each of the reduced connectivity matrices must have  
699 negative eigenvalues to produce the instability of the HSS. as  $f_1 g_2 g'_1 f'_2 < 0$  and  $g_1 g_2 f'_1 g'_3 < 0$ .  
700 Critically, the HSS instability inequality (3.32) highlights that as the layer-wise activator/receptor  
701 polarity increases, i.e.  $w_1^{[i]} \ll w_2^{[i]}$ , the potential to induce laminar patterns also increases in the  
702 quotient system. Then by the spectral retention property of the equitable partition  $\pi_2$ , we have  
703 that laminar patterns must exist in the pattern space of the associated large-scale system.

704 To illustrate the application Theorem 3.1 to the IO system (3.20 - 3.24) numerical verification  
705 of the polarity parameter regime for laminar pattern existence determined by inequality (3.32) is  
706 given in Figure 7.

707 As demonstrated in Example 3.1, the method of pattern templating for contrasting solutions  
708 between cells in opposing layers can be used to show the existence of layer-wise differing steady  
709 states via polarity-driven instabilities. However, the associated large-scale systems may have  
710 many locally stable steady states that produce the pattern space of the IO system which could  
711 have been lost during the dimension reducing transformation by the partition,  $\pi_2$  [33]. Therefore,

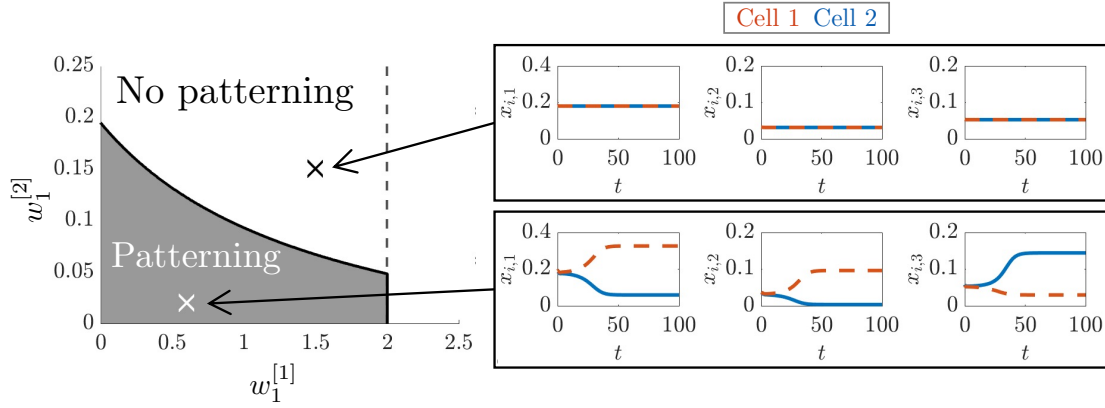


FIGURE 7. Polarity parameter regimes for the existence of laminar patterns in the IO system (3.20-3.24). For fixed  $w_2^{[1]} = w_2^{[2]} = 1$ , inequality (3.32) in addition to the monotone polarity conditions  $w_1^{[1]} \leq w_2^{[1]}$  and  $w_1^{[2]} \leq 2w_2^{[2]}$  to define a regions in  $(w_1^{[1]}, w_1^{[2]})$ -space for the existence of laminar patterns. The dashed line in the  $(w_1^{[1]}, w_1^{[2]})$ -space corresponds to the monotone condition  $w_1^{[1]} \leq 2w_2^{[1]}$ . Example simulations are given for polarity parameter values inside the pattern region,  $(0.6, 0.02)$ , and outside the pattern region  $(1.5, 0.15)$ . Initial conditions were given as small random perturbations about the HSS,  $\mathbf{x}^* = [0.18, 0.03, 0.05]^T$ . IO system (3.20-3.24) parameter values and details on simulations are given in Appendix B.

712 in the following section, we investigate the spectral properties of the bilayer connectivity graphs  
 713 to ensure that the laminar patterns produced by Theorem 3.1 are indeed globally dominant.

### 714 3.3. Spectral links between quotient and large-scale bilayer connectivity graphs.

715 For linearised dynamical systems near steady state, the local solution trajectories are a linear  
 716 combination of the associated eigenvectors scaled by the corresponding exponent of the eigenval-  
 717 ues [57]. Thus, in the instance of steady-state instability, all trajectories close to the steady-state  
 718 will locally tend in the direction of the eigenvector associated with the largest real-part eigen-  
 719 value. Critically, to ensure the monotone convergence of laminar patterns in the reduced IO  
 720 systems in Theorem 3.1, we transformed the polarity-dependent eigenvector to be directed in  
 721 the positive orthant, conforming to the behaviour of cooperative dynamics. Thus, motivated by  
 722 this positive direction transformation, we seek to understand when the eigenvalue associated with  
 723 laminar pattern formation dominates the large-scale spectra. This ensures the perturbed trajec-  
 724 tories from the HSS to be preferably pointed in the direction to achieve layer-wise contrasting  
 725 states in the large-scale IO systems.

726 Previous studies on pattern formation using IO systems have imposed the sufficient condition  
 727 that the large-scale and quotient multilayer connectivity graphs  $\mathcal{G}_k$  are bipartite, as this generates  
 728 monotone dynamics with respect to the bipartition vector [29, 33–35]. Namely, a graph  $\mathcal{G}_k$  is  
 729 said to be bipartite if the vertices  $v \in V$  can be partitioned into two independent sets  $V_1$  and  
 730  $V_2$  such that no two vertices in the same set are adjacent [38]. Example bipartite bilayer graphs  
 731 are given in Figure 8A. However, it can be demonstrated that for bipartite bilayer graphs,  
 732 the polarity-dependent eigenvalue,  $\bar{\lambda}_{k,2}$ , associated with laminar pattern formation cannot be  
 733 dominant.

734 **Lemma 3.5.** *Let  $\mathcal{G}_k$  be a bipartite bilayer graph with weighted adjacency matrix  $\mathbf{W}_k \in \mathcal{W}$ .*  
 735 *Then for any  $w_1^{[k]}, w_2^{[k]} > 0$  the polarity-dependent eigenvalue  $\bar{\lambda}_{k,2}$  associated with the reduced*  
 736 *adjacency matrix  $\bar{\mathbf{W}}_k$  satisfies*

$$737 \quad \bar{\lambda}_{k,2} \neq \min(\text{Spec}(\mathbf{W}_k)). \quad (3.33)$$

738 *Proof.* Consider  $\lambda_{k,j} \in \text{Spec}(\mathbf{W}_k)$ , then by the spectral symmetry of bipartite graphs about the  
 739 origin we have that  $-\lambda_{k,j} \in \text{Spec}(\mathbf{W}_k)$  [38]. As  $\mathbf{W}_k \in \mathcal{W}$  then  $\lambda_{k,1} = \max(\text{Spec}(\mathbf{W}_k)) = 1$  by  
 740 the connected and row-stochastic properties of  $\mathbf{W}_k$  [40]. Consequently,  $-\lambda_{k,1} = \min(\text{Spec}(\mathbf{W}_k)) =$   
 741  $-1$ . However, the minimal eigenvalue of the reduced adjacency matrix  $\bar{\mathbf{W}}_k$  defined by the lam-  
 742 inar pattern partition,  $\pi_2$ , must be of the form  $\bar{\lambda}_{k,2} = a_k + b_k - 1$  for  $a_k, b_k \in (0, 1)$  by Lemma  
 743 3.3. Critically, this implies that  $\bar{\lambda}_{k,2} \in (-1, 1)$  and therefore  $\bar{\lambda}_{k,2} \neq \min(\text{Spec}(\mathbf{W}_k))$  for any  
 744 layer-wise polarity values  $w_1^{[k]}, w_2^{[k]} > 0$ .  $\square$

745 A direct consequence of Lemma 3.5 is that if the large-scale IO system (2.1) is spatially coupled  
 746 by a bipartite bilayer graph  $\mathcal{G}_k$  then any trajectory initiated from a small perturbation of an  
 747 unstable HSS will not be dominantly travelling in the direction of the eigenvector associated  
 748 with laminar patterning. Critically, there will always exist a greater instability mode of the  
 749 IO system (2.1). Figure 8B demonstrates the consequences of Lemma 3.5, and for the given  
 750 bipartite graphs, the laminar patterning polarity-dependent eigenvalue  $\bar{\lambda}_{k,2}$  defines a spectral  
 751 gap about the origin which is proved in Appendix C.

752 Following Lemma 3.5, we focus our attention on the spectral investigation of non-bipartite  
 753 semi-regular bilayer graphs. As we are interested in the polarity-driven pattern events using a  
 754 pre-defined pattern template,  $\pi_2$ , we seek layer-wise polarity conditions in which  $\bar{\lambda}_{k,2}$  becomes  
 755 minimal. Subsequently, we considered a variety of non-bipartite graphs each with different edge  
 756 connectivity structure and varied the same-layer weighting parameter  $w_1^{[k]}$  for fixed  $w_2^{[k]} = 1$ ,  
 757 measuring the position of  $\bar{\lambda}_{k,2}$  in terms of the ascending spectrum of the associated large-scale  
 758 graph. A summary of the non-bipartite connectivity structures that were considered are in given  
 759 Table 2 in Appendix B.

760 For each of the non-bipartite bilayer graphs that were considered, we observed that decreasing  
 761 same-layer weighting parameter,  $w_1^{[k]}$ , shifted the eigenvalue  $\bar{\lambda}_{k,2}$  associated with laminar pattern  
 762 formation towards the minimum of the spectrum (Figure 9). Furthermore, we demonstrate that  
 763  $\bar{\lambda}_{k,2} = \min(\text{Spec}(\mathbf{W}_k))$  for values of  $w_1^{[k]} < w_2^{[k]}$ , noting that this was achieved for higher values of  
 764  $w_1^{[k]}$  in the graphs with more cross-layer connections than same-layer connections,  $n_{1,\mathcal{L}_i} < n_{2,\mathcal{L}_i}$ .  
 765 Critically, Figure 9 highlights that there exists large-scale non-bipartite connectivity graphs  
 766 that have the capacity to be fully characterised by the extrema of the spectrum of the laminar  
 767 quotient graph by control of the amount of polarity in the system. That is, with high layer-wise  
 768 polarity,  $w_1^{[k]} \ll w_2^{[k]}$ , we have  $\min(\text{Spec}(\bar{\mathbf{W}}_k)) = \min(\text{Spec}(\mathbf{W}_k))$  and  $\max(\text{Spec}(\bar{\mathbf{W}}_k)) =$   
 769  $\max(\text{Spec}(\mathbf{W}_k))$ .

