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Cell Orientation under Stretch: Stability of a Linear Viscoelastic Model

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Abstract

The sensitivity of cells to alterations in the microenvironment and in particular to external mechanical stimuli is significant in many biological and physiological circumstances. In this regard, experimental assays demonstrated that, when a monolayer of cells cultured on an elastic substrate is subject to an external cyclic stretch with a sufficiently high frequency, a reorganization of actin stress fibers and focal adhesions happens in order to reach a stable equilibrium orientation, characterized by a precise angle between the cell major axis and the largest strain direction. To examine the frequency effect on the orientation dynamics, we propose a linear viscoelastic model that describes the coupled evolution of the cellular stress and the orientation angle. We find that cell orientation oscillates tending to an angle that is predicted by the minimization of a very general orthotropic elastic energy, as confirmed by a bifurcation analysis. Moreover, simulations show that the speed of convergence towards the predicted equilibrium orientation presents a changeover related to the viscous-elastic transition for viscoelastic materials. In particular, when the imposed oscillation period is lower than the characteristic turnover rate of the cytoskeleton and of adhesion molecules such as integrins, reorientation is significantly faster.

Keywords: Cell orientation · Cell stretching · Stress fibers · Cell mechanosensing · Viscoelasticity · Bifurcations

2020 Mathematics Subject Classification: 74D05 · 74L15 · 92C10 · 92C37

1 Introduction

During their life cycle, cells are constantly exposed to numerous stimuli coming from the surrounding microenvironment. The nature of these cues is wide-ranging: among them, a significant role is played by mechanical prompts, since many experiments demonstrated that they trigger a cellular response [1, 6, 9, 15, 22, 29, 33, 47, 65, 74]. Cell sensitivity to mechanical actions is relevant in many biological and physiological circumstances, such as growth, differentiation,

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1 motility, apoptosis and tissue fibrosis [7, 29, 65]. Its precise understanding has then gathered
2 increasing research attention, since it could be helpful to acquire a deeper knowledge of some
3 diseases and of morphogenesis, just to mention a few examples. For instance, an altered percep-
4 tion of mechanical stimuli due to cell-cell contact inhibition and to cell tensile stress is known
5 to have a role in tumourigenesis and cancer development [5, 8, 32, 36], and it is also related to
6 epithelial-mesenchymal transition [31, 58] in neoplastic tissues.

7 Moreover, the active response of the cell to mechanical interactions with the environment is
8 involved in cell culturing, development, and tissue engineering. In particular, during embryo-
9 genesis, the formation of residual stresses and active forces is believed to drive heart formation
10 and looping [60]. Cardiac cell cultures also display enhanced hypertrophy, proliferation and
11 alignment when subject to static or cyclic stress [64]. Notably, experimental tests carried out
12 on several types of cells (like fibroblasts, myofibroblasts, cardiomyocytes and endothelial cells)
13 showed that alignment in response to a deformation is a common feature which proves their
14 capability to adapt after mechanical stimuli [1, 9, 15, 40, 46, 47, 48]. In detail, when a monolayer
15 undergoes a cyclic deformation, cells lying on the substrate tend to change their orientation in a
16 precise way, until they reach a stable configuration characterized by a well-defined angle between
17 their major axis and the direction of largest stretching. In this process, a fundamental role is
18 played by the cytoskeleton [27, 47, 66, 67]: focal adhesions (FAs), i.e. protein complexes which
19 provide cell contact with the substrate and the extracellular matrix, sense the mechanical stress
20 and induce a remodelling of the cytoskeletal structure, through the formation of oriented actin
21 stress fibers (SFs). These fiber bundles are able to develop contractile forces: when submitted
22 to an external stretch, the cell reorganizes the SF structure, disrupting and rebuilding them in
23 a specific direction to relieve the stress.

24 Several works, both on the experimental [1, 11, 15, 23, 27, 30, 33, 40, 47, 68] and on the
25 theoretical side [3, 9, 17, 18, 19, 67, 70], have tried to address the problem of cell reorientation
26 under mechanical stretch. It is recognized that this mechanism is actively performed by the cell
27 [69], and that it is induced by mechanical strain deforming the substrate to which they adhere.
28 Moreover, there is common agreement on the fact that cells on a plane substrate undergoing
29 a cyclic deformation orient their stress fibers in a direction which is oblique or in some cases
30 perpendicular to the applied strain [9, 27, 40, 45]. *Indeed, when the substrate deformation is
31 transmitted to the cell cytoskeleton through FAs, a reorganization of SF structure happens: they
32 are disassembled and rebuilt in a precise direction [56], fostering changes in shape and orientation
33 of the whole cell. In addition, FAs themselves form clusters at the ends of aligned SFs, giving
34 the cell an elongated and clearly oriented morphology (see Fig.1). Therefore it is possible to
35 define an equilibrium orientation angle, θ_{eq} , that is the angle formed by the cell major axis
36 and the direction of stretching when cell orientation does not evolve anymore. In this respect,
37 mathematical models trying to predict this equilibrium orientation angle and the driving force
38 of such a behaviour have been proposed, using different approaches but mainly in a linear elastic
39 framework. For instance, the first attempts to describe cell orientation suggested a preference for
40 the minimal strain or minimal stress directions [18, 19, 23, 40, 66, 68]. Looking closely at biaxial
41 tests, Livne and coworkers [40] found a linear relationship between $\cos^2 \theta_{eq}$ and a parameter
42 quantifying the biaxiality of the deformation.*

43 Remarkably, some experimental assays were performed applying deformations for which using
44 linear elasticity should be theoretically inaccurate (e.g., up to 24% in [40] and up to 32% in [23]).
45 Starting from this experimental evidence, Lucci and Preziosi [42] proved that a generalization
46 of the linear relationship found in the linear elastic case by Livne et al. [40] also holds for
47 a very large class of nonlinear constitutive orthotropic models. In the nonlinear framework,
48 the squared cosine of the orientation angle is linearly dependent on a parameter which is the
49 natural generalization of the one found in [40], with a slope depending on a combination of elastic

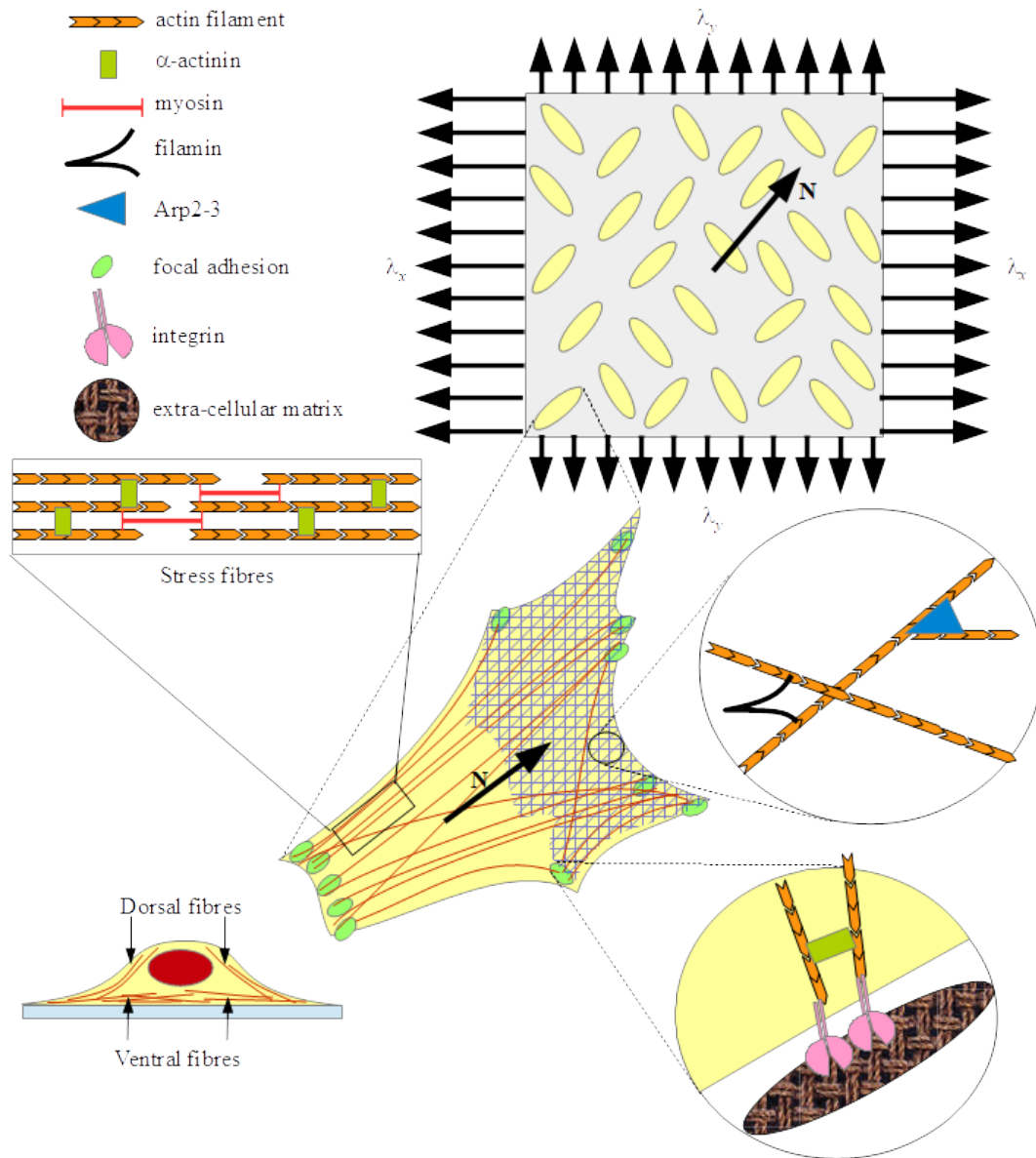


Figure 1: Sketch of experimental set-up, of the inner structure of a typical cell, and of its adhesion to the substrate.

