Counting constraints in tissue mechanics

1. **Multicellular Rosettes Drive Fluid-solid Transition in Epithelial Tissues**
   Authors: Le Yan and Dapeng Bi
   Phys. Rev. X 9, 011029 (2019)

2. **Bayesian inference of force dynamics during morphogenesis**
   Authors: Shuji Ishihara and Kaoru Sugimura
   Journal of Theoretical Biology 313 (2012) 201–211

Recommended with a Commentary by Silke Henkes, School of Mathematics, University of Bristol

Understanding the mechanics of tissues is crucial to understanding multicellular life: All existing animals undergo *gastrulation*, where the embryo turns inside-out in the first step of the formation of the germ layers that will later become different parts of the body, like the brain, the skin or inner organs [1]. Two-dimensional, one cell thick epithelial cell sheets are at the heart of this transformation, and understanding the mechanics of these cobble-stone pavement like structures is necessary to understand embryo development and cell mechanics in general.

In recent years, physics has started to contribute models of epithelial tissues. The most celebrated (but not the only) of these models is the Vertex model [2, 3], which in its most compact form corresponds to an energy functional

$$V = \sum_I \frac{K}{2}(A_I - A_0)^2 + \frac{\Gamma}{2}(P_I - P_0)^2. \tag{1}$$

Here, the sum is over model cells I that form a two dimensional planar polygonal tesselation (see Figure 1) where individual cells have area A_I and perimeter P_I. The first term, the area term punishes deviations from a target area A_0 with stiffness parameter K, and the second similarly punishes perimeter fluctuations with stiffness Γ. Inspired by foam models, this energy functional can be seen as a quadratic expansion of an effective free energy near a (mechanical) equilibrium state. The biological relevance of this precise form of the energy has been under evaluation since the Vertex model was first formulated, and a large number of variants with additional terms like specific junction springs exist [4]. I will return to biological relevance, in particular the role of dynamics, below.

Recently, the physics of this model has attracted a lot of attention [5, 6, 7]. Of particular interest, there is an apparent rigidity transition between a solid and a liquid in this model as a function of the shape parameter $p_0 = P_0/\sqrt{A_0}$ (or equivalently the isoperimetric quotient s_0) around the value of $p_0^* \approx 3.81$.

The first recommended paper by Yan and Bi approaches this rigidity transition carefully using constraint counting, bolstered by an explicit calculation of the Hessian and its null space as well as an effective medium theory approach. Counting such constraints has previously been attempted in two communities: Initially, by the theoretical biology community in the context of force inference methods. The second recommended paper by Ishihara and Sugimura carefully counts the independent force components and the degrees of freedom in the system, and then uses a bayesian statistics framework on the inverse problem of finding the most likely forces and pressures in the tissue. Second, the problem has also been approached by the soft matter community, in particular Merkel et al [8, 9], as an extension of the study of network rigidity [10], and by Moshe et al [11] in a continuum nonlinear elastic problem. The emerging consensus is that a geometric incompatibility between the area and perimeter terms leads to frozen-in self stresses in much of parameter space which can stabilise the tissue. This is the same mechanism as tensegrity (as popularised by Buckminster Fuller), and an established mechanism for spring network mechanics. Therefore unlike e.g. for rigidity problems in granular materials [12], the tension on junctions is emerging as a crucial variable.

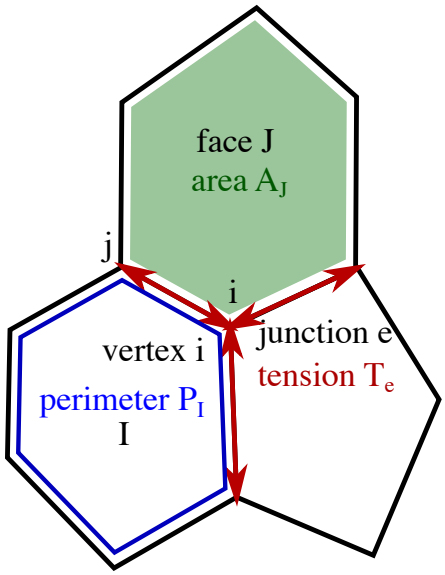

Figure 1: Polygonal cell tiling at the heart of vertex model formulations.

At the heart of all of these approaches is the following Maxwell constraint counting arithmetic: Consider an epithelial tissue such as in Fig. 1, with F cells, E junctions and V vertices. The Euler topological criterion requires $V - E + F = 0$, assuming there are no holes of boundaries for simplicity. For each of its V vertices, there are 2 degrees of freedom. For the system to be rigid (at least at a mean field level, see rigidity percolation problems for exceptions), there need to be a sufficient number of constraints to fix these degrees of freedom. The problem lies in counting them: A naive argument would count F constraints from each of the area and perimeter terms, leading to $N_0 = 2V - 2F$ zero modes. In a regular tissue where each vertex has Z junctions i.e. $E = Z/2V$, this suggests $N_0 = (4 - Z)V$, or for $Z < 4$ a heavily *underconstrained* material which leads to the manifestly incorrect conclusion that a standard hexagonal tissue is never rigid.