770 By Theorem 3.1 we demonstrated that the existence of laminar patterns with competitive  
 771 kinetics is dependent on the existence of connectivity polarity within the quotient connectivity  
 772 graphs to induce both HSS instability and monotonicity of solutions. Therefore, in the following  
 773 section, we explore whether solution behaviours observed in the reduced systems are preserved in  
 774 the associated large-scale systems when the quotient graphs preserve the extrema of the spectra  
 775 of the large-scale graphs. Namely, we show that the analysis conducted on the reduced IO  
 776 systems yields global pattern convergence in high polarity regimes. Specifically, we say that the

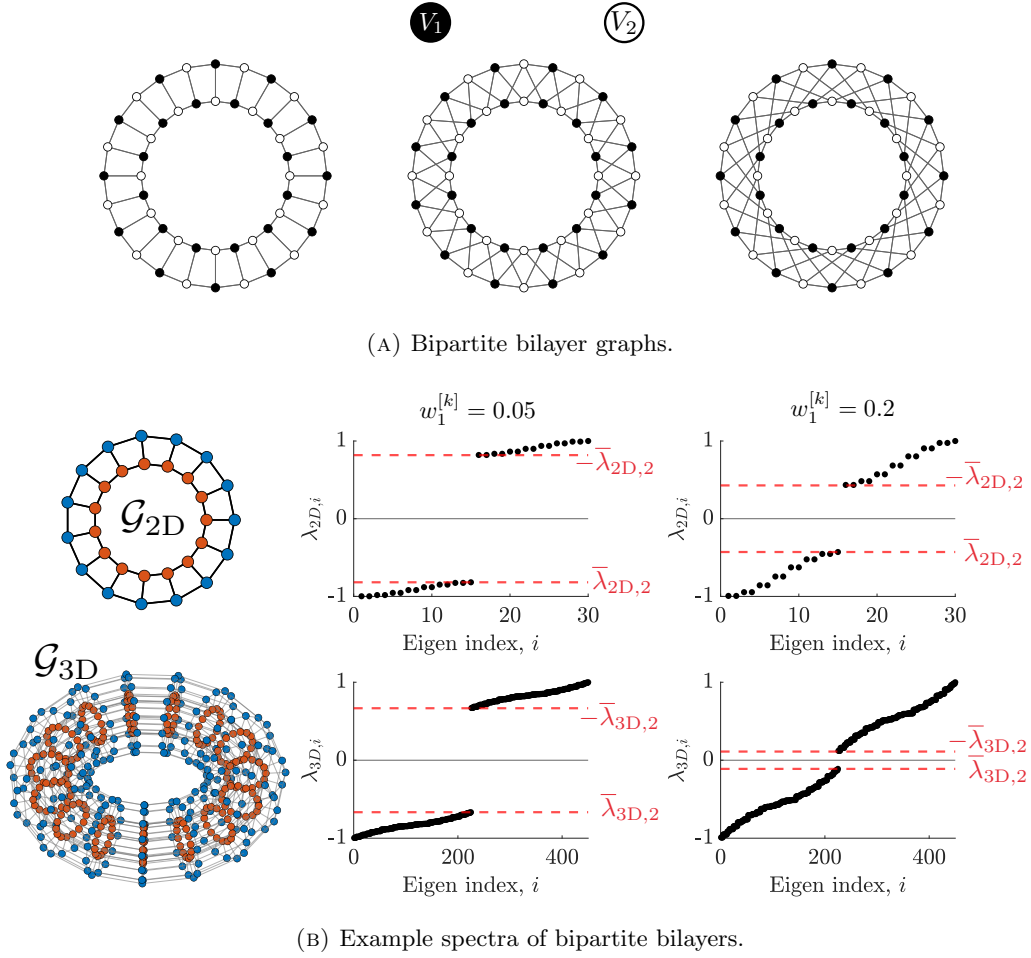


FIGURE 8. Structure and spectra of bipartite bilayer connectivity graphs. (A) Example regular bipartite graphs where vertices are coloured with respect to the bipartition sets  $V_1$  and  $V_2$  in black and white, respectively. (B) Spectra of two bipartite graphs  $\mathcal{G}_{2D}$  and  $\mathcal{G}_{3D}$  is shown for  $w_1^{[k]} = 0.05$  and  $w_1^{[k]} = 0.2$  for fixed  $w_2^{[k]} = 1$  ( $k \in \{2D, 3D\}$ ) where the eigen index refers to the position of the eigenvalue when listed in ascending order. The dashed red lines correspond to the polarity-dependent eigenvalue  $\bar{\lambda}_{k,2}$  highlighting its position with respect to the ascending spectrum of the associated large-scale graph. The vertices of the graphs are coloured layer-wise to emphasise their bilayer structure.

777 large-scale IO system (2.1) globally converges to laminar patterns if  $\mathbf{x}$  conforms to Definition  
 778 2.1 for all sufficiently small perturbations about the HSS,  $\mathbf{x}^*$ .

### 779 3.4. Polarity induced laminar pattern formation derived by quotient systems for 780 large-scale bilayer geometries.

781 In this section, we investigate the conditions in which the patterns predicted using the dimension  
 782 reduction technique of quotient templating are the globally dominant patterns produced in the  
 783 large-scale IO systems. We have demonstrated in Section 3.3 that the spectra of non-bipartite  
 784 semi-regular bilayer connectivity graphs have the capacity to be bounded by the extrema of the  
 785 spectra of the associated quotient graphs defined by  $\pi_2$ . This implies that the polarity-driven  
 786 HSS instability imposed by the pattern existence condition of Theorem 3.1 in the quotient  
 787 systems must also exist in the large-scale systems and can become dominant in high-polarity

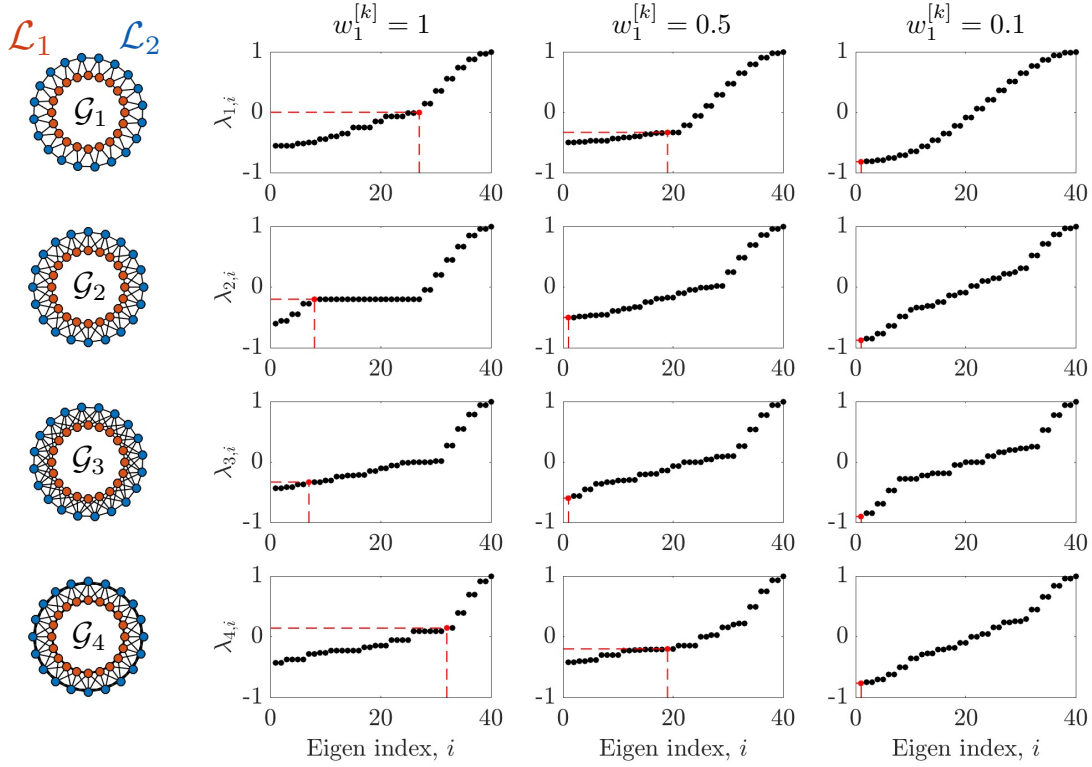


FIGURE 9. Eigenvalues associated with laminar pattern trajectories tend to the minimum of the large-scale spectra as polarity increases in non-bipartite bilayer graphs. The non-bipartite graphs  $\mathcal{G}_k$  ( $k = 1, 2, 3, 4$ ) are shown on the left with vertices coloured layer-wise. The spectrum of each graph is then shown in ascending order for same-layer polarity values  $w_1^{[k]} = 1$ ,  $w_1^{[k]} = 0.5$  and  $w_1^{[k]} = 0.1$ , where the eigen index corresponds to the increasing ordering of the eigenvalues. In each of the plots,  $\bar{\lambda}_{k,2}$  is highlighted in red with dashed lines included to emphasise the value position in the ascending ordering of eigenvalues. Details on the connectivity structures each of the graphs is provided in Appendix B.

788 regimes. Therefore, we now focus our attention on whether the large-scale IO system is monotone  
 789 with respect to the eigenvector locally directing solutions to laminar patterns, thus preserving  
 790 trajectory direction.

791 **Lemma 3.6.** *Consider the large-scale IO system (2.1) spatially coupled by the global adjacency*  
 792 *matrix  $\mathbf{P}$  (2.3) where  $\mathbf{W}_k \in \mathcal{W}$  for  $k \in \{1, \dots, r\}$ . Assuming that (A1) is satisfied and the*  
 793 *laminar pattern partition,  $\pi_2$ , is simultaneously equitable. If all connectivity graphs  $\mathcal{G}_k$  are highly*  
 794 *polarised,  $w_1^{[k]} \ll w_2^{[k]}$ , the large-scale IO system (2.1) generates monotone solutions in the*  
 795 *direction of laminar patterns.*

796 *Proof.* Similar to Theorem 3.1 we consider the large-scale auxiliary system

$$797 \quad \begin{bmatrix} \dot{z}_1 \\ \vdots \\ \dot{z}_N \end{bmatrix} = - \begin{bmatrix} z_1 \\ \vdots \\ z_N \end{bmatrix} + \mathbf{P} \begin{bmatrix} \mathbf{T}(z_1) \\ \vdots \\ \mathbf{T}(z_N) \end{bmatrix} := \mathbf{F}(z), \quad (3.34)$$

798 which has the identical behaviour to the large-scale IO system (2.1) by Lemma 2.1 yet the aux-  
 799 iliary system (3.34) only explicitly considers the spatially dependent components of the model.  
 800 First, we will construct the sign structure of the eigenvector associated with laminar patterns in  
 801 the large-scale graphs. Then, by transforming the auxiliary system (3.34) to ensure the positivity  
 802 of the laminar pattern eigenvector, we demonstrate that the large-scale IO system (2.1) has the  
 803 capacity to become type K for high polarity bilayers.