1 coefficients characterizing the nonlinear strain energy.

2 Nevertheless, there are other factors influencing the orientation of the cell, the most relevant
3 of which is probably the frequency of the applied cyclic deformation [30, 38]. In fact, it has been
4 observed that, in order to trigger such a response, the period of the stretching cycle must be
5 sufficiently small [28, 30]. As specified in [30], this threshold seems to be cell-type dependent,
6 leading to minimum frequencies that go from 0.01 Hz for rat embryonic fibroblasts to 0.1 Hz for
7 human dermal fibroblasts. This mechanism cannot be covered by the purely elastic descriptions
8 discussed above, and calls for the introduction of a characteristic response time that needs to be
9 compared with the periodic deformation time scale. The existence of such a characteristic time
10 might be related to the reorganization of the acto-myosin cytoskeleton and of the ensemble of focal
11 adhesions with the substrate. Indeed, it is known that the characteristic turnover times of both
12 phenomena are of the order of tens of seconds, or even minutes (see, for instance, [11, 50, 51, 63]).

13 On the basis of this observation, in this paper we propose a viscoelastic model for cell pref-
14 erential orientations, in order to describe reorganization processes occurring inside the cell and
15 between the cell and its microenvironment when a mechanical deformation is applied to the
16 substrate. To our knowledge, previous viscoelastic descriptions of cell stress fiber dynamics have
17 been mainly focused on the microscopic scale [35, 55], while in this article we treat the monolayer
18 as a continuum. In particular, we introduce an anisotropic viscoelastic description that couples
19 the evolution of the SF orientation angle with the mechanical stress exerted on the cell as a con-
20 sequence of cyclic stretching. Hence, the ensemble of cells lying on the substrate is considered
21 as a Maxwell orthotropic fluid with a single relaxation time. We prove that, for high stretching
22 frequencies, the cell cytoskeleton does not have enough time to reorganize and behaves elastically,
23 while for slow processes the viscous character emerges.

24 Furthermore, after having showed that the steady angles are predicted by an energy mini-
25 mization, we work with a very general orthotropic material. An extensive bifurcation analysis
26 is then performed, discussing the role of elastic parameters and finding the conditions under
27 which a certain angle of cell orientation is stable. We find that also in this general set up there
28 exists a linear relationship between $\cos^2 \theta_{eq}$ and a combination of parameters of the orthotropic
29 elasticity tensor. [The slope of the straight line fitting experimental data suggests that, among](#)
30 [all coefficients, a more relevant role is played by those in charge of describing the cell response](#)
31 [to elongation along its orientation axis and to shear.](#)

32 Finally, we perform some numerical simulations using the complete viscoelastic model, study-
33 ing the reorientation dynamics in the high frequency and low frequency cases together with stress
34 evolution. It is found that the cell orientation angle evolves toward the steady state predicted
35 by the linear stability analysis, with a speed which depends on the elastic or viscous character
36 of the system. Moreover, in accordance with the observation in [28, 30], simulations show that
37 the speed of reorientation towards the equilibrium angle sensibly depends on the frequency of
38 imposed oscillations. In particular, it presents a transition for values of the ratio of the oscilla-
39 tion period and the characteristic time of viscoelasticity close to 2π , so that the time required to
40 observe reorientation is of the order of days for smaller frequencies, saturating to one hour for
41 larger frequencies.

42 In detail, the paper is organized as follows. In Section 2 the general mathematical model
43 is introduced, deriving the equations for the viscoelastic system and studying their significant
44 limits, i.e. the high-frequency and low-frequency cases. Section 3 is devoted to a detailed
45 bifurcation analysis of the model for an orthotropic energy density, deriving conditions under
46 which equilibrium orientations are stable. In Section 4 we discuss the implementation and report
47 some numerical results of our model, showing both the elastic and the viscous behaviour of the
48 system. Finally, Section 5 is dedicated to a summary of the results and to the discussion of some
49 open issues, which may be of interest for future research. In Appendix A we report some details

1 related to the possible presence of a symmetry breaking phenomenon.

2 Viscoelastic Model

3 We consider a two-dimensional substrate seeded of cells that is stretched biaxially. While the
 4 response of the extracellular material is in general isotropic and elastic, the mechanical behaviour
 5 of the ensemble of cells can be regarded as anisotropic and viscoelastic. The viscoelastic character
 6 is due to the reorganization of the acto-myosin network inside the cell and to the rearrangement
 7 of focal adhesions (FAs), performed through repeated detachments and attachments of integrin
 8 bonds with the substrate, especially under stretch, to relax the perceived stress (see, for instance,
 9 [54]). Instead, anisotropy derives from the fact that, when subject to a mechanical deformation,
 10 cells tend to build properly oriented actin stress fibers (SFs) within their cytoskeleton [29, 66].
 11 In addition, these SFs are linked by a network of proteins (such as fascin, fimbrin, α -actinin,
 12 filamin, ARP2-3 [14, 24, 57, 67]) that spans them orthogonally with respect to the fiber bundles
 13 or at well defined angles, as in the case of ARP2-3, as sketched in Fig. 1. As a consequence,
 14 the cell responds differently to stretches and stresses along its major axis with respect to the
 15 transversal axis and to shear as well.

16 The main orientation of SFs, which will be identified by a unit vector \mathbf{N} , can change in
 17 time due to several cues, among which mechanical deformations. We will here consider, as in
 18 experiments, that the specimen is subject to a biaxial stretch and take the x -axis aligned to the
 19 direction of maximal stretch. Then, the angle formed by \mathbf{N} and the x -axis will be denoted by θ .

20 Resorting to Lagrangian mechanics, we can relate the evolution in time of the orientation
 21 angle θ with the changes in the virtual work \mathcal{L} done by the stress acting on the cell due to SF
 22 alignment. Considering an overdamped regime, which corresponds to neglecting inertial effects,
 23 we can then write

$$0 = -\eta \frac{d\theta}{dt} - \frac{\partial \mathcal{L}}{\partial \theta}, \quad (1)$$

24 where $\mathcal{L} := \mathbb{T} : \mathbb{E}$, being \mathbb{T} the excess Cauchy stress tensor and \mathbb{E} the infinitesimal deformation
 25 tensor, is the work done by the stress, assuming that the mechanical behaviour is linear. More-
 26 over, $\eta > 0$ is a viscous-like coefficient measuring cell resistance to realignment. Since we are
 27 interested in deformation tests, where a periodic deformation is imposed to the specimen, \mathbb{E} is
 28 assumed to be independent of θ and externally imposed. It is then convenient to rearrange Eq.
 29 (1) to get the following evolution equation for $\theta(t)$:

$$\frac{d\theta}{dt}(t) = - \frac{1}{K\lambda_\theta} \frac{\partial \mathbb{T}}{\partial \theta}(t|\theta) : \mathbb{E}(t), \quad (2)$$

30 where we identified K as the characteristic Young modulus of the material and $\lambda_\theta := \eta/K$ as a
 31 parameter related to the time the cell takes to reorient itself. The notation $\mathbb{T}(t|\theta)$ reads as the
 32 stress at time t given the history of orientations θ .

33 Equation (2) implies that, for a given deformation \mathbb{E} , θ tends to assume a value such that the
 34 variation of \mathbb{T} with respect to θ either vanishes or becomes orthogonal to \mathbb{E} .

35 Focusing on \mathbb{T} , we assume here that the stress in the elastic substrate and the viscoelastic
 36 cellular component embedded in it is given by

$$\mathbb{T}(t|\theta) = \int_{-\infty}^t \mathbf{C}(\theta(\tau); t - \tau) [\mathbb{E}(t) - \mathbb{E}(\tau)] d\tau, \quad (3)$$

37 (see, for instance, [2] for the isotropic case and [52, 53] for the anisotropic case). We notice
 38 that the elements of the fourth-order tensor \mathbf{C} are all bounded and that, in the isotropic case,

1 \mathbf{C} reduces to the derivative of the so-called relaxation kernel (apart from the sign) times the
 2 identity tensor. The kernel \mathbf{C} depends on the alignment direction, i.e. on the orientation angle
 3 θ , which during the history of deformation can evolve in time. On the other hand, the second
 4 part of the kernel dependence takes into account the weight of past orientations (at time τ) on
 5 the present state of stress and represents memory effects of the viscoelastic material. We will
 6 assume that such a dependence is exponential with a single relaxation time λ [52, 53], that is,

$$\mathbf{C}(\theta(\tau); t - \tau) = \frac{1}{\lambda} \mathbf{C}_0(\theta(\tau)) e^{-(t-\tau)/\lambda}, \quad (4)$$

7 where $\mathbf{C}_0(\theta(\tau))$, which is the fourth-order elasticity tensor depending on the orientation direction
 8 θ at time τ , inherits from \mathbf{C} the boundedness and regularity properties. Therefore, we can write

$$\mathbb{T}(t|\theta) = \int_{-\infty}^t \frac{1}{\lambda} e^{-(t-\tau)/\lambda} \mathbf{C}_0(\theta(\tau)) [\mathbb{E}(t) - \mathbb{E}(\tau)] d\tau. \quad (5)$$

9 As usual in rheology, for this type of kernels it is useful to differentiate (5) and to rewrite the
 10 constitutive equation in the following differential form:

$$\lambda \frac{d\mathbb{T}}{dt}(t|\theta) + \mathbb{T}(t|\theta) = \mathcal{C}_0(t|\theta) \frac{d\mathbb{E}}{dt}(t), \quad (6)$$

11 where

$$\mathcal{C}_0(t|\theta) := \int_{-\infty}^t e^{-(t-\tau)/\lambda} \mathbf{C}_0(\theta(\tau)) d\tau = \int_0^{+\infty} \lambda e^{-s} \mathbf{C}_0(\theta(t - \lambda s)) ds, \quad (7)$$

12 is a functional on the exponentially weighted history of past orientations. We observe that, in
 13 the isotropic case, \mathcal{C}_0 is twice the so-called elastic viscosity, i.e., the area under the relaxation
 14 kernel (times the identity tensor).