Yan and Bi ultimately come to the same conclusion as the other soft matter approaches: the prestresses, i.e. the tension T_e in the junctions, is responsible for rigidity. However, their approach is a tour de force which yields much more precise results: By computing the Hessian matrix analytically, the authors are able to compute its nullspace. The calculation hinges on the rank of the $2V \times 2V$ Hessian. The salient terms are F stiffness terms coming from the perimeter term, and an additional but hard to calculate number from the area term. In addition to these terms already present in the naive counting, and following arguments that

have been recently made in the glassy materials community [13], every edge under tension contributes positive definite term to the Hessian, thus increasing its rank by 1. Then there will be an additional $E - E_0$ contributions, one from each junction with positive tension. Ultimately, the number of null-modes at $K = 0$ (no area terms) which sets the rigidity boundary is given by

$$N_0 = 2V - [(E - E_0) + F] \tag{2}$$

As alluded to in the title, the paper specifically applies this approach to cell tilings where 4-vertices and higher order vertices (rosettes) are present. This can be parametrised by $Z \geq 3$, the mean vertex coordination as $N_0 = (3 - Z)V - E_0$, and the $Z = 3$ point then corresponds to marginal stability when all junction forces are positive (i.e. $E_0 = 0$). This is precisely at the point where area and perimeter terms start to become incompatible, $p_0^* = 3.72$ for a hexagonal ordered tissue, corresponding to an unstressed regular hexagon, and empirically $p_0^* \approx 3.81$ for a disordered one. The phase boundary of rigidity then shifts linearly upwards to $Z > 3$ for larger p_0. The authors then turn to effective medium theory to compute the shear modulus of the system, for a network with given Z and a prescribed fraction of non force-bearing junctions. The authors find excellent agreement between the predicted rigidity line and the counting argument. In conceptual agreement, Merkel et al [9], for networks, are also able to compute the shear modulus and its jump across the rigidity transition, using the point in edge length where tensions become positive.

However, at this point one has to return back to biological plausibility: The vertex model is not expected to be a particularly realistic approximation of tissue mechanics, and thus counting arguments that rely on the specific form of its potential are not generic. Ishihara and Sugimura set out to develop a formalism for force inference on a given network, with more generic assumptions. The counting argument of Ishihara and Sugimura is as thus: Based on the experimentally accessible variables, every junction edge length l_e enters the definition of the potential, and the area of every cell A_I, leading to a generic potential $V(\{A_I\}, \{l_e\})$.

One writes the general form of the force on each vertex, $\mathbf{F}_i = \hat{\mathbf{A}} \cdot \mathbf{P}$, where $\mathbf{P} = (\{\partial U/\partial l_e\}, \{\partial U/\partial A_I\}) = (\{T_e\}, \{P_I\})$, the $F + E$ length vector of individual junction tensions and cell pressures. The compatibility matrix (or rather its inverse) is $\hat{\mathbf{A}}_{ie} = \partial l_e/\partial \mathbf{r}_i$ and $\hat{\mathbf{A}}_{iI} = \partial A_I/\partial \mathbf{r}_i$, with dimensions $2V \times (F + E)$. In the quasistatic limit, the force on every vertex is zero, and force inference is then equivalent to finding the most likely solution to $\hat{\mathbf{A}} \cdot \mathbf{P} = 0$. The counting here is identical to Yan and Bi: The number of unknown force components is given by $(F+E)-2V = -N_0$, assuming positive tensions on junctions. Much like in an approach developed for granular packings, the force network ensemble [14], force inference then presumes that the network is overconstrained, i.e. that there are a sufficient number of force components to fix vertices, $N_0 < 0$ in the notation above, and that therefore there is a $|N_0|$-dimensional space of allowed forces (or equivalently self-stresses). As observed, this system is very nearly isostatic for $Z = 3$, and then a careful counting identifies a force space with dimension $\sim R$, the number of boundary cells of the observed tissue. As for Yan and Bi, and as required by the Euler criterion, higher order vertices contribute one extra force component / constraint each to the counting. While the force network ensemble then seeks to compute allowed force distributions based on a flat measure, force inference uses a Bayesian approach to find the most likely force configuration.

This approach is then thoroughly tested on vertex model simulated data, with excellent

agreement, and also already applied to experimental data from drosophila embryos. It reveals strongly heterogeneous junction forces and the presence of shear stresses in the system. Force inference has become an established technique, in particular for drosophila, where the quasistatic assumption is a good approximation [15]. The results of both recommended papers then lend weight to constraint counting to be ultimately as follows, with observed biological tissues being on the *rigid* side of constraint counting:

- Two degrees of freedom per vertex ($2V$)
- One constraint per cell due to pressure (F). In the absence of an area term, a coupling (perimeter) term will also provide this set of constraints.
- One constraint for every junction tension ($E - E_0$),

What are the consequences of this counting for actual tissues? In the first instance, an unjamming transition has been observed in airway epithelia around p_0^* [16]. Tissues at higher p_0, believed to be fluid, could instead be rigid due to the presence of higher order vertices / rosettes. More subtle, but more relevant to development, are the ways tissue mechanics is an integral part of shaping an embryo. Mechanical signalling and active dynamics refer to force transmission and active response through the actomyosin network of each cell in an embryo. This feedback between junction tensions and actomyosin concentration then controls the local tissue response through oriented rearrangements (T1 transitions), cell shape changes, divisions and ingressions [17, 18]. The active driving force in this soft active material is given by the actomyosin-generated tension in the junctions itself, suggesting a rich and complex interplay between active driving, rigidity and flow. To understand these materials at the core of multicellular life, we will need to build on models [19, 20] that incorporate the rigid mechanics *and* realistic active junction dynamics.

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