804 Linearising the auxiliary system (3.34) about a generic point  $\mathbf{z} \in \mathbb{R}_{\geq}^{rN}$  yields

$$\frac{\partial \mathbf{F}}{\partial \mathbf{z}} = -\mathbf{I}_{rN} + \mathbf{P} \begin{bmatrix} \mathcal{DT}(\mathbf{z}_1) & & \\ & \ddots & \\ & & \mathcal{DT}(\mathbf{z}_N) \end{bmatrix} \quad (3.35)$$

806 where  $\mathbf{T}(\cdot)$  satisfies (A1) and thus  $\text{sgn}(\mathcal{DT}) = \mathcal{S}_1$  or  $\text{sgn}(\mathcal{DT}) = \mathcal{S}_2$ . As in the proof of Theorem  
 807 (3.1), the transformation  $\mathbf{M} = \mathbf{I}_N \otimes \tilde{\mathbf{M}}$ , where  $\tilde{\mathbf{M}}$  as given in equation (3.13), demonstrates  
 808 the equivalence of the sign structures. That is, if  $\text{sgn}(\mathcal{DT}) = \mathcal{S}_1$  then  $\text{sgn}(\tilde{\mathbf{M}}^T \mathcal{DT} \tilde{\mathbf{M}}) = \mathcal{S}_2$ .  
 809 Therefore we continue assuming  $\text{sgn}(\mathcal{DT}) = \mathcal{S}_2$ , critically that  $\text{diag}(\mathcal{DT}(\mathbf{z}_1), \dots, \mathcal{DT}(\mathbf{z}_N))$  is a  
 810 non-positive matrix.

811 The reduced graphs associated with the laminar pattern template  $\mathcal{G}_{k,\pi_2}$  have eigenvalues  
 812  $\bar{\lambda}_{k,1} = 1$  and  $\bar{\lambda}_{k,2} = a_k + b_k - 1$  with eigenvectors  $\bar{\mathbf{v}}_{k,1} = [1, 1]^T$  and  $\bar{\mathbf{v}}_{k,2} = [1, (b_k - 1) / (1 - a_k)]^T$ ,  
 813 noting that  $a_k, b_k \in (0, 1)$  by definition of the reduced adjacency matrix  $\bar{\mathbf{W}}_k$  (3.5). Subsequently,  
 814 the polarity dependent eigenvector has sign structure  $\text{sgn}(\bar{\mathbf{v}}_{k,2}) = [+,-]^T$ . Furthermore, as  $\pi_2$   
 815 is equitable for all graphs  $\mathcal{G}_k$ , then there exists a matrix  $\mathbf{L} \in \{0, 1\}^{N \times 2}$  that maps the large-scale  
 816 graph into the quotient graph such that

$$\mathbf{L} \bar{\mathbf{W}}_k = \mathbf{W}_k \mathbf{L} \quad (3.36)$$

818 where  $\mathbf{L}$  allocates the vertices of the large-scale system into the reduced groups associated with  
 819 the laminar pattern template [38]. Owing to the layer-wise vertex indexing as constructed in  
 820 Section 2.1, we have that

$$\mathbf{L} = \begin{bmatrix} \mathbf{1}_{|\mathcal{L}_1| \times 1} & \mathbf{0}_{|\mathcal{L}_1| \times 1} \\ \mathbf{0}_{|\mathcal{L}_2| \times 1} & \mathbf{1}_{|\mathcal{L}_2| \times 1} \end{bmatrix}. \quad (3.37)$$

822 From the quotient to large-scale algebraic relation (3.36), we have that  $\mathbf{L} \bar{\mathbf{v}}_{k,2}$  is an eigenvector  
 823 of  $\mathbf{W}_k$  with eigenvalue  $\bar{\lambda}_{k,2}$ . Specifically, this implies that the eigenvector associated with lami-  
 824 nar patterning in the large-scale graphs has the sign structure  $\text{sgn}(\mathbf{L} \bar{\mathbf{v}}_{k,2}) = [+,\dots,+,-,\dots,-]^T$   
 825 which has  $|\mathcal{L}_1|$  and  $|\mathcal{L}_2|$  positive and negative entries, respectively. Hence, the matrix  $\tilde{\mathbf{R}} =$   
 826  $\text{diag}(1, \dots, 1, -1, \dots, -1)$  orientates the laminar patterning eigenvector  $\mathbf{L} \bar{\mathbf{v}}_{k,2}$  in the positive or-  
 827 thant, i.e.,  $\tilde{\mathbf{R}} \mathbf{L} \bar{\mathbf{v}}_{k,2} > 0$ .

We next introduce the transformation  $\mathbf{w} = \mathbf{R} \mathbf{z}$  where  $\mathbf{R} = \tilde{\mathbf{R}} \otimes \mathbf{I}_r$ , noting that  $\mathbf{R}^{-1} =$   
 $\mathbf{R}$ . Following this change of variables, let  $\mathbf{X}_1 = \text{diag}(\mathcal{DT}(\mathbf{z}_1), \dots, \mathcal{DT}(\mathbf{z}_{|\mathcal{L}_1|}))$  and  $\mathbf{X}_2 =$   
 $\text{diag}(\mathcal{DT}(\mathbf{z}_{|\mathcal{L}_1|+1}), \dots, \mathcal{DT}(\mathbf{z}_N))$  be non-positive matrices, then in combination with the layer-  
 wise block formulation of the bilayer adjacency matrices,  $\mathbf{W}_k$ , the linearised auxiliary system

(3.35) has the form

$$\begin{aligned}
\mathbf{R} \frac{\partial \mathbf{F}}{\partial \mathbf{z}} \mathbf{R} &= -\mathbf{I}_{rN} + \mathbf{R} \mathbf{P} \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_2 \end{bmatrix} \mathbf{R}, \\
&= -\mathbf{I}_{rN} + \left( \sum_{i=1}^r \tilde{\mathbf{R}} \mathbf{W}_i \otimes \mathbf{D}_i \right) \cdot \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & -\mathbf{X}_2 \end{bmatrix}, \\
&= -\mathbf{I}_{rN} + \left( \sum_{i=1}^r \begin{bmatrix} \widehat{\mathbf{W}}_{1, \mathcal{L}_1}^{[i]} \otimes \mathbf{D}_i & \widehat{\mathbf{W}}_{2, \mathcal{L}_1}^{[i]} \otimes \mathbf{D}_i \\ -\left(\widehat{\mathbf{W}}_{2, \mathcal{L}_1}^{[i]}\right)^T \otimes \mathbf{D}_i & -\widehat{\mathbf{W}}_{1, \mathcal{L}_2}^{[i]} \otimes \mathbf{D}_i \end{bmatrix} \right) \cdot \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & -\mathbf{X}_2 \end{bmatrix}, \\
&= -\mathbf{I}_{rN} + \sum_{i=1}^r \begin{bmatrix} \left(\widehat{\mathbf{W}}_{1, \mathcal{L}_1}^{[i]} \otimes \mathbf{D}_i\right) \mathbf{X}_1 & -\left(\widehat{\mathbf{W}}_{2, \mathcal{L}_1}^{[i]} \otimes \mathbf{D}_i\right) \mathbf{X}_2 \\ -\left(\widehat{\mathbf{W}}_{2, \mathcal{L}_1}^{[i]}\right)^T \otimes \mathbf{D}_i \mathbf{X}_1 & \left(\widehat{\mathbf{W}}_{1, \mathcal{L}_2}^{[i]} \otimes \mathbf{D}_i\right) \mathbf{X}_2 \end{bmatrix}, \tag{3.38}
\end{aligned}$$

828 by the mixed-product and block-product properties of the Kronecker product [58]. The trans-  
829 formed auxiliary system (3.38) is monotone if the off-diagonal row-sum is non-negative by Lemma  
830 3.1. Namely, if  $\tau(i) = (i-1) \bmod r + 1$  then

$$\sum_{j \neq i} \left( \mathbf{R} \frac{\partial \mathbf{F}}{\partial \mathbf{z}} \mathbf{R} \right)_{ij} = \begin{cases} \hat{w}_1^{[\tau(i)]} \sum_{j=1, j \neq i}^{r|\mathcal{L}_1|} (\mathbf{X}_1)_{\tau(i)j} - \hat{w}_2^{[\tau(i)]} \sum_{j=1, j \neq i}^{r|\mathcal{L}_2|} (\mathbf{X}_2)_{\tau(i)j} & 1 \leq i \leq r|\mathcal{L}_1|, \\ -\hat{w}_2^{[\tau(i)]} \sum_{j=1, j \neq i}^{r|\mathcal{L}_1|} (\mathbf{X}_1)_{\tau(i)j} + \hat{w}_1^{[\tau(i)]} \sum_{j=1, j \neq i}^{r|\mathcal{L}_2|} (\mathbf{X}_2)_{\tau(i)j} & r|\mathcal{L}_1| + 1 \leq i \leq rN. \end{cases} \tag{3.39}$$

831  
832 In particular, as  $\text{sgn}(\mathbf{DT}) = \mathcal{S}_2$ , then all positive and negatives components of the row-sum are  
833 scaled by  $w_2^{[k]}$  and  $w_1^{[k]}$ , respectively. Then for sufficiently small values of  $w_1^{[k]}$  combined with  
834 relatively large values  $w_2^{[k]}$ , confirmed by  $w_1^{[k]} \ll w_2^{[k]}$ , we have that,

$$\sum_{j \neq i} \left( \mathbf{R} \frac{\partial \mathbf{F}}{\partial \mathbf{z}} \mathbf{R} \right)_{ij} \geq 0 \tag{3.40}$$

836 for all  $1 \leq i \leq rN$ . Therefore the auxiliary system (3.34) is type K by Lemma 3.1 and so  
837 is monotone in the direction for solutions associated with laminar patterning in high polarity  
838 regimes.  $\square$

839 Applying the cooperative transformation in high-polarity regimes to an IO system (2.1) where  
840 the extrema of the spectra are preserved in the quotient mapping guarantees the global con-  
841 vergence of laminar patterns in the large-scale systems. Critically, this extends the existence  
842 statement of Theorem 3.1 to sufficient conditions for large-scale laminar patterning.