15 In Eq. (2) and (6) there are two intrinsic characteristic times: λ refers to the viscous behaviour
 16 of cells due, for instance, to the continuous renewal of adhesion bonds with the substrate, while
 17 λ_θ is related to the characteristic time of reorganization of stress fibers and consequently to the
 18 change in cell orientation. It is known that both remodelling phenomena occur on time scales
 19 of tens of seconds or even minutes [11, 50, 51, 63]. Now, given that in mechanical tests cells are
 20 often subject to cyclic strains, it is useful to discuss how the model behaves when the imposed
 21 oscillation period T is much shorter or longer than the characteristic times mentioned above. In
 22 order to do so, we observe that, for a periodic deformation $\mathbb{E}(t) = \mathbb{E}_0 e^{i\omega t}$, the expression of the
 23 stress (5) can be rewritten as

$$\begin{aligned} \mathbb{T}(t|\theta) &= \frac{1}{\lambda} \left[\int_{-\infty}^t e^{-(t-\tau)/\lambda} \mathbf{C}_0(\theta(\tau)) \left(1 - e^{-i\omega(t-\tau)/\lambda} \right) d\tau \right] \mathbb{E}_0 e^{i\omega t} \\ &= \left[\int_0^{+\infty} e^{-s} \mathbf{C}_0(\theta(t - \lambda s)) \left(1 - e^{-i\lambda\omega s} \right) ds \right] \mathbb{E}_0 e^{i\omega t}. \end{aligned} \quad (8)$$

24 2.1 High Frequency Regime

25 First of all, we consider a high frequency regime with $\lambda, \lambda_\theta \gg T = 2\pi/\omega$, so that the relaxation
 26 times are much longer than the oscillation period of the deformation, i.e., the reorganization

1 process is slower than the imposed cyclic strain. In this case, it is useful to split the integral in
 2 Eq. (8) as

$$\mathbb{T}(t|\theta) = \left[\int_0^{+\infty} e^{-s} \mathbf{C}_0(\theta(t-\lambda s)) ds \right] \mathbb{E}_0 e^{i\omega t} - \left[\int_0^{+\infty} e^{-s} \mathbf{C}_0(\theta(t-\lambda s)) e^{-i\lambda\omega s} ds \right] \mathbb{E}_0 e^{i\omega t}. \quad (9)$$

3 We observe that, as stated before, the coefficients of tensor $\mathbf{C}_0(\theta(t))$ are regular in θ . In
 4 particular, they are bounded as well as their derivatives. Hence, by Riemann-Lebesgue lemma,
 5 the second integral in the r.h.s. of Eq. (9), which can be regarded as the unilateral Fourier
 6 transform of the L^1 function $e^{-s} \mathbf{C}_0(\theta(t-\lambda s))$, vanishes in the limit of high frequencies.

As regards the first term, integrating by parts and exploiting Eq. (2) we have

$$\begin{aligned} \mathbb{T}(t|\theta) &= \left[\mathbf{C}_0(\theta(t)) + \frac{\lambda}{K\lambda_\theta} \int_0^{+\infty} e^{-s} \frac{\partial \mathbf{C}_0}{\partial \theta}(\theta(t-\lambda s)) \frac{\partial \mathbb{T}}{\partial \theta}(t-\lambda s|\theta) : \mathbb{E}_0 e^{i\omega(t-\lambda s)} ds \right] \mathbb{E}_0 e^{i\omega t} \\ &= \mathbf{C}_0(\theta(t)) \mathbb{E}_0 e^{i\omega t} + \left[\frac{\lambda}{K\lambda_\theta} \int_0^{+\infty} e^{-s} \frac{\partial \mathbf{C}_0}{\partial \theta}(\theta(t-\lambda s)) \frac{\partial \mathbb{T}}{\partial \theta}(t-\lambda s|\theta) : \mathbb{E}_0 e^{-i\lambda\omega s} ds \right] \mathbb{E}_0 e^{i2\omega t}. \end{aligned}$$

7 Provided that, denoting by $H(s)$ the Heaviside function, the tensorial function

$$\mathbf{f}(s) = H(s) \frac{\partial \mathbf{C}_0}{\partial \theta}(\theta(t-\lambda s)) \frac{\partial \mathbb{T}}{\partial \theta}(t-\lambda s|\theta) : \mathbb{E}_0 e^{-s} \in L^1(\mathbb{R}),$$

8 as we expect because of the boundedness of the derivative of the coefficients in \mathbf{C}_0 , the integral
 9 in the stress expression corresponds to the Fourier transform of $\mathbf{f}(s)$, which again vanishes in the
 10 limit of high frequencies. Hence, in the high frequency regime we are left with

$$\mathbb{T}(t|\theta) \approx \mathbf{C}_0(\theta(t)) \mathbb{E}_0 e^{i\omega t}. \quad (10)$$

11 Such a constitutive equation corresponds to an anisotropic linear elastic response of the
 12 material, where $\mathbf{C}_0(\theta(t))$ is the fourth-order elasticity tensor depending on the orientation θ .

13 So, in the high frequency regime Eq. (2) can be simplified to

$$\frac{d\theta}{dt} = -\frac{1}{\eta} \left[\frac{\partial \mathbf{C}_0}{\partial \theta} \mathbb{E} \right] : \mathbb{E} = -\frac{2}{K\lambda_\theta} \frac{\partial U}{\partial \theta}, \quad (11)$$

14 where

$$U(t, \theta) := \frac{1}{2} \mathbb{E}(t) : \mathbf{C}_0(\theta) \mathbb{E}(t), \quad (12)$$

15 is the elastic strain energy. Therefore, in the high frequency regime, the change in cell orientation
 16 is driven by the minimization of an elastic energy with respect to the orientation angle, coherently
 17 with previous models and experimental results [40, 42].

18 2.2 Low Frequency Regime

19 In a low frequency regime, in which the period of the cyclic strain imposed to the specimen is
 20 much longer than the characteristic time λ of cell relaxation, the reorientation process is faster
 21 than the external oscillations. Therefore, taking into account the approximation $\lambda \ll T = 2\pi/\omega$,
 22 or equivalently $\lambda\omega \ll 1$, we have that

$$1 - e^{-i\lambda\omega s} \approx i\lambda\omega s,$$

1 and the stress can be expressed using Eq. (8) through

$$\mathbb{T}(t|\theta) \approx i\lambda\omega \left[\int_0^{+\infty} se^{-s} \mathbf{C}_0(\theta(t-\lambda s)) ds \right] \mathbb{E}_0 e^{i\omega t}, \quad (13)$$

2 which, defining

$$\bar{\mathbf{C}}_0(t|\theta) := \int_0^{+\infty} se^{-s} \mathbf{C}_0(\theta(t-\lambda s)) ds,$$

3 shows an anisotropic viscous-like response characterized by the constitutive equation

$$\mathbb{T}(t|\theta) \approx \lambda \bar{\mathbf{C}}_0(t|\theta) \frac{d\mathbb{E}}{dt}(t). \quad (14)$$

Essentially, if the imposed oscillations are sufficiently slow, the system behaviour is similar to the one of a viscous fluid with anisotropy induced by oriented cells. Moreover, since we are mostly interested in steady orientations, given that $\mathbf{C}_0(\theta(t-\lambda s))$ is an analytic function on \mathbb{R} it is possible to write

$$\begin{aligned} \mathbf{C}_0(\theta(t-\lambda s)) &= \mathbf{C}_0(\theta(t)) + \mathbf{C}'_0(\theta(t)) \frac{d\theta}{dt}(t)(-\lambda s) \\ &+ \frac{1}{2} \left[\mathbf{C}''_0(\theta(t)) \left(\frac{d\theta}{dt}(t) \right)^2 + \mathbf{C}'_0(\theta(t)) \frac{d^2\theta}{dt^2}(t) \right] (-\lambda s)^2 + \dots \end{aligned}$$

4 and therefore, at the equilibrium orientations,

$$\mathbf{C}_0(\theta(t-\lambda s)) \approx \mathbf{C}_0(\theta(t)). \quad (15)$$

5 By means of this approximation, we can write the steady state oscillatory stress in the low
6 frequency regime using Eq. (14) and Eq. (15) as

$$\mathbb{T}(t|\theta) \approx \lambda \mathbf{C}_0(\theta(t)) \frac{d\mathbb{E}}{dt}(t). \quad (16)$$

7 Comparing the latter with the stress in the high frequency case (10), we observe that they
8 essentially differ for a factor $\lambda\omega$, as will be highlighted in the simulations.