843 **Theorem 3.2** (Global convergence of laminar patterns in highly-polarised regimes). *Consider*  
844 *the large-scale IO system (2.1) spatially coupled by the global adjacency matrix  $\mathbf{P}$  (2.3) where*  
845  *$\mathbf{W}_k \in \mathcal{W}$  for  $k \in \{1, \dots, r\}$ . Assuming that (A1) is satisfied, the laminar pattern partition  $\pi_2$*   
846 *is simultaneously equitable, and each connectivity graph,  $\mathcal{G}_k$ , is highly polarised,  $w_1^{[k]} \ll w_2^{[k]}$ .*  
847 *If  $\bar{\lambda}_{k,2} = \min(\text{Spec}(\mathbf{W}_k))$  such that the laminar pattern existence criterion, Theorem 3.1, is*  
848 *satisfied, then laminar patterns are globally convergent in the large-scale IO system (2.1).*

849 *Proof.* Following Theorem 3.1, by analysing the quotient graphs there exists  $\bar{\Lambda}_2$  such that the  
850 HSS instability condition (2.17) is satisfied. In addition, Lemma 3.6 guarantees that the IO  
851 system (2.1) generates monotone solutions in the direction of laminar patterns, such that the

852 eigenvector associated with  $\bar{\lambda}_{k,2}$  is directed in the positive orthant,  $\tilde{\mathbf{R}}\mathbf{L}\bar{\mathbf{v}}_{k,2} > 0$ . Furthermore,  
 853 Lemma 3.4 ensures that the linearised IO system is irreducible and thus the IO system (2.1) is  
 854 strongly monotone by Lemma 3.2.

855 By the identical arguments of Theorem 3.1, the corresponding large-scale auxiliary system  
 856 (3.35) has bounded solutions, which induces the convergence of solutions to steady-state  $\mathbf{Rz}^{**} \neq$   
 857  $\mathbf{Rz}^*$  by the Cooperative Irreducible Convergence Theorem (Theorem 4.3.3 in [47]). Critically,  
 858 mapping back to the original coordinating system guarantees that vertices in different layers  
 859 have contrasting solutions.  $\square$

860 The sufficient conditions for large-scale laminar patterning outlined in Theorem 3.2 ensure  
 861 that the behaviour observed in the quotient systems is preserved in the corresponding large-scale  
 862 systems. Subsequently, this enables an analytic approach to pattern prediction as we can fully  
 863 determine the spectra of the quotient graphs  $\mathcal{G}_2$  independently and without imposing commu-  
 864 tativity conditions on the reduced adjacency matrices. The following example demonstrates the  
 865 accessibility of the analysis for large-scale IO systems spatially coupled multilayer connectivity  
 866 graphs.

867 **Example 3.2.** *We revisit Example 3.1 to seek a polarity regime that guarantees the global*  
 868 *convergence of laminar patterns using analysis conducted in the quotient systems when templating*  
 869 *the large-scale system using the equitable partition,  $\pi_2$ . Namely, in conjunction with the results*  
 870 *of applying Theorem 3.1 to the DIDO system (3.20-3.24) as in Example 3.1, we also invoke*  
 871 *Theorem 3.2 to isolate regions of polarity parameter values for  $w_1^{[1]}$  and  $w_1^{[2]}$  in which the extrema*  
 872 *of the quotient graph spectra are the extrema of the large-scale graphs.*

873 *As each of the connectivity large-scale and quotient graphs are row-stochastic, we always have*

$$\max(\text{Spec}(\bar{\mathbf{W}}_k)) = \max(\text{Spec}(\mathbf{W}_k)) = 1, \quad (3.41)$$

875 *therefore, the quotient graphs retain the maximum eigenvalues, and so now we focus on the*  
 876 *preservation of the minimal eigenvalues.*

877 *In Figure 9 we have demonstrated that for  $w_1^{[k]} < 0.5$  and  $w_2^{[k]} = 1$ , the quotient connectivity*  
 878 *graphs for short-range diffusion and contact-dependent signalling mechanism,  $\mathcal{G}_{1,\pi_2}$  and  $\mathcal{G}_{2,\pi_2}$*   
 879 *(which are denoted  $\mathcal{G}_1$  and  $\mathcal{G}_3$  in Figure 9, respectively) have the capacity to bound the spectra of*  
 880 *the large-scale graphs  $\mathcal{G}_1$  and  $\mathcal{G}_2$  from below. Critically, this implies that for any  $w_1^{[k]} < 0.5$  with*  
 881 *fixed  $w_2^{[k]} = 1$ , which induced HSS instability, solutions will be locally directed towards laminar*  
 882 *patterning and so following from Theorem 3.2, for sufficiently small  $w_1^{[k]} < 0.5$ , the large-scale*  
 883 *DIDO system (3.20-3.24) will converge to laminar patterns.*

884 *To highlight the results of applying both theorems 3.1 and 3.2 to the example DIDO system*  
 885 *(3.20-3.24), regions of pattern convergence were found numerically in Figure 10 which includes*  
 886 *examples of large-scale simulations for which laminar patterns are and are not dominant. It*  
 887 *is worth noting that the magnitude of the difference between  $w_1^{[k]}$  and  $w_2^{[k]}$  is dependent on*  
 888 *the magnitude of the entries of  $\mathcal{DT}(\mathbf{u}_i)$  and thus assuming that  $w_1^{[k]} \ll w_2^{[k]}$  is sufficient for*  
 889 *the monotonicity of the large-scale system but is not necessary to satisfy the type K criteria*  
 890 *(Lemma 3.1). Subsequently, simply selecting polarity parameters in which both the HSS instability*  
 891 *condition for the reduced system (3.32) and  $\min(\text{Spec}(\bar{\mathbf{W}}_k)) = \min(\text{Spec}(\mathbf{W}_k))$  are satisfied*  
 892 *resulted in the large-scale system converging to laminar patterns without requiring significant*  
 893 *layer-wise polarity.*



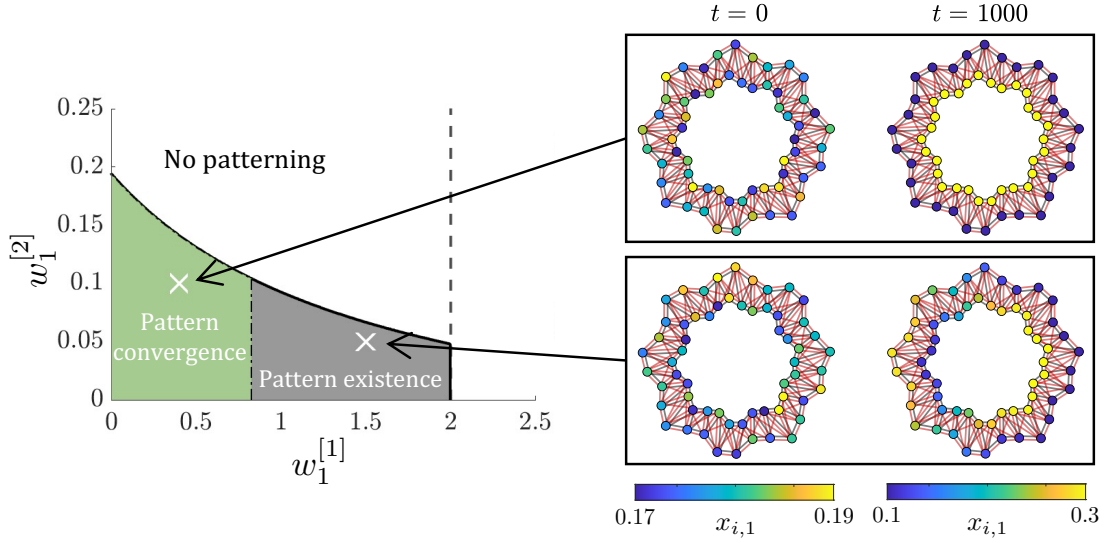


FIGURE 10. Polarity parameter regimes for the existence and convergence of laminar pattern in the large-scale IO system (3.20-3.24). The grey existence region is determined using the quotient system analysis and is defined by polarity-driven HSS instability inequality (3.32) for fixed  $w_2^{[1]} = w_2^{[2]} = 1$ . The green convergence region highlights the subset of grey region in  $(w_1^{[1]}, w_1^{[2]})$ -space in which  $\min(\text{Spec}(\overline{\mathbf{W}}_k)) = \min(\text{Spec}(\mathbf{W}_k))$ . Example large-simulations are shown for polarity parameters inside the convergence region, (0.4,0.1), and inside the existence region (1.5,0.05). Large-scale bilayer graphs are shown with both  $\mathcal{G}_1$  and  $\mathcal{G}_2$  embedded in the same vertex set with edges in black and red, respectively. Vertex colour corresponds to the values of  $x_{i,1}$  in each  $v_i$ . Simulations were initiated from small random perturbations about the HSS of the IO system (3.20-3.24) and first and final states are shown following trajectory convergence. IO system (3.20-3.24) parameter values and details on simulations are given in Appendix B.

894 As highlighted in Example 3.2, theorems 3.1 and 3.2 facilitate the analytic study of laminar  
 895 pattern formation in large-scale interconnected dynamical systems, independent of the number  
 896 of cells in the system or physical dimension owing to the topological definition of the connec-  
 897 tivity graphs. Hence the pattern analysis conducted on the quotient systems can evolve from  
 898 explorative- which geometries enable laminar patterning, to constructive- how much edge weight  
 899 manipulation is required to robustly generate laminar patterns.

900

#### 4. DISCUSSION

901 In this study, we have developed analytic methods for exploring the interplay of cellular  
 902 polarity and multiple signalling mechanisms in the emergence of laminar patterns in bilayer tis-  
 903 sues independent of the precise intracellular kinetics. To facilitate such analysis we focused on  
 904 methods of dimension reduction of large interconnected dynamical systems that preserve funda-  
 905 mental cellular behaviour. Specifically, we demonstrate that cell signalling transfer dynamics can  
 906 be treated as a proxy for intracellular components, reducing the dimensionality of the spatially  
 907 discrete ODE systems by analysing only the spatially dependent intracellular components, which  
 908 enabled us to provide sufficient conditions for the existence and uniqueness of the homogeneous  
 909 steady state.

910 In addition, we use properties of commuting graphs to decompose large MIMO systems into  
 911 lower-order interconnected systems, decoupling the spatial and temporal components. This not  
 912 only has advantages in reducing the computational cost associated with large-scale eigenvalue  
 913 problems but also enables the direct analysis of the influence each signalling mechanism has on  
 914 driving spatial instabilities of the homogeneous steady state. From a practical standpoint, the  
 915 requirement of commuting graphs of cell signalling currently limits the applications of the large-  
 916 scale HSS instability results in general pattern formation problems as there exist no analytically  
 917 tractable methods for checking these conditions for large graphs. Therefore developing a proce-  
 918 dure for constructing commuting families of large signalling graphs is critical to broadening the  
 919 scope of these modelling approaches.