9 Finally, recalling Eq. (2), we have that the angle θ tends to assume, as already stated, the
10 configuration such that $\frac{\partial \mathbb{T}}{\partial \theta} \perp \mathbb{E}$, which in the low frequency limit writes as

$$\left(\frac{\partial \mathbf{C}_0}{\partial \theta} \frac{d\mathbb{E}}{dt} \right) \perp \mathbb{E}.$$

11 However, since the deformation is periodic, the last condition can be rephrased as

$$i\omega \left(\frac{\partial \mathbf{C}_0}{\partial \theta} \mathbb{E}_0 \right) : \mathbb{E}_0 = 0 \quad \implies \quad \frac{\partial U}{\partial \theta} = 0,$$

12 where U is the elastic energy defined in (12). Therefore, we conclude that in the low frequency
13 regime also in the viscoelastic case the steady cell configurations are predicted by a minimization
14 with respect to the orientation angle θ of the energy introduced in the elastic case.

15
16 We remark that, even though our model predicts that the equilibrium orientation of the cell
17 is the same in both regimes, the characteristic time of reorientation is highly influenced by the
18 frequency, leading therefore to different final orientation angles of the cell in the two regimes,
19 considering the time of the biological experiment, as will be shown in Section 4.

3 Bifurcation Analysis

In this Section, we study equilibrium orientations and their bifurcations. Our goal is to describe the monolayer subject to a periodic stretch through its elastic energy, since the steady orientation of the cells is predicted by its minimization as discussed above. This allows us to study in detail the equilibrium angles in terms of a very general strain energy, looking for those orientations which minimize it for a fixed deformation. Finally, we draw bifurcation diagrams in terms of a parameter that quantifies the biaxiality of the deformation, putting in evidence the conditions under which the preferential orientations exist and are stable. This is in agreement with previous works showing that an energetic approach allows to reproduce experimental data of cell orientation more accurately [40].

3.1 Elastic Energy and Deformation

We consider the most general elastic energy density U depending on the classical first three invariants $I_1 := \text{tr } \mathbb{C}$, $I_2 := \frac{1}{2} [(\text{tr } \mathbb{C})^2 - \text{tr } \mathbb{C}^2]$ and $I_3 := \det \mathbb{C}$ (where $\mathbb{C} = \mathbb{F}^T \mathbb{F}$ is the right Cauchy-Green deformation tensor and \mathbb{F} is the deformation gradient) representing the isotropic response of the material, and on the anisotropic invariants [49]

$$\begin{aligned} I_4 &:= \mathbf{N} \cdot \mathbb{C}\mathbf{N} = |\mathbb{F}\mathbf{N}|^2, & I_5 &:= \mathbf{N} \cdot \mathbb{C}^2\mathbf{N} = |\mathbb{C}\mathbf{N}|^2, \\ I_6 &:= \mathbf{N}_\perp \cdot \mathbb{C}\mathbf{N}_\perp = |\mathbb{F}\mathbf{N}_\perp|^2, & I_7 &:= \mathbf{N}_\perp \cdot \mathbb{C}^2\mathbf{N}_\perp = |\mathbb{C}\mathbf{N}_\perp|^2, \\ I_8 &:= \mathbf{N}_\perp \cdot \mathbb{C}\mathbf{N} = (\mathbb{F}\mathbf{N}_\perp) \cdot \mathbb{F}\mathbf{N}. \end{aligned} \quad (17)$$

Then, the general energy functional can be written as

$$U = U_i(I_1, I_2, I_3) + U_\ell(I_1, I_2, I_3, I_4, I_5, I_6, I_7, I_8) + U_q(I_4, I_5, I_6, I_7, I_8), \quad (18)$$

where U_i is the purely isotropic contribution, U_q is the purely anisotropic one and U_ℓ includes a coupling between isotropic and anisotropic terms. However, since the invariants I_1, I_2, I_3 do not depend on the orientation angle, the inclusion of U_i will not influence the following discussion. Henceforth, the energy dependence upon it will not be explicitly mentioned anymore, though one should recall that this term might appear in an irrelevant way as an extra contribution in the energy that does not alter our results and conclusions.

Considering the limit of small deformations and denoting by \mathbb{E} the infinitesimal strain tensor, one has

$$\begin{aligned} I_4 &\approx 1 + 2\mathbf{N} \cdot \mathbb{E}\mathbf{N}, & I_5 &\approx 1 + 4\mathbf{N} \cdot \mathbb{E}\mathbf{N}, \\ I_6 &\approx 1 + 2\mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}_\perp, & I_7 &\approx 1 + 4\mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}_\perp, \\ I_8 &\approx 2\mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}, \end{aligned}$$

so that in linear elasticity it is impossible to discriminate the dependence on I_4 (resp. I_6) from the one on I_5 (resp. I_7), since they both merge in a dependence on $\mathbf{N} \cdot \mathbb{E}\mathbf{N}$ (resp. $\mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}_\perp$). As a consequence, working in a linear framework, from now on we will consider the following dependences:

$$U_q = U_q(\hat{I}_4, \hat{I}_6, I_8) \quad \text{and} \quad U_\ell = U_\ell(\hat{I}_i \hat{I}_4, \hat{I}_i \hat{I}_6, \hat{I}_i I_8), \quad i = 1, 2, 3,$$

1 where we have defined

$$\begin{aligned} \hat{\mathbf{I}}_1 &:= \mathbf{I}_1 - 2, & \hat{\mathbf{I}}_2 &:= \mathbf{I}_2 - 2, & \hat{\mathbf{I}}_3 &:= \mathbf{I}_3 - 1, \\ \hat{\mathbf{I}}_4 &:= \mathbf{I}_4 - 1 \propto \mathbf{N} \cdot \mathbb{E}\mathbf{N}, & \hat{\mathbf{I}}_6 &:= \mathbf{I}_6 - 1 \propto \mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}_\perp. \end{aligned} \quad (19)$$

2 Consider now a biaxial extension experiment. We assume that the deformation inside the speci-
3 men is homogeneous, so that in two dimensions the deformation gradient reads

$$\mathbb{F} = \begin{pmatrix} \lambda_x & 0 \\ 0 & \lambda_y \end{pmatrix},$$

4 where we take $\lambda_x > \lambda_y$ since in our notation, as stated before, the maximum stretching is
5 performed along the x -direction. For future comparison we will denote $\lambda_x = 1 + \varepsilon$ and $\lambda_y = 1 - r\varepsilon$,
6 so that for small ε - i.e. for small deformations - the infinitesimal strain tensor writes

$$\mathbb{E} = \begin{pmatrix} \varepsilon & 0 \\ 0 & -r\varepsilon \end{pmatrix}, \quad (20)$$

7 where the parameter r is often referred to as the *biaxiality ratio*. We also observe that the
8 assumption $\lambda_x > \lambda_y$ implies $r + 1 > 0$. Finally, we remark that the particular case $\lambda_x = \lambda_y$,
9 corresponding to $r = -1$, will not be discussed explicitly and is not really interesting from the
10 practical point of view as experimental evidence showed that, under equi-biaxial stretch, cells do
11 not orient in a specific direction in the plane of the deformation [67].

12 Recalling that θ is the angle formed by the average cell orientation direction and the x -axis,
13 then $\mathbf{N} = (\cos \theta, \sin \theta)$ and one has

$$\begin{aligned} \mathbf{N} \cdot \mathbb{E}\mathbf{N} &= (\cos^2 \theta - r \sin^2 \theta) \varepsilon = [(r + 1) \cos^2 \theta - r] \varepsilon, \\ \mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}_\perp &= [1 - (r + 1) \cos^2 \theta] \varepsilon, \\ \mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N} &= -(r + 1) \sin \theta \cos \theta \varepsilon, \end{aligned}$$

14 and therefore, in the small deformation approximation, we can write both terms U_ℓ and U_q of
15 the elastic energy as functions of θ .

16 As regards U_q , the most general elastic constitutive model for linear elasticity takes the
17 following quadratic form:

$$\begin{aligned} U_q(\hat{\mathbf{I}}_4, \hat{\mathbf{I}}_6, \mathbf{I}_8) &= \frac{1}{2} K_{\parallel} (\mathbf{N} \cdot \mathbb{E}\mathbf{N})^2 + \frac{1}{2} K_{\perp} (\mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}_\perp)^2 + \frac{1}{2} K_s (\mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N})^2 \\ &+ K_{\parallel\perp} (\mathbf{N} \cdot \mathbb{E}\mathbf{N})(\mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}_\perp) + K_{\parallel s} (\mathbf{N} \cdot \mathbb{E}\mathbf{N})(\mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}) + K_{\perp s} (\mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}_\perp)(\mathbf{N}_\perp \cdot \mathbb{E}\mathbf{N}), \end{aligned} \quad (21)$$

18 where K_{\parallel} is a coefficient related to the stiffness to stretching in the direction of cell orientation,
19 K_{\perp} to the one in the orthogonal direction, and K_s to the one related to shear. The other
20 coefficients are due to mixing effects among these three. We remark that the coefficient K_s
21 weights the response to shear: then, at the microscopic level, it quantifies the resistance to a
22 change in angle between stress fibers, which also involves the cross-linking network of proteins
23 like filamin, Rho/Rac GTPases, and ARP2/3 mentioned above.

In terms of θ , the anisotropic part of the energy can then be written as

$$\begin{aligned} U_q(\theta) &= \frac{1}{2} \varepsilon^2 \left\{ K_{\parallel} [\xi(\theta) - r]^2 + K_{\perp} [1 - \xi(\theta)]^2 + K_s \xi(\theta) [r + 1 - \xi(\theta)] \right. \\ &\quad \left. + 2K_{\parallel\perp} [\xi(\theta) - r] [1 - \xi(\theta)] - 2K_{\parallel s} [\xi(\theta) - r] (r + 1) \sin \theta \cos \theta \right\} \end{aligned}$$

$$-2K_{\perp s}[1 - \xi(\theta)](r + 1) \sin \theta \cos \theta \}, \quad (22)$$

1 where $\xi(\theta) := (r + 1) \cos^2 \theta$. We remark that, here and in the remainder of this Section, we have
 2 dropped the explicit energy dependence on t , since we are interested in the steady orientations
 3 of the cells which do not depend on time.

Moreover, we point out that, in the following, we will take $K_{\parallel s} = K_{\perp s} = 0$ due to symmetry
 requirements on the energy. Indeed, for the problem at hand, biological observations suggest
 that the energy must be symmetric with respect to $\theta = 0$ and $\theta = \pi/2$, that is,

$$U(-\theta) = U(\pi - \theta) = U(\theta) \quad \forall \theta.$$

4 These symmetries are biologically reasonable and not surprising: in absence of other directional
 5 stimuli, there is no reason why the cell should prefer the configuration $-\theta$ instead of the one
 6 characterized by the angle θ , as well as $\pi - \theta$ instead of θ , since they are the same up to a change
 7 in the viewpoint. In this context, the cell does not own a real orientation with a head and a
 8 tail [68], but rather a direction along which it reorients its stress fibers and focal adhesions as a
 9 consequence of mechanical stretch. Such a fact is translated in the energy symmetries, leading to
 10 configurations which are energetically equivalent. It is clear that, among the invariants appearing
 11 in the energy, the one which can lead to symmetry issues is I_8 , because it gives rise to terms like
 12 $\sin \theta \cos \theta$ which do not preserve the above symmetries. Hence, it is reasonable to assume that U_q
 13 depends on I_8 only through its square, leading to $K_{\parallel s} = K_{\perp s} = 0$. For the sake of completeness,
 14 in Appendix A we also discuss the case $K_{\parallel s}, K_{\perp s} \neq 0$, showing that their introduction provokes
 15 a symmetry breaking which is not biologically feasible, unless one needs to account for other
 16 directional cues.

17
 18 Instead, concerning U_ℓ , we firstly drop the dependence on I_8 for the same symmetry reasons
 19 discussed above. Moreover, we observe that, in the linearized case, the contribution of \hat{I}_3 is
 20 equivalent to the one of \hat{I}_1 . In fact, since $\mathbb{C} \approx \mathbb{I} + 2\mathbb{E}$, we have that

$$\hat{I}_1 = \text{tr}(\mathbb{C}) - 2 \approx 2 \text{tr}(\mathbb{E}) \approx \hat{I}_3,$$

neglecting terms of higher order. Consequently, the dependence on \hat{I}_3 can be dropped and merged
 with the one on \hat{I}_1 . Finally, since we are considering a quadratic approximation of the energy in
 the linear regime, the only admissible couplings between the other invariants are $\hat{I}_1 \hat{I}_4$ and $\hat{I}_1 \hat{I}_6$,
 because products involving \hat{I}_2 would have a higher order. Therefore, the dependence on \hat{I}_2 can
 be neglected as well, and the most general expression of the coupling term becomes

$$U_\ell \left(\hat{I}_1, \hat{I}_4, \hat{I}_6 \right) = 2K_{14}(\text{tr } \mathbb{E})(\mathbf{N} \cdot \mathbb{E} \mathbf{N}) + 2K_{16}(\text{tr } \mathbb{E})(\mathbf{N}_\perp \cdot \mathbb{E} \mathbf{N}_\perp),$$

or, as a function of θ ,

$$U_\ell(\theta) = 2\varepsilon^2(1 - r) \left[(K_{14} - K_{16})\xi(\theta) + (K_{16} - rK_{14}) \right],$$

21 where K_{14} and K_{16} are coefficients that weigh the coupling between the three invariants involved.

22 Since we want to study in more detail the equilibrium orientations and their stability, we take
 23 the first derivative of the overall energy with respect to θ and obtain

$$\begin{aligned} U'(\theta) &= U'_q(\theta) + U'_\ell(\theta) \\ &= \varepsilon^2 \left\{ K_{\parallel} [\xi(\theta) - r] + K_{\perp} [\xi(\theta) - 1] + \left(\frac{1}{2} K_s + K_{\parallel\perp} \right) [r + 1 - 2\xi(\theta)] \right\} \end{aligned}$$

$$+2(K_{14} - K_{16})(1 - r)\xi'(\theta). \quad (23)$$

1 Before going on, we notice that such a derivative vanishes for $\theta = \pi/4$ when $r = 1$, since in this
 2 case $\xi(\pi/4) = 1$. This fact is coherent with experimental observations [40] suggesting that $\pi/4$ is
 3 an equilibrium orientation when the biaxiality ratio amounts to 1, i.e., $\lambda_x = 1 + \epsilon$ and $\lambda_y = 1 - \epsilon$.
 4 In order to rewrite the expression (23) in a more compact form, we define

$$\widehat{K}_{\parallel} := K_{\parallel} + 4K_{14}, \quad \widehat{K}_{\perp} := K_{\perp} + 4K_{16}, \quad K_m := \frac{1}{2}K_s + K_{\parallel\perp} + 2K_{14} + 2K_{16}, \quad (24)$$

so that

$$\begin{aligned} U'(\theta) &= \varepsilon^2 \left\{ \widehat{K}_{\parallel} [\xi(\theta) - r] + \widehat{K}_{\perp} [\xi(\theta) - 1] + K_m [1 + r - 2\xi(\theta)] \right\} \xi'(\theta) \\ &= \varepsilon^2 [A\xi(\theta) - B(r + 1) + C] \xi'(\theta), \end{aligned} \quad (25)$$

5 setting

$$A := \widehat{K}_{\parallel} + \widehat{K}_{\perp} - 2K_m, \quad B := \widehat{K}_{\parallel} - K_m, \quad C := \widehat{K}_{\parallel} - \widehat{K}_{\perp}. \quad (26)$$

6 Since, under mechanical stretch, cell stress fibers are mainly aligned to the preferred direction,
 7 coherently with [6] in the following we will take $\widehat{K}_{\parallel} > \widehat{K}_{\perp}$. As a consequence, C is always
 8 positive, while the sign of A and B cannot be determined a priori, since it depends on the
 9 relative magnitude of the various coefficients involved.

10 Finally, to study the stability of the equilibrium orientations we will need to examine the sign
 11 of the second derivative of the energy, which reads

$$U''(\theta) = \varepsilon^2 \left\{ A\xi'(\theta)^2 + [A\xi(\theta) - B(r + 1) + C] \xi''(\theta) \right\}. \quad (27)$$

12 3.2 Equilibrium Orientations and Stability

13 Recalling (25), the equilibrium orientations are given by

$$\theta : \xi'(\theta) = 0 \quad \text{i.e.} \quad \theta = k\pi/2, \quad k \in \mathbb{Z} \quad \text{or} \quad \theta : A\xi(\theta) - B(r + 1) + C = 0,$$

14 the latter meaning

$$\cos^2 \theta = \frac{B}{A} - \frac{C}{A} \frac{1}{1+r} = \frac{1}{2} + \mathcal{K} \left(\frac{1}{2} - \frac{1}{1+r} \right), \quad (28)$$

15 where we have defined

$$\mathcal{K} := \frac{C}{A} = \frac{\widehat{K}_{\parallel} - \widehat{K}_{\perp}}{\widehat{K}_{\parallel} + \widehat{K}_{\perp} - 2K_m}. \quad (29)$$

16 So, in addition to the angles $\theta = k\frac{\pi}{2}$, one might have other four symmetric equilibrium angles
 17 given by (28) that depend only on the combination of parameters contained in \mathcal{K} . For simplicity,
 18 we will denote these configurations as *oblique* equilibria, while those with $\theta = k\pi$ will be referred
 19 to as *parallel* equilibria and those with $\theta = \frac{2k+1}{2}\pi$ as *perpendicular* equilibria, where the definition
 20 of parallel and perpendicular obviously refers to the stretching direction.