920 Combining methods of multilayer graph partitions with monotone dynamical systems the-  
 921 ory, we demonstrate the existence of laminar pattern formation with competitive kinetics relies  
 922 on the amount of signalling polarisation present within each graph. Critically, the application  
 923 of equitable partitions to the connectivity structures where layer-wise symmetries are present  
 924 enables drastic dimensionality reductions of the global dynamical system when seeking contrast-  
 925 ing steady-states between the bilayer of cells. Thereby exploiting the spectral structure of the  
 926 quotient graphs we demonstrate the instability conditions derived for large-scale interconnected  
 927 dynamical systems that can be applied to the reduced system, independent of commutativity  
 928 of the quotient graphs, which facilitates the investigation of whether the pre-defined contrast-  
 929 ing states are achievable with the given kinetics. The symmetry requirements of the equitable  
 930 partitions need not be restricted to globally regular cell-cell interaction graphs. We only require  
 931 regularity within each partition which therefore permits the application of semi-regular graphs  
 932 for dimension reduction. Such graphs can then capture characteristic traits of the biological sys-  
 933 tem such as subpopulation phenotypes and tissue curvature, and their influence on intracellular  
 934 behaviour.

935 These methods of prescribing patterns allude to studying the inverse problem, specifically,  
 936 starting with the desired pattern of the tissue and then defining constraints for the intracellu-  
 937 lar kinetics that have the potential to induce such instabilities, as previously demonstrated in  
 938 spatially continuous Turing systems [59]. Additionally, as the full and quotient system analy-  
 939 sis depends only on the topology of the connectivity networks, the results from this study are  
 940 immediately applicable to more biologically relevant 3D morphologies. Classically, introducing  
 941 3D structures drastically increases the computational complexity in pattern formation analysis  
 942 [19, 60, 61], yet the topological approach allows for the transition between physical dimension  
 943 with no additional requirements as discussed in [37].

944 Investigating the link between the reduced and large-scale dynamical systems when seeking  
 945 laminar patterns, we demonstrate the statements of laminar existence derived using pattern-  
 946 templating have the capacity to be globally convergent in the corresponding large-scale inter-  
 947 connected system in high polarity regimes. To show the existence of a monotone transformation  
 948 we imposed weak but sufficient conditions that  $w_1^{[k]} \ll w_2^{[k]}$ , highlighting the requirement of  
 949 edge weight anisotropy for laminar pattern formation. However, we suspect that this condition  
 950 can be significantly refined by illustrating a dependence on the magnitude of entries of  $\mathcal{DT}(\mathbf{u}_i)$   
 951 when applying the type K criterion for monotone solution behaviour, namely, having *a priori*  
 952 estimations of the size of the cellular output signals for given input signal regimes.

953 Furthermore, to explore these links between the quotient and large-scale systems, we impose  
 954 the restrictive semi-regular bilayer structure with identical edge weights. This ensures the ex-  
 955 istence of an equitable partition to generate low-dimensional dynamical representations with  
 956 common spectra which is guided by a graphical perspective. However, these vertex symmetries  
 957 are not the only be a subset of the graph topologies in which preserve spectra between quotient  
 958 and large-scale systems. These results may be generalised to less restrictive spatial domains  
 959 which are more realistic in biological applications by investigating the lifting maps of the pat-  
 960 tern representing quotient graphs as previously considered in synchronised subspaces of balanced  
 961 systems [53]. In addition, our methods of ensuring desired pattern convergence in the large-scale  
 962 dynamics by exploiting the simple block structure of the weighted adjacency matrices  $\mathbf{W}_k \in \mathcal{W}$   
 963 can also be generalised by the row-sum constructions of block weighted adjacency matrices in  
 964 general liftings as highlighted in [52, 53], such that the structures considered in this study present  
 965 as a special case of those discussed previously. Subsequently, further work is required to isolate  
 966 desired vertex pattern modes from generic lifted weighted adjacency matrices, specifically under  
 967 the control of edge weights to satisfy the type K criterion to guarantee global convergence within  
 968 these more general graph structures.

969 As discussed in the previous interconnected monotone systems studies of pattern formation  
 970 [29, 34, 35], the most limiting assumption in large-scale systems analysis is the existence of  
 971 competitive to cooperative monotone kinetics transformation which previously has relied on the  
 972 sufficient requirement of the connectivity graphs being bipartite. However, in Section 3.4 we not  
 973 only demonstrate that laminar patterns are not the dominant pattern of bipartite bilayer graphs  
 974 but also manipulating graph edge weights of non-bipartite graphs enables competitive to coop-  
 975 erative kinetics transformations for laminar pattern formation. The key feature of cooperative  
 976 dynamics used in these pattern formation studies is the guarantee of non-periodic solutions when  
 977 considering bounded kinetics [47]. Therefore another promising direction to ensure such solution  
 978 behaviour is the study of variational families associated with the interconnected systems [62],  
 979 that is, applying Lyapunov methods for non-oscillatory dynamics to enable the investigation of  
 980 intracellular crosstalk inference in biologically relevant morphologies.

981 Throughout this study, we have reserved the precise definitions of the intracellular kinetics  
 982 and associated signalling mechanisms to consider general competitive MIMO dynamics. Sub-  
 983 sequently, the generality of results presented here enables the investigation of crosstalk of key  
 984 molecular pathways with multiple spatially dependent intracellular signalling components, such  
 985 as the well-established Wnt-Notch interactions that have been observed in both intestinal and  
 986 mammary epithelia [63]. Both the Wnt and Notch pathways are involved in cell-fate determi-  
 987 nation and have been observed to have active apical-basal polarity mechanisms during tissue  
 988 development [64, 65]. However existing models have previously been limited to analysis of one  
 989 or two cells [66, 67], the methods we provide here allow us to study how the geometry of the  
 990 tissue influences such cell-fate choices, specifically within the bilayer structures commonly found  
 991 in mammary glands.

992

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## 1156 APPENDIX A. ADDITIONAL PROPERTIES OF INTERWOVEN MATRICES

1157 Here, we present further properties of interwoven matrices which have particular applications  
1158 in dynamical network theory of mixed kernels. Specifically, an interwoven matrix  $\mathbf{P}$  is composed  
1159 of the sequence of real matrices  $\mathcal{M} = \{\mathbf{M}_i \mid \mathbf{M}_i \in \mathbb{R}^{N \times N}\}$  called constructor matrices such that  
1160 the rows and columns of each matrix  $\mathbf{M}_i$  are uniformly separated by zero elements, preserving  
1161 the structure of  $\mathbf{M}_i$ , where the order of  $\mathcal{M}$  defines the sequence of spacing. Formally we define  
1162 an interwoven matrix  $\mathbf{P}$  by

$$1163 \quad \mathbf{P} = \sum_{i=1}^r \mathbf{M}_i \otimes \text{diag}(\delta_{i,1}, \dots, \delta_{i,r}), \quad (\text{A.1})$$

1164 where  $\otimes$  is the Kronecker product and  $\delta_{i,j}$  is the Kronecker delta function (2.4) as defined in  
1165 Section 2.1. Here we do not assume that the constructor matrices  $\mathbf{M}_i$  are nonnegative.

1166 The following statement provides a constructive diagonal decomposition of the  $\mathbf{P}$ , illuminating  
 1167 the spectral structure of  $\mathbf{P}$ .

1168 **Lemma A.1.** *Let  $\mathbf{P}$  be the interwoven matrix defined as defined in equation (A.1) and let  $\mathbf{Q}$*   
 1169 *be the permutation matrix such that*

$$1170 \quad \mathbf{Q} = [\mathbf{I}_N \otimes \mathbf{e}_1, \dots, \mathbf{I}_N \otimes \mathbf{e}_r]. \quad (\text{A.2})$$

1171 where  $\mathbf{e}_i = [\delta_{i,1}, \dots, \delta_{i,r}]^T$ . Then

$$1172 \quad \mathbf{Q}^T \mathbf{P} \mathbf{Q} = \bigoplus_{i=1}^r \mathbf{M}_i, \quad (\text{A.3})$$

1173 where  $\oplus$  is the direct sum of tensors.

1174 *Proof.* Consider the permutation map  $\tau : \{1, \dots, rN\} \rightarrow \{1, \dots, rN\}$  such that

$$1175 \quad \tau(x) = ((x-1) \bmod r)N + \left\lfloor \frac{x-1}{r} \right\rfloor + 1 \quad (\text{A.4})$$

1176 which permutes the rows and columns of  $\mathbf{P}$  so any row and columns  $\mathbf{M}_i$  of  $\mathbf{P}$  become adjacent  
 1177 for  $1 \leq i \leq r$ . In cycle notation,  $\tau(x)$  defines the mapping

$$1178 \quad \begin{pmatrix} 1 & 2 & \dots & r & r+1 & \dots & rN \\ 1 & N+1 & \dots & (r-1)N+1 & 2 & \dots & (r-1)N+N \end{pmatrix} \quad (\text{A.5})$$

1179 which represents the column and row permutation of  $\mathbf{P}$ . The cycle (A.5) defined by equation  
 1180 (A.4) yields the following matrix representation

$$1181 \quad \mathbf{Q} = [\mathbf{I}_N \otimes \mathbf{e}_1, \dots, \mathbf{I}_N \otimes \mathbf{e}_r], \quad (\text{A.6})$$

1182 namely,  $\mathbf{Q}_{i,\tau(i)} = 1$  and zero entries else. Therefore applying the transformation  $\mathbf{Q}$  to  $\mathbf{P}$  produces  
 1183 the block diagonal representation where

$$1184 \quad \mathbf{Q}^T \mathbf{P} \mathbf{Q} = \text{blkdiag}(\mathbf{M}_1, \dots, \mathbf{M}_r) \quad (\text{A.7})$$

1185 which is by definition the direct sum of matrices  $\mathbf{M}_i$ . □

1186 The block diagonal representation of  $\mathbf{P}$  following from Lemma A.1 motivates the subsequent  
 1187 properties involving the spectra and inverse of the interwoven matrix  $\mathbf{P}$ .