21 As we shall see, the discussion will depend on the sign of \mathcal{K} (i.e., whether A is positive or
 22 negative, having observed that $C > 0$) and whether $|\mathcal{K}|$ is smaller or larger than 1. For this

1 purpose it is useful to define

$$\begin{aligned}
\rho_{\parallel} &:= \frac{\mathcal{K} - 1}{2\mathcal{K}} = \frac{K_m - \widehat{K}_{\perp}}{\widehat{K}_{\parallel} - \widehat{K}_{\perp}}, \\
\rho_{\perp} &:= \frac{\mathcal{K} + 1}{2\mathcal{K}} = \frac{\widehat{K}_{\parallel} - K_m}{\widehat{K}_{\parallel} - \widehat{K}_{\perp}}, \\
\rho &:= \frac{\mathcal{K} + 1}{\mathcal{K} - 1} = \frac{\widehat{K}_{\parallel} - K_m}{K_m - \widehat{K}_{\perp}}.
\end{aligned} \tag{30}$$

2 Then, the existence of the oblique equilibrium angle depends on the value of the biaxiality ratio
3 r . Namely, referring to Fig. 2, the equilibrium orientation defined by (28) exists if

$$\begin{aligned}
\rho_{\parallel} < \frac{1}{1+r} < \rho_{\perp} & \quad \text{or} \quad \frac{1}{\rho} < r < \rho & \quad \text{when} \quad \mathcal{K} > 1, \\
0 < \frac{1}{1+r} < \rho_{\perp} & \quad \text{or} \quad r > \frac{1}{\rho} & \quad \text{when} \quad 0 < \mathcal{K} < 1, \\
0 < \frac{1}{1+r} < \rho_{\parallel} & \quad \text{or} \quad r > \rho & \quad \text{when} \quad -1 < \mathcal{K} < 0, \\
\rho_{\perp} < \frac{1}{1+r} < \rho_{\parallel} & \quad \text{or} \quad \rho < r < \frac{1}{\rho} & \quad \text{when} \quad \mathcal{K} < -1.
\end{aligned}$$

4 Looking at the stability of this orientation, recalling (27) one readily has that the second
5 derivative evaluated in this configuration is $U''(\theta) = \varepsilon^2 A \xi'(\theta)^2$, which is positive provided that
6 $A > 0$. So, if the coefficient A is positive, or equivalently if $\mathcal{K} > 0$, the oblique equilibrium
7 angle turns out to be stable. Otherwise, if the combination of elastic coefficients in A becomes
8 negative, the oblique orientation is unstable.

9 Looking instead at parallel orientations, e.g., $\theta = 0$, we have that

$$U''(0) = -2\varepsilon^2(r+1)[(A-B)(r+1) + C]. \tag{31}$$

10 Therefore, referring again to Fig. 2 and observing that $A - B = -\rho_{\parallel}C$, such an orientation is
11 stable if

$$\frac{1}{1+r} < \rho_{\parallel}. \tag{32}$$

12 Consequently, if $\rho_{\parallel} > 0$ (i.e. if $\mathcal{K} < 0$ or $\mathcal{K} > 1$) the parallel orientation is stable under the
13 condition (32), while if $\rho_{\parallel} < 0$ (i.e. if $0 < \mathcal{K} < 1$) it is always unstable.

14 Finally, the perpendicular orientations, e.g. $\theta = \pi/2$, are stable if

$$U''\left(\frac{\pi}{2}\right) = -2\varepsilon^2(r+1)[B(r+1) - C] > 0, \tag{33}$$

15 leading to the condition

$$\frac{1}{1+r} > \rho_{\perp}, \tag{34}$$

16 or equivalently $r < 1/\rho$. However, if $\mathcal{K} \in (-1, 0)$ the r.h.s. of (34) is negative. So, in this range
17 the perpendicular orientation is always stable, while outside the aforementioned interval stability
18 is granted whenever r satisfies (34).

19 Taken together, these results show that, as shown in Figs. 2(b), 2(d) for any quadratic
20 orthotropic elastic energy in a linear regime, oblique equilibrium angles follow a straight line in

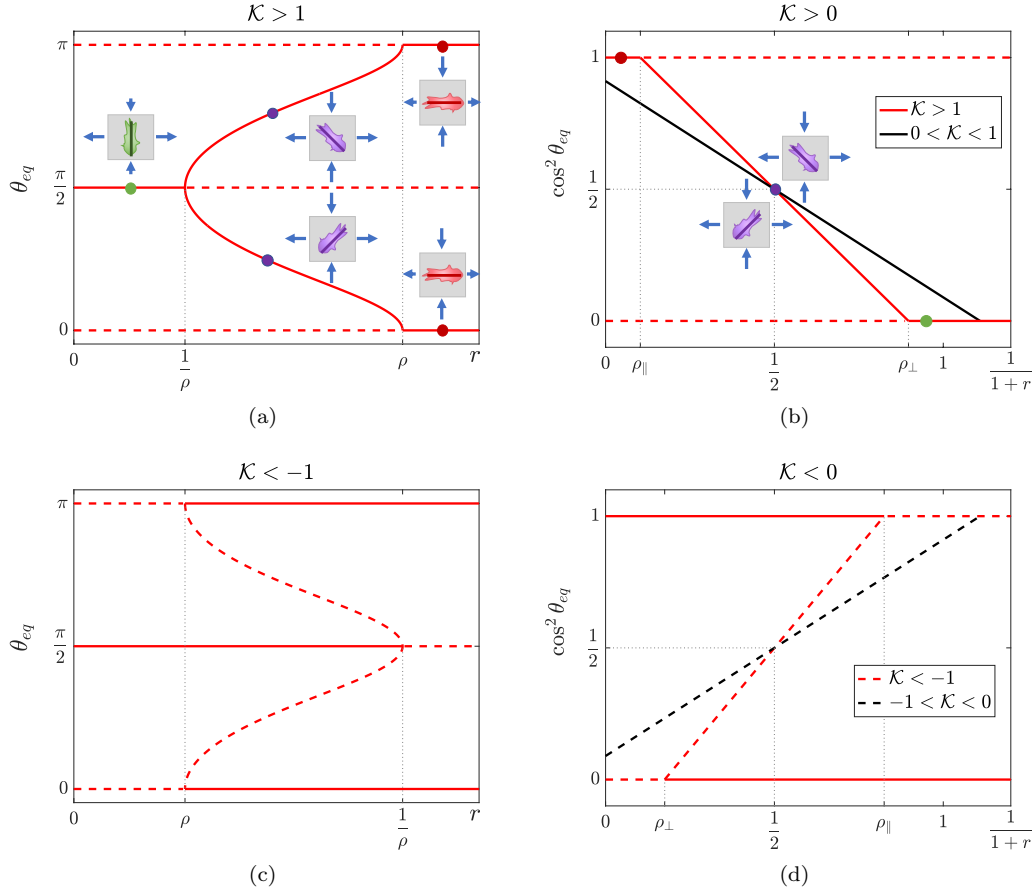


Figure 2: Bifurcation diagrams for positive \mathcal{K} (top) and negative \mathcal{K} (bottom). The bifurcation values are obtained for $(1+r)^{-1} = \rho_{\parallel} = (K_m - \hat{K}_{\perp})/(\hat{K}_{\parallel} - \hat{K}_{\perp})$ and $(1+r)^{-1} = \rho_{\perp} = (\hat{K}_{\parallel} - K_m)/(\hat{K}_{\parallel} - \hat{K}_{\perp})$. The insets and dots in (a) and (b) show representative cellular orientations: perpendicular (green), oblique (purple) and parallel (red).

1 the $((1+r)^{-1}, \cos^2 \theta)$ plane upon changes in the values of the biaxiality ratio. This is confirmed
 2 by experimental assays: in the set-up of the experiments by Livne et al. [40], collected data of
 3 the oblique orientation seem to align along a straight line with $\mathcal{K} = 1.26 \pm 0.08$.

4 Figure 2 summarizes the above discussion : in Fig. 2(a) and 2(c) we show the bifurcation
 5 diagram in the (r, θ) plane for $\mathcal{K} > 1$ and $\mathcal{K} < -1$ respectively, while in Fig. 2(b) and 2(d)
 6 we report the straight lines in the $((1+r)^{-1}, \cos^2 \theta)$ plane, for $\mathcal{K} > 0$ and $\mathcal{K} < 0$ respectively.
 7 It can be observed that if $\mathcal{K} > 1$ there are two supercritical pitchfork bifurcation points for
 8 $r = 1/\rho$ and $r = \rho$. So, for any r there is only one stable equilibrium angle in the interval $[0, \frac{\pi}{2}]$,
 9 and its symmetric counterpart with respect to $\pi/2$ if $r \in (\rho^{-1}, \rho)$. Hence, changing r one can
 10 smoothly pass from a configuration with the cell axis aligned along the stretching direction to
 11 one perpendicular to the stretching direction. We observe however that the range of values tested
 12 in the experiments is $(1+r)^{-1} \in [\frac{1}{2}, 1]$, because the substrate is not compressed along y more
 13 than it is stretched along x , which would correspond to values of $r > 1$. At the same time, it is
 14 not extended simultaneously along x and y , which would lead to negative values of r . This is the
 15 reason why, if $0 < \mathcal{K} < 1$, only the oblique equilibrium orientation is stable, while the parallel and
 16 perpendicular ones turn out to be always unstable in the experimental range of variation of r .
 17 We observe that, since for instance in Livne's experiments they find $\mathcal{K} \approx 1.26$ [40], a constraint
 18 can be inferred among the three coefficients appearing in (29), or equivalently among the six
 19 parameters in (24). In particular, the fact that $\mathcal{K} > 1$ assures that K_m cannot be neglected,
 20 because otherwise \mathcal{K} in (29) would always be smaller than 1. Starting from this observation and
 21 recalling that $\widehat{K}_{\parallel} > \widehat{K}_{\perp}$, looking for the minimum number of coefficients necessary to satisfy the
 22 experimental values, one finds that, on the other hand, \widehat{K}_{\perp} could be neglected, so that \mathcal{K} can be
 23 rewritten in terms of the ratio $K_m/\widehat{K}_{\parallel}$ as

$$\frac{1}{\mathcal{K}} \approx 1 - 2 \frac{K_m}{\widehat{K}_{\parallel}}.$$

24 Actually, on the basis of the experimental slope, we can argue that, if $\widehat{K}_{\perp} = 0$ (i.e. $K_{\perp} = K_{16} =$
 25 0), then

$$\frac{K_m}{\widehat{K}_{\parallel}} = \frac{\frac{1}{2}K_s + K_{\parallel\perp} + 2K_{14}}{K_{\parallel} + 4K_{14}} \approx 0.103,$$