1188 **Lemma A.2.** *Let  $\mathbf{P}$  be the interwoven matrix as defined in equation (A.1). Then  $\mathbf{P}$  has the*  
 1189 *following properties:*

(i)

$$1190 \quad \text{Spec}(\mathbf{P}) = \bigcup_{i=1}^r \text{Spec}(\mathbf{M}_i) \quad (\text{A.8})$$

1191 including multiplicities;

1192 (ii) if  $\mathbf{M}_i$  is invertible for all  $1 \leq i \leq k$ , then the inverse of the interwoven matrix  $\mathbf{P}$  is the  
 1193 interweave of the inverse of the construction matrices. That is,

$$1194 \quad \mathbf{P}^{-1} = \sum_{i=1}^r \mathbf{M}_i^{-1} \otimes \text{diag}(\delta_{i,1}, \dots, \delta_{i,r}), \quad (\text{A.9})$$



1195 (iii) the trace of the interwoven matrix is the sum of the traces of the constructor matrices

$$1196 \quad \operatorname{tr}(\mathbf{P}) = \sum_{i=1}^r \operatorname{tr}(\mathbf{M}_i), \quad (\text{A.10})$$

1197 (iv) the determinant of the interwoven matrix is the product of the determinant of the con-  
1198 structor matrices

$$1199 \quad \det(\mathbf{P}) = \prod_{i=1}^r \det(\mathbf{M}_i). \quad (\text{A.11})$$

1200 *Proof.* Let  $\lambda_{k,j} \in \operatorname{Spec}(\mathbf{M}_k)$  with its associated eigenvector  $\mathbf{v}_{k,j}$ . Then define the interweave  
1201 extension of  $\mathbf{v}_{k,j}$  by

$$1202 \quad \tilde{\mathbf{v}}_{k,j} = \mathbf{v}_{k,j} \otimes \begin{bmatrix} \delta_{k,1} \\ \vdots \\ \delta_{k,r} \end{bmatrix}. \quad (\text{A.12})$$

For brevity, denote the Kronecker diagonal matrix by  $\mathbf{D}_i = \operatorname{diag}(\delta_{i,1}, \dots, \delta_{i,r})$  and then by direct computation we have

$$\begin{aligned} \mathbf{P}\tilde{\mathbf{v}}_{k,j} &= \left( \sum_{i=1}^r \mathbf{M}_i \otimes \mathbf{D}_i \right) \tilde{\mathbf{v}}_{k,j}, \\ &= \left( \sum_{i=1}^r \mathbf{M}_i \otimes \mathbf{D}_i \right) \left( \mathbf{v}_{k,j} \otimes [\delta_{k,1}, \dots, \delta_{k,r}]^T \right), \\ &= \left( \sum_{i=1}^r \mathbf{M}_i \mathbf{v}_{k,j} \otimes \mathbf{D}_i [\delta_{k,1}, \dots, \delta_{k,r}]^T \right), \\ &= \mathbf{M}_k \mathbf{v}_{k,j} \otimes [\delta_{k,1}, \dots, \delta_{k,r}]^T, \end{aligned} \quad (\text{A.13})$$

1203 where the last two equalities follow from the mixed product property of the Kronecker product  
1204 and that direct multiplication of the Kronecker matrix and vector are non-zero only if  $i = j$ .  
1205 Therefore we have that

$$1206 \quad \mathbf{P}\tilde{\mathbf{v}}_{k,j} = \mathbf{M}_k \mathbf{v}_{k,j} \otimes [\delta_{k,1}, \dots, \delta_{k,r}]^T = \lambda_{k,j} \mathbf{v}_{k,j} \otimes [\delta_{k,1}, \dots, \delta_{k,r}]^T = \lambda_{k,j} \tilde{\mathbf{v}}_{k,j}, \quad (\text{A.14})$$

1207 thus  $\lambda_{k,j}$  is an eigenvalue of  $\mathbf{P}$  with associated eigenvector  $\tilde{\mathbf{v}}_{k,j}$ .

1208 Next, there exists  $\mathbf{M}_i^{-1}$  for all  $1 \leq i \leq r$  from the assumption in (ii). Note that

$$1209 \quad \mathbf{D}_i \mathbf{D}_j = \begin{cases} \mathbf{D}_i & i = j, \\ \mathbf{0}_{r \times r} & i \neq j, \end{cases} \quad (\text{A.15})$$

then consider the following matrix  $\mathbf{R}$  defined by the multiplication

$$\begin{aligned} \mathbf{R} &= \left( \sum_{i=1}^r \mathbf{M}_i \otimes \mathbf{D}_i \right) \left( \sum_{i=1}^r \mathbf{M}_i^{-1} \otimes \mathbf{D}_i \right), \\ &= (\mathbf{M}_1 \otimes \mathbf{D}_1) \left( \sum_{i=1}^r \mathbf{M}_i^{-1} \otimes \mathbf{D}_i \right) + \dots + (\mathbf{M}_r \otimes \mathbf{D}_r) \left( \sum_{i=1}^r \mathbf{M}_i^{-1} \otimes \mathbf{D}_i \right). \end{aligned} \quad (\text{A.16})$$

From the mixed-product property of the Kronecker product and equation (A.15), we have that (A.16) reduces to

$$\begin{aligned} \mathbf{R} &= \mathbf{M}_1 \mathbf{M}_1^{-1} \otimes \mathbf{D}_1 + \dots + \mathbf{M}_r \mathbf{M}_r^{-1} \otimes \mathbf{D}_r, \\ &= \sum_{i=1}^r \mathbf{I}_n \otimes \mathbf{D}_i = \mathbf{I}_{rN}, \end{aligned} \quad (\text{A.17})$$

1210 hence the inverse of  $\mathbf{P}$  is given by  $\mathbf{P}^{-1} = \sum_{i=1}^r \mathbf{M}_i^{-1} \otimes \mathbf{D}_i$  as required for (ii).

The trace of a Kronecker product is the product of the trace [45] such that  $\text{tr}(\mathbf{M}_i \otimes \mathbf{D}_i) = \text{tr}(\mathbf{M}_i) \text{tr}(\mathbf{D}_i)$ . Therefore applying the trace to the definition of  $\mathbf{P}$  (A.1) yields

$$\text{tr}(\mathbf{P}) = \text{tr} \left( \sum_{i=1}^r \mathbf{M}_i \otimes \mathbf{D}_i \right) = \sum_{i=1}^r \text{tr}(\mathbf{M}_i \otimes \mathbf{D}_i) = \sum_{i=1}^r \text{tr}(\mathbf{M}_i) \text{tr}(\mathbf{D}_i) = \sum_{i=1}^r \text{tr}(\mathbf{M}_i), \quad (\text{A.18})$$

1211 where the second equality holds by the trace of the sum of matrices [45] and the fourth holds by  
1212  $\text{tr}(\mathbf{D}_i) = 1$  for all  $1 \leq i \leq r$ .

1213 Property (iv) follows immediately from (i) by expressing the determinant of a matrix as  
1214 the product of the eigenvalues including multiplicities [45]. From (i) we have that  $\text{Spec}(\mathbf{P}) =$   
1215  $\text{Spec}(\mathbf{M}_1) \cup \dots \cup \text{Spec}(\mathbf{M}_r)$  including multiplicities and so we know the eigenvalues of  $\mathbf{P}$  are  
1216 all the eigenvalues of each  $\mathbf{M}_i$ . Subsequently, the determinant of  $\mathbf{P}$  must be the product of all  
1217 these eigenvalues which leads to the required representation

$$\det(\mathbf{P}) = \left( \prod_{j=1}^N \lambda_{1,j} \right) \dots \left( \prod_{j=1}^N \lambda_{r,j} \right) = \det(\mathbf{M}_1) \dots \det(\mathbf{M}_r) = \prod_{i=1}^r \det(\mathbf{M}_i). \quad (\text{A.19})$$

1219

□

1220 A direct consequence of Lemma A.2 is that if  $\mathbf{M}_j$  are nonnegative, then the spectral radius,  
1221  $\rho$ , of interwoven matrix  $\mathbf{P}$  is a real eigenvalue and is defined by

$$\rho(\mathbf{P}) = \max_i (\rho(\mathbf{M}_i)) = \max \left( \bigcup_{i=1}^r \text{Spec}(\mathbf{M}_i) \right) \quad (\text{A.20})$$

1223 from the Perron-Frobenius theorem for nonnegative matrices [47].

1224 In addition to its spectral properties, the interwoven matrix (A.1) also has the following  
1225 exponent property.

1226 **Lemma A.3.** *Let  $\mathbf{P}$  be the interwoven matrix defined as defined in equation (A.1). Then for*  
1227 *all  $n \in \mathbb{N}$*

$$\mathbf{P}^n = \sum_{i=1}^r \mathbf{M}_i^n \otimes \mathbf{D}_i. \quad (\text{A.21})$$

1228

*Proof.* The result follows by induction. Assume for some  $k \in \mathbb{N}$  that equation (A.21) holds. Consider the case for  $k + 1$ ,

$$\begin{aligned} \left( \sum_{i=1}^r \mathbf{M}_i \otimes \mathbf{D}_i \right)^{k+1} &= \left( \sum_{i=1}^r \mathbf{M}_i \otimes \mathbf{D}_i \right) \left( \sum_{i=1}^r \mathbf{M}_i \otimes \mathbf{D}_i \right)^k, \\ &= \left( \sum_{i=1}^r \mathbf{M}_i \otimes \mathbf{D}_i \right) \left( \sum_{i=1}^r \mathbf{M}_i^k \otimes \mathbf{D}_i \right), \end{aligned} \quad (\text{A.22})$$

where the second equality follows from the inductive hypothesis. Applying the multiplication property of the of Kronecker matrix (A.15), expansion of equation (A.22) and the mixed-product property of the Kronecker product leads to the following cancellations,

$$\begin{aligned} \left( \sum_{i=1}^r M_i \otimes D_i \right) \left( \sum_{i=1}^r M_i^k \otimes D_i \right) &= (M_1 \otimes D_1) \left( \sum_{i=1}^r M_i^k \otimes D_i \right) + \cdots + (M_r \otimes D_r) \left( \sum_{i=1}^r M_i^k \otimes D_i \right), \\ &= M_1 M_1^k \otimes D_1 + \cdots + M_r M_r^k \otimes D_r, \\ &= \sum_{i=1}^r M_i^{k+1} \otimes D_i. \end{aligned} \tag{A.23}$$

1229 That is, the inductive hypothesis is satisfied and thus equation (A.21) holds for all  $n \in \mathbb{N}$  by the  
1230 principle of induction.

1231

□

1232

## APPENDIX B. COMPUTATIONAL METHODS

1233 The ODE systems in this study were solved numerically using the `ODE15s` solver in Matlab  
1234 (R2021a). Simulations were performed over a total of 1000 time units in addition to an stopping  
1235 event applied to the ODE solver to check for solution convergence. Namely, if all trajectories  
1236 varied less than  $1 \times 10^{-4}$  over four consecutive iterations, then we assume that the system has  
1237 converged to a steady state. We note that all simulations presented in this study satisfied the  
1238 convergence criteria. The intracellular kinetics parameter values of the IO system (3.20-3.24)  
1239 used in all simulations are given in Table 1 below.

Parameter	$a_1$	$a_2$	$b_1$	$b_2$	$b_3$	$k_1$	$k_2$	$h_1$	$h_2$	$h_3$
Value	0.01	1	100	100	100	2	2	2	2	1

TABLE 1. Parameter values used in the simulations of the IO system (3.20-3.24).

1240 Random initial conditions were sampled from a uniform distribution using the `rand` func-  
1241 tion. The homogeneous steady state of the system was calculated using the `fsolve` function  
1242 that implements the trust-region-dogleg minimisation algorithm [68]. In addition, heterotypic  
1243 weighting parameters was set to  $w_2^{[k]} = 1$  for all simulations. Both quotient and large-scale ODE  
1244 systems where solved using the same kinetics functions where respective adjacency matrices were  
1245 introduced as an argument to these kinetics functions to ensure solution consistency.

1246 To visualise the approximate cell membranes in the large-scale simulation Voronoi diagrams  
1247 were drawn around graph vertices using the `delaunayTriangulation` and `voronoi` functions  
1248 within the Computational Geometry toolbox in Matlab (R2021a). Ghost vertices were intro-  
1249 duced to ensure that each graph vertex has a closed boundary.

1250 Eigenvalues of the adjacency matrices were calculated using the `eig` function from the Linear  
1251 Algebra toolbox in Matlab (R2021a). The edge structures of the semi-regular non-bipartite  
1252 graphs used in the numerical spectral investigation are given in Table 2. These graphs were  
1253 confirmed non-bipartite by violating the spectral symmetry property of bipartite graphs.

1254 Source code for the simulations presented in this study can be found at [https://github.com/joshwillmoore1/Mixed\\_Signal\\_mechanisms](https://github.com/joshwillmoore1/Mixed_Signal_mechanisms).  
1255

	$n_{1,\mathcal{L}_1}$	$n_{2,\mathcal{L}_1}$	$n_{1,\mathcal{L}_2}$	$n_{2,\mathcal{L}_2}$
$\mathcal{G}_1$	2	2	2	2
$\mathcal{G}_2$	2	3	2	3
$\mathcal{G}_3$	2	4	2	4
$\mathcal{G}_4$	4	3	4	3

TABLE 2. Summary of the bilayer edge connectivity structures for the graphs used in the numerical investigation of non-bipartite spectra in Figure 9.

1256 APPENDIX C. PROOF OF  $\pi_2$ -DEPENDENT SPECTRAL GAP FOR  $\mathcal{G}_{2D}$  AND  $\mathcal{G}_{3D}$  FROM FIGURE 8B

1257 Bipartite graphs have many particularly convenient algebraic properties due to the existence  
 1258 of a simple canonical form of the respective adjacency matrix. Namely, for any bipartite graph  
 1259  $\mathcal{G}_k$  with adjacency matrix  $\mathbf{W}_k$  there exists a permutation matrix  $\mathbf{U}$  that re-indexes the vertices  
 1260 with respect to the independent sets  $V_1$  and  $V_2$  such that

$$1261 \quad \mathbf{U}^T \mathbf{W}_k \mathbf{U} = \begin{bmatrix} \mathbf{0} & \mathbf{X}_k \\ \mathbf{X}_k^T & \mathbf{0} \end{bmatrix}, \quad (\text{C.1})$$

1262 where  $\mathbf{X}_k$  is the biadjacency matrix [38]. Subsequently, the spectra of the bipartite graphs have  
 1263 a distinct structure such that there is a symmetry of eigenvalues respective to the biadjacency  
 1264 matrices, i.e.,  $\text{Spec}(\mathbf{W}_k) = \text{Spec}(\mathbf{X}_k) \cup \text{Spec}(-\mathbf{X}_k)$ . Leveraging the spectral symmetry of  
 1265 bipartite graphs and the spectral retention of equitable partitions, we demonstrate that for the  
 1266 bipartite bilayer graphs  $\mathcal{G}_{2D}$  and  $\mathcal{G}_{3D}$  in Figure 8, the smallest eigenvalue of  $\mathbf{X}_k$  is  $-\bar{\lambda}_{k,2}$ , the  
 1267 polarity driven eigenvalue associated with laminar pattern template  $\pi_2$ .

1268 **Lemma C.1.** *Let  $\mathcal{G}_k$  be a regular bipartite bilayer graph with associated row-stochastic weighted*  
 1269 *adjacency matrix  $\mathbf{W}_k$  (2.8) for 2D or 3D tissues as shown in Figure 8 ( $k = 2D, 3D$ ). Consider*  
 1270 *the equitable partition  $\pi_2$  such that the quotient graph,  $\mathcal{G}_{k,\pi_2}$ , consists of only two representative*  
 1271 *vertices in each layer of  $\mathcal{G}_k$  and has the reduced adjacency matrix  $\bar{\mathbf{W}}_k$  (3.5). Then biadjacency*  
 1272 *matrix  $\mathbf{X}_k$  associated with  $\mathbf{W}_k$  satisfies*

$$1273 \quad -\bar{\lambda}_{k,2} = \min(\text{Spec}(\mathbf{X}_k)), \quad (\text{C.2})$$

1274 where  $\bar{\lambda}_{k,2}$  is the smallest eigenvalue of  $\bar{\mathbf{W}}_k$  with associated eigenvector  $\bar{\mathbf{v}}_{k,2}$ .

1275 *Proof.* We present the proof for  $\mathcal{G}_{2D}$  as the following argument holds immediately for  $\mathcal{G}_{3D}$  also. As  
 1276 we make use of the biadjacency form of  $\mathbf{W}_{2D}$ , we first construct the biadjacency transformation  
 1277  $\mathbf{U}$ . The bipartite bilayer graph  $\mathcal{G}_{2D}$  has vertex indices in layer-wise order as defined in Section  
 1278 2.1 with block adjacency matrices given in Example 2.2. To reorder the vertices of  $\mathcal{G}_{2D}$  such  
 1279 that vertex groups  $V_1$  and  $V_2$  are ordered consecutively, we define the permutation matrix

$$1280 \quad \mathbf{U} = \begin{bmatrix} \mathbf{I}_{|\mathcal{L}_1|/2} \otimes \mathbf{D}_1 & \mathbf{I}_{|\mathcal{L}_1|/2} \otimes \mathbf{D}_2 \\ \mathbf{I}_{|\mathcal{L}_2|/2} \otimes \mathbf{D}_2 & \mathbf{I}_{|\mathcal{L}_2|/2} \otimes \mathbf{D}_1 \end{bmatrix}, \quad (\text{C.3})$$

where  $|\mathcal{L}_1| = |\mathcal{L}_2|$  as each layer has the same number of vertices. In particular we have the biadjacency form

$$\begin{aligned} \mathbf{U}^T \mathbf{W}_{2D} \mathbf{U} &= \begin{bmatrix} \mathbf{I}_{|\mathcal{L}_1|/2} \otimes \mathbf{D}_1 & \mathbf{I}_{|\mathcal{L}_1|/2} \otimes \mathbf{D}_2 \\ \mathbf{I}_{|\mathcal{L}_2|/2} \otimes \mathbf{D}_2 & \mathbf{I}_{|\mathcal{L}_2|/2} \otimes \mathbf{D}_1 \end{bmatrix} \begin{bmatrix} \widehat{\mathbf{W}}_{1,\mathcal{L}_1} & \widehat{\mathbf{W}}_{2,\mathcal{L}_1} \\ \widehat{\mathbf{W}}_{2,\mathcal{L}_1}^T & \widehat{\mathbf{W}}_{1,\mathcal{L}_2} \end{bmatrix} \begin{bmatrix} \mathbf{I}_{|\mathcal{L}_1|/2} \otimes \mathbf{D}_1 & \mathbf{I}_{|\mathcal{L}_1|/2} \otimes \mathbf{D}_2 \\ \mathbf{I}_{|\mathcal{L}_2|/2} \otimes \mathbf{D}_2 & \mathbf{I}_{|\mathcal{L}_2|/2} \otimes \mathbf{D}_1 \end{bmatrix}, \\ &= \begin{bmatrix} 0 & \mathbf{X}_{2D} \\ \mathbf{X}_{2D} & 0 \end{bmatrix}, \end{aligned} \quad (\text{C.4})$$

1281 for  $\mathbf{X}_{2D}$  in cyclic tridiagonal form

$$\mathbf{X}_{2D} = \begin{bmatrix} \hat{w}_2^{[2D]} & \hat{w}_1^{[2D]} & 0 & \cdots & 0 & \hat{w}_1^{[2D]} \\ \hat{w}_1^{[2D]} & \hat{w}_2^{[2D]} & \hat{w}_1^{[2D]} & & & \\ & & \ddots & & & \\ & & & \ddots & & \\ & & & & \hat{w}_1^{[2D]} & \hat{w}_2^{[2D]} & \hat{w}_1^{[2D]} \\ \hat{w}_1^{[2D]} & 0 & \cdots & 0 & \hat{w}_1^{[2D]} & \hat{w}_2^{[2D]} \end{bmatrix}, \quad (\text{C.5})$$

1283 noting that  $\mathbf{X}_{2D}^T = \mathbf{X}_{2D}$  by the regularity of  $\mathcal{G}_{2D}$  and therefore  $\mathbf{U}^T \mathbf{W}_{2D} \mathbf{U}$  is symmetric.

1284 As the laminar pattern template partition  $\pi_2$  is equitable there exists a lifting matrix  $\mathbf{L} \in$   
1285  $\{0, 1\}^{N \times 2}$  that maps the large-scale adjacency matrix  $\mathbf{W}_{2D}$  into its reduced form  $\overline{\mathbf{W}}_{2D}$  such that

$$1286 \quad \mathbf{W}_{2D} \mathbf{L} = \mathbf{L} \overline{\mathbf{W}}_{2D}, \quad (\text{C.6})$$

1287 as demonstrated in [69]. The lifting transformation is constructed by grouping vertices associated  
1288 with the partition  $\pi_2$  on  $\mathbf{W}_{2D}$  for example  $L_{ij} = 1$  if  $v_i \in \mathcal{L}_j$ . Owing to the block structure of  
1289  $\mathbf{W}_{2D}$  (2.8) which follows from the layer-wise vertex indexing, we have that

$$1290 \quad \mathbf{L} = \begin{bmatrix} \mathbf{1}_{|\mathcal{L}_1|,1} & \mathbf{0}_{|\mathcal{L}_1|,1} \\ \mathbf{0}_{|\mathcal{L}_2|,1} & \mathbf{1}_{|\mathcal{L}_2|,1} \end{bmatrix}. \quad (\text{C.7})$$

1291 Critically, the lifting matrix  $\mathbf{L}$  provides the algebraic link between the quotient and large-scale  
1292 graphs.