26 that entails $\widehat{K}_{\parallel} \gg K_m$, i.e., the coefficient related to the stiffness to stretching in the direction
 27 of cell orientation is higher than all the other parameters. We highlight again that, even though
 28 the contribution of K_m is smaller than the one of \widehat{K}_{\parallel} , the former parameter is fundamental to
 29 obtain a biologically relevant response.

30 Looking now at the case $\mathcal{K} < -1$, as in Fig. 2(c), which might for instance occur if K_m
 31 is large with respect to \widehat{K}_{\parallel} and \widehat{K}_{\perp} , then the pitchfork bifurcations become subcritical and
 32 one jumps from the parallel to the perpendicular equilibria, since the oblique one is always
 33 unstable. So, imagining to operate on K_m , when $2K_m$ passes from being smaller to being larger
 34 than $\widehat{K}_{\parallel} + \widehat{K}_{\perp}$, corresponding respectively to $\rho_{\parallel} < \rho_{\perp}$ and $\rho_{\parallel} > \rho_{\perp}$, there is a switch from
 35 supercritical to subcritical bifurcations. In Fig. 2(d) we also plot the case $-1 < \mathcal{K} < 0$, in
 36 which one has the same bistable behaviour for all the experimental values of r , while the oblique
 37 orientation loses its stability. This could be an explanation of why the oblique orientation might
 38 not be observed in the case $\mathcal{K} < 0$, that is $\widehat{K}_{\parallel} + \widehat{K}_{\perp} < 2K_m$. Differently from previous models,
 39 our bifurcation analysis includes this possibility, which however needs to be validated precisely
 40 through experimental data.

41 Moreover, even though experiments are commonly performed in a range of biaxiality ratio
 42 $r \in [0, 1]$, our model is able to foresee the behaviour of the cell even for values of $r > 1$, i.e.

1 when the substrate is more compressed in the y -direction than it is stretched in the x -direction,
 2 a condition not tested yet experimentally.

3 We finally observe that the presence of pitchfork bifurcations is not surprising, since they
 4 often arise in one-dimensional dynamical systems that present some symmetries: this is indeed
 5 our case, since we took an energy functional which is even and symmetric with respect to $\pi/2$ in
 6 order to match some biological considerations. As a matter of fact, the introduction of $K_{\parallel s}$ and
 7 $K_{\perp s}$, discussed in Appendix A, leads to a symmetry breaking and therefore to the appearance
 8 of turning points.

9 4 Simulations of the Viscoelastic Model

10 After having discussed the equilibrium orientations in Section 3, here we focus on the dynamics
 11 of cell reorientation in response to the viscoelastic model presented in Section 2, performing some
 12 numerical simulations. More specifically, we consider the system of equations which describes
 13 the time evolution of the Cauchy stress tensor \mathbb{T} and the reorientation dynamics of the angle
 14 θ . As regards the former, its evolution is governed by the viscoelastic constitutive equation (6)
 15 described in Section 2; concerning the angle, as in Eq. (2) we assume that changes in orientation
 16 are driven by a dissipative process in which the cell tries to find the direction which minimizes
 17 the virtual work done by the Cauchy stress. Consequently, the system of equations is

$$\begin{cases} \dot{\theta} = -\frac{1}{\widehat{K}_{\parallel}\lambda_{\theta}}\frac{\partial\mathbb{T}}{\partial\theta}:\mathbb{E}, & (35a) \\ \dot{\mathbb{T}} + \frac{1}{\lambda}\mathbb{T} = \frac{1}{\lambda}\mathcal{C}_0\dot{\mathbb{E}}, & (35b) \end{cases}$$

18 where \mathcal{C}_0 is the functional that accounts for the exponentially weighted history of past orientations
 19 defined in (7), depending on the elasticity tensor \mathbf{C}_0 . The components of \mathbf{C}_0 can be written in
 20 terms of θ as

$$(\mathbf{C}_0)_{xxxx} = \widehat{K}_{\parallel}\cos^4\theta + \widehat{K}_{\perp}\sin^4\theta + 2K_m\sin^2\theta\cos^2\theta,$$

$$(\mathbf{C}_0)_{yyyy} = \widehat{K}_{\parallel}\sin^4\theta + \widehat{K}_{\perp}\cos^4\theta + 2K_m\sin^2\theta\cos^2\theta,$$

$$(\mathbf{C}_0)_{xxyy} = K_m - \frac{1}{2}K_s + (\widehat{K}_{\parallel} + \widehat{K}_{\perp} - 2K_m)\sin^2\theta\cos^2\theta.$$

21 We consider a specimen stretched in the x -direction uniformly with a fixed biaxiality ratio r ,
 22 such that the infinitesimal deformation tensor is given by (20) with

$$\varepsilon(t) = \frac{1}{2}\varepsilon_0(1 - \cos\omega t),$$

23 for different angular frequencies. Compared to experiments, in the simulations we do not assume
 24 that the oscillation period is smaller or greater than the characteristic relaxation time λ or
 25 reorientation time λ_{θ} , in order to put in evidence both the elastic and the viscous behaviour of
 26 the system.

We now solve Eq. (35) for a range of values of r to check the theoretical predictions obtained
 through the bifurcation analysis. In particular, a numerical algorithm has been implemented
 using MATLAB[®]. As regards Eq. (35b), its discretization was performed through the explicit

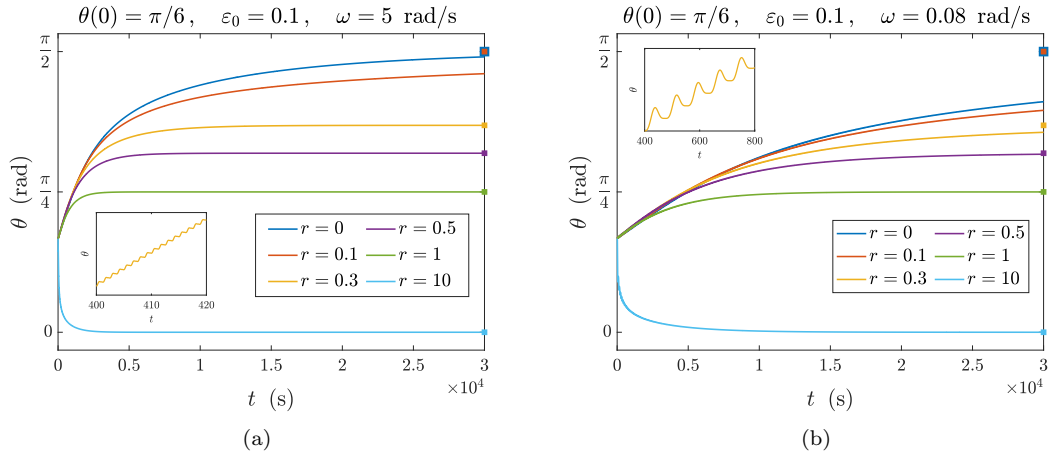


Figure 3: Evolution of θ according to (35) in the high frequency (a) and low frequency (b) cases, for $\mathcal{K} = 1.26$ and $\varepsilon_0 = 0.1$, while the biaxiality ratio r is varied. As initial condition, we take in both cases $\theta(0) = \pi/6$. The squares on the right of each plot highlight the steady orientations predicted by the bifurcation analysis. Moreover, all curves display an oscillatory behaviour, as shown in the insets for the specific case $r = 0.3$.

Euler method. It is equivalent to two scalar equations for the components T_{xx}, T_{yy} of the Cauchy tensor \mathbb{T} : the integrals in the r.h.s. of (35b) have been approximated observing that, for instance in the case $r = 0$,

$$\begin{aligned}
 (\mathcal{C}_0^{k+1})_{xxxx} &= \int_{-\infty}^{t_{k+1}} e^{-(t_{k+1}-\tau)/\lambda} (\mathbf{C}_0)_{xxxx}(\theta(\tau)) d\tau \\
 &= e^{-(t_{k+1}-t_k)/\lambda} (\mathcal{C}_0^k)_{xxxx} + \int_{t_k}^{t_{k+1}} e^{-(t_{k+1}-\tau)/\lambda} (\mathbf{C}_0)_{xxxx}(\theta(\tau)) d\tau.
 \end{aligned} \tag{36}$$

1 Then, with an analogous procedure, all the integral terms can be evaluated from the value
 2 at the previous time instant plus the discretization of the remaining integral in (36), which
 3 was performed through the trapezoidal rule. The generalization to the case $r \neq 0$ is then
 4 straightforward. Finally, concerning the virtual work term in Eq. (35a), the derivative of the
 5 stress with respect to θ was approximated using a centered finite difference. In all our simulations
 6 we take $\lambda = \lambda_\theta = 6.6$ s and to have coherence with experimental data we consider a value of
 7 $\mathcal{K} = 1.26$ [40]. Instead, we focus on the effect of variations of r, ε_0 and ω to evaluate their impact
 8 on the reorientation dynamics of the cell.