1293 Following from the regular structure of  $\mathcal{G}_{2D}$  and direct computation, the eigenvector associated  
1294 with  $\bar{\lambda}_{2D,2}$  has the form  $\bar{\mathbf{v}}_{2D,2} = [1, -1]^T$ . The spectral retention property of the equitable  
1295 partition,  $\pi_2$ , guarantees that  $\bar{\lambda}_{2D,2} \in \text{Spec}(\mathbf{W}_{2D})$  where  $\mathbf{L} \bar{\mathbf{v}}_{2D,2}$  is the corresponding eigenvector  
1296 for the large-scale graph  $\mathcal{G}_{2D}$  (by Theorem 9.3.3 in [38]). Explicitly, we have that the lifted  
1297 eigenvector is of the form

$$1298 \quad \mathbf{L} \bar{\mathbf{v}}_{2D,2} = \begin{bmatrix} \mathbf{1}_{|\mathcal{L}_1|,1} \\ -\mathbf{1}_{|\mathcal{L}_2|,1} \end{bmatrix}, \quad (\text{C.8})$$

1299 with associated eigenvalue  $\bar{\lambda}_{2D,2}$ . In the biadjacency matrix form (C.4), the corresponding  
1300 eigenvector has the transformed representation

$$1301 \quad \boldsymbol{\nu} := \mathbf{U}^T \mathbf{L} \bar{\mathbf{v}}_{2D,2} = \underbrace{\begin{bmatrix} 1 & -1 & \cdots & 1 & -1 \end{bmatrix}}_{|\mathcal{L}_1|} \underbrace{\begin{bmatrix} 1 & -1 & \cdots & 1 & -1 \end{bmatrix}}_{|\mathcal{L}_2|}^T. \quad (\text{C.9})$$

1302 The spectral symmetry of bipartite graphs ensures that if there exists an eigenpair  $(\bar{\lambda}_{2D,2}, \boldsymbol{\nu} = [\mathbf{x}, \mathbf{y}^T])$   
1303 then there must also exist the eigenpair  $(-\bar{\lambda}_{2D,2}, \tilde{\boldsymbol{\nu}} = [\mathbf{x}, -\mathbf{y}^T])$  [38]. Therefore the eigenvector  
1304 associated with  $-\lambda_{2D,2}$  has biadjacency form

$$1305 \quad \tilde{\boldsymbol{\nu}} = \underbrace{\begin{bmatrix} 1 & -1 & \cdots & 1 & -1 \end{bmatrix}}_{|\mathcal{L}_1|} \underbrace{\begin{bmatrix} -1 & 1 & \cdots & -1 & 1 \end{bmatrix}}_{|\mathcal{L}_2|}^T. \quad (\text{C.10})$$

1306 which negates the signs of those entries associated with the latter half of the vertices in  $\mathcal{G}_{2D}$ .  
 1307 Subsequently, the first  $|\mathcal{L}_1|$  entries of  $\tilde{\mathbf{v}}$  are an eigenvector of  $\mathbf{X}_{2D}$  with eigenvalue  $-\bar{\lambda}_{2D,2}$  fol-  
 1308 lowing from the canonical bidjacency representation of  $\mathbf{W}_{2D}$  (C.4). We denote this reduced  
 1309 eigenvector in normalised form

$$\tilde{\mathbf{v}}_1 = \frac{1}{\sqrt{|\mathcal{L}_1|}} \underbrace{\begin{bmatrix} 1 & -1 & \cdots & 1 & -1 \end{bmatrix}}_{|\mathcal{L}_1|}^T, \quad (\text{C.11})$$

1311 and therefore it remains to show that the eigenpair  $(-\bar{\lambda}_{2D,2}, \tilde{\mathbf{v}}_1)$  is minimal in the spectrum of  
 1312  $\mathbf{X}_{2D}$ .

1313 The Rayleigh quotient for  $\mathbf{X}_{2D}$  is defined by

$$R_{\mathbf{X}_{2D}}(\mathbf{y}) = \frac{\mathbf{y}^T \mathbf{X}_{2D} \mathbf{y}}{\mathbf{y}^T \mathbf{y}} \quad (\text{C.12})$$

1315 and as  $\mathbf{X}_{2D}$  is real and symmetric by the Min-Max theorem the Rayleigh quotient is bounded by  
 1316 the maximal and minimal eigenvalues of the matrix,  $R_{\mathbf{X}_{2D}}(\mathbf{y}) \in [\lambda_{\min}, \lambda_{\max}]$  [38]. In particular,  
 1317  $R_{\mathbf{X}_{2D}}(\mathbf{y})$  generates the eigenvalues of  $\mathbf{X}_{2D}$  when  $\mathbf{y}$  is the respective eigenvector. Hence we show  
 1318 that  $\tilde{\mathbf{v}}_1$  minimises  $R_{\mathbf{X}_{2D}}(\mathbf{y})$ , namely

$$\arg \min_{\substack{\mathbf{y} \in \mathbb{R}^{|\mathcal{L}_1|} \\ \|\mathbf{y}\|=1}} (R_{\mathbf{X}_{2D}}(\mathbf{y})) = \tilde{\mathbf{v}}_1. \quad (\text{C.13})$$

1320 where the normality constraint follows from  $\mathbf{X}_{2D}$  being real and symmetric and so the eigenvec-  
 1321 tors of  $\mathbf{X}_{2D}$  are orthonormal with real eigenvalues.

The normalised form of  $\tilde{\mathbf{v}}_1$  yields  $\tilde{\mathbf{v}}_1^T \tilde{\mathbf{v}}_1 = 1$  and therefore the Rayleigh quotient evaluated at  
 $\tilde{\mathbf{v}}_1$  simplifies to  $R_{\mathbf{X}_{2D}}(\tilde{\mathbf{v}}_1) = \tilde{\mathbf{v}}_1^T \mathbf{W}_{2D} \tilde{\mathbf{v}}_1$ . By direct computation we have that

$$\begin{aligned}
 \tilde{\mathbf{v}}_1^T \mathbf{X}_{2D} \tilde{\mathbf{v}}_1 &= \sum_{k=1}^{|\mathcal{L}_1|} \sum_{j=1}^{|\mathcal{L}_1|} (\mathbf{X}_{2D})_{k,j} (\tilde{\mathbf{v}}_1)_k (\tilde{\mathbf{v}}_1)_j \\
 &= \sum_{i=1}^{|\mathcal{L}_1|} (\mathbf{X}_{2D})_{ii} (\tilde{\mathbf{v}}_1)_i^2 + \sum_{i=2}^{|\mathcal{L}_1|-1} (\tilde{\mathbf{v}}_1)_i \left( (\mathbf{X}_{2D})_{i,i-1} (\tilde{\mathbf{v}}_1)_{i-1} + (\mathbf{X}_{2D})_{i,i+1} (\tilde{\mathbf{v}}_1)_{i+1} \right) \\
 &\quad + (\tilde{\mathbf{v}}_1)_1 \left( (\mathbf{X}_{2D})_{1,2} (\tilde{\mathbf{v}}_1)_2 + (\mathbf{X}_{2D})_{1,|\mathcal{L}_1|} (\tilde{\mathbf{v}}_1)_{|\mathcal{L}_1|} \right) \\
 &\quad + (\tilde{\mathbf{v}}_1)_{|\mathcal{L}_1|} \left( (\mathbf{X}_{2D})_{|\mathcal{L}_1|,1} (\tilde{\mathbf{v}}_1)_1 + (\mathbf{X}_{2D})_{|\mathcal{L}_1|,|\mathcal{L}_1|-1} (\tilde{\mathbf{v}}_1)_{|\mathcal{L}_1|-1} \right)
 \end{aligned} \quad (\text{C.14})$$

1322 by the cyclic tridiagonal form of  $\mathbf{X}_{2D}$  (C.5). Critically as  $(\mathbf{X}_{2D})_{i,j} \geq 0$  for all  $i, j$ , then  $\tilde{\mathbf{v}}_1^T \mathbf{X}_{2D} \tilde{\mathbf{v}}_1$   
 1323 is minimised when  $\text{Sgn}((\tilde{\mathbf{v}}_1)_k) \neq \text{Sgn}((\tilde{\mathbf{v}}_1)_{k+1})$  for all  $k \in \{1, \dots, |\mathcal{L}_1| - 1\}$  which is satisfied by  
 1324 definition of  $\tilde{\mathbf{v}}_1$ . Furthermore, the orthonormal property of the eigenbasis of  $\mathbf{X}_{2D}$  ensures that  
 1325 no other eigenvector has this alternating sign structure which implies that  $-\bar{\lambda}_{2D,2}$  is the smallest  
 1326 eigenvalue of  $\mathbf{X}_{2D}$ .

1327 □

1328 A consequence of Lemma C.1 is the existence of a spectral gap about the origin for  $\mathcal{G}_{2D}$  and  
 1329  $\mathcal{G}_{3D}$ .

1330 **Theorem C.1.** *Let  $\mathcal{G}_k$  and  $\mathcal{G}_{k,\pi_2}$  be defined as in Lemma C.1 and let  $\lambda_{k,j} \in \text{Spec}(\mathbf{W}_k)$ . If*  
 1331  *$\bar{\lambda}_{k,2} < 0$  then  $\lambda_{k,j} \notin (\bar{\lambda}_{k,2}, -\bar{\lambda}_{k,2})$ .*

1332 *Proof.* From Lemma C.1 we have that  $-\bar{\lambda}_{k,2} = \min(\mu_{k,j} : \mu_{k,j} \in \text{Spec}(\mathbf{X}_k))$  and thus  $-\bar{\lambda}_{k,2} > 0$ .  
1333 From the symmetry of the spectrum of bipartite graphs we have that  $\bar{\lambda}_{k,2} < 0$  is the maximum  
1334 of the negative eigenvalues of  $\mathbf{W}_k$  therefore defining a region about the origin bounded by  $\bar{\lambda}_{k,2}$   
1335 and  $-\bar{\lambda}_{k,2}$  that contains no eigenvalues.  $\square$