9 In Figure 3(a) we show the evolution of the orientation angle in the high frequency case,
 10 starting from an initial condition $\theta(0) = \pi/6$, for different values of r . We see that the angle
 11 approaches the value obtained in Section 3.2 in the stationary case (identified by a coloured
 12 marker on the the right side of the box) and reported in the bifurcation diagrams in Fig. 2.
 13 More specifically, for low values of r the final orientation is almost orthogonal to the direction
 14 of stretching. Increasing the biaxiality ratio r makes the equilibrium angle decrease, reaching
 15 the expected value given by the bifurcation diagrams: in particular, we observe that the steady
 16 angle is $\pi/4$ when $r = 1$, as predicted by the theory and by the experiments. For the sake of
 17 completeness, we also showed a case in which $r \gg 1$, even if no experimental data are available

1 in this case: in this situation one has $\theta \rightarrow 0$, coherently with the study carried out in Section 3.
2 We also studied the behaviour of the angle reorientation for a different initial condition greater
3 than $\pi/2$, that is $\theta(0) = 5\pi/6$ (not shown in the Figures): as before, the evolution towards the
4 steady angle is predicted by the bifurcation diagram for all values of r . In fact, such a choice
5 for the initial condition makes the system go to the other mirror-image orientation, greater than
6 $\pi/2$ and finally, for large values of r , to $\theta = \pi$. We recall however that the last configuration is
7 biologically equivalent to $\theta = 0$ because of the discussed symmetries of the energy, implying that
8 the cell is aligned with the x -axis.

9 Conversely, in Figure 3(b), we report the plots of θ in the low frequency regime for the same
10 initial condition, choosing $\omega = 0.08$ rad/s which is slightly above the experimental reorienta-
11 tion threshold of 0.06 rad/s suggested for experiments. The dynamics is coherent with model
12 predictions: we have the same equilibria as in the high frequency case, even if the convergence
13 towards the steady angle is slower due to the presence of viscous effects. As shown in the inset
14 plots in Fig. 3(a) and Fig. 3(b), all curves display an oscillatory behaviour as expected, since we
15 are imposing a periodic deformation to the specimen. Hence, the orientation angle progressively
16 increases through small oscillations until it reaches the predicted orientation. In particular, such
17 oscillations are smaller in amplitude and faster in the elastic case, while they have a greater
18 amplitude and are slower in the viscous limit.

19 To compare our results with reorientation frequencies and thresholds from Jungbauer et al.
20 [30], we performed some simulations for their same experimental biaxiality ratio and amplitude,
21 changing instead the value of ω . As shown in Fig. 4(a), angular frequencies below a minimum
22 threshold (which can be quantified in about 0.01 Hz \approx 0.06 rad/s, which is coherent with exper-
23 iments on some cell types [28, 30]) do not induce a significant or significantly fast response. In
24 this case, the reorientation is so slow that it cannot be seen on time scales comparable with the
25 cell cycle, and in the experimental case the process is destroyed by random fluctuations. Instead,
26 higher frequencies induce reorientation, with a characteristic time that, coherently with [30],
27 decreases with the frequency. This is true until ω reaches a second threshold of about 2π rad/s
28 (i.e. 1 Hz), after which a further increase in the frequency does not substantially accelerate the
29 reorientation process towards the expected equilibrium angle. This is confirmed and summarized
30 by the results in Fig. 4(b), where we report the average speed of reorientation v_m , calculated
31 over a suitable interval where each curve can be approximated by a line, as a function of the
32 frequency of the imposed deformation. We find that the speed of reorientation is very low when
33 $\omega < 0.01$ rad/s, corresponding to evolution times of the order of days. Then, there is a sudden
34 transition interval for $\omega \in [0.01, 1]$ rad/s with an inflection point close to $\omega \approx 0.15$ rad/s, i.e.
35 when $\lambda\omega \approx 1$, related to the viscous-elastic transition in the material. Finally, for higher values
36 of ω , the speed of reorientation saturates to values corresponding to experimental times of the
37 order of an hour. To make a further comparison with experiments, we define the characteristic
38 time of reorientation for our model as $\tau := (\theta_{eq} - \theta_0)/v_m$, assuming that the evolution curves for
39 θ as a function of time are approximated by a saturating exponential. Then, in Fig. 4(c) we plot
40 this characteristic time together with data for rat embryonic fibroblasts (REF cells) from [30].
41 As discussed before, such a time decreases with the frequency until a threshold, above which it
42 remains almost constant, and the model predictions show a good agreement with experimental
43 data.

44 In Figure 5 we studied instead the influence of the stretch amplitude ε_0 , while the angular
45 frequency is kept high in Fig. 5(a) and low in Fig. 5(b), fixing $r = 1$ and therefore $\theta_{eq} = \pi/4$.
46 As one could expect, the equilibrium orientation for a given initial condition $\theta(0)$ and biaxiality
47 ratio r is not altered by variations of ε_0 and remains equal to $\pi/4$ in this case. Changes in
48 the amplitude only influence the speed of convergence towards the predicted equilibrium angle.
49 Indeed, if we scale times with $1/\omega$, the stress tensor with $\hat{K}_{\parallel}\varepsilon_0$, and the strain tensor with ε_0 ,

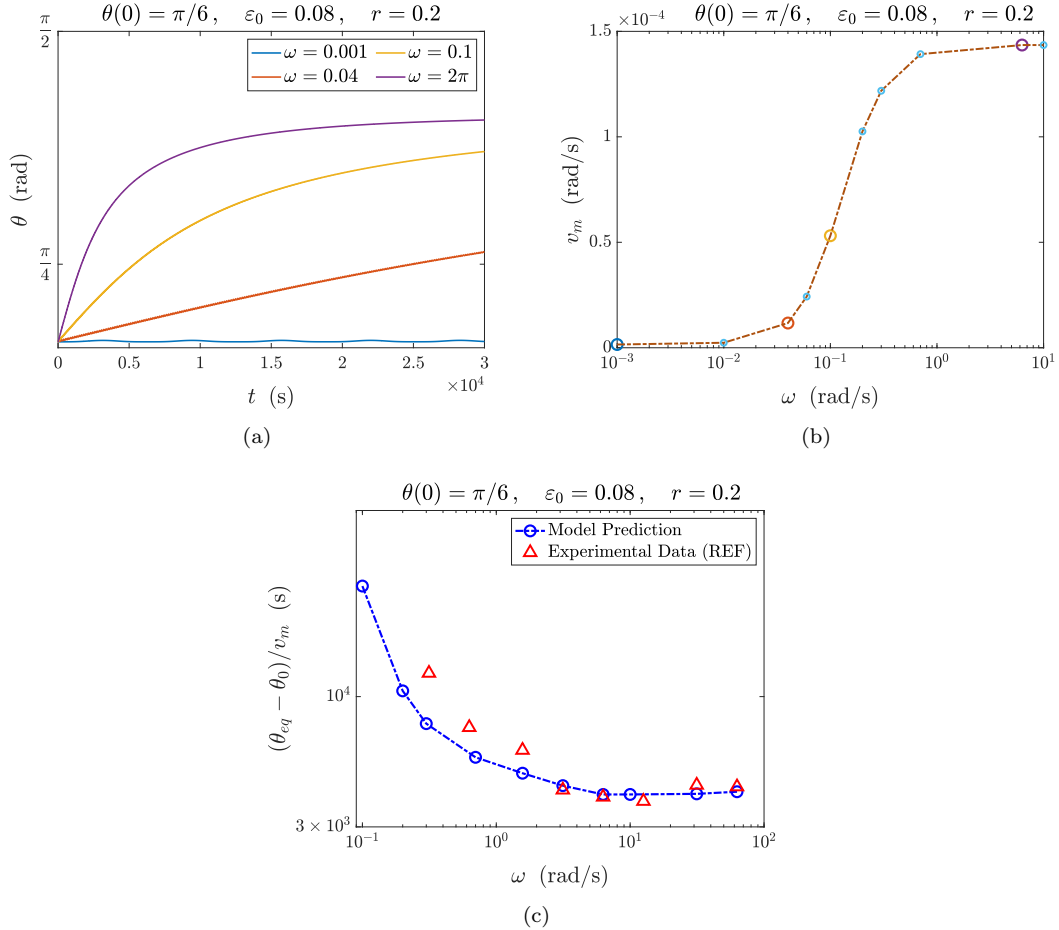


Figure 4: (a): Evolution of θ according to (35) for a fixed biaxiality ratio and different angular frequencies. We observe that low frequencies (approximately below a minimum threshold of 0.06 rad/s, i.e., about 0.01 Hz, coherently with experimental results [28, 30]) do not induce a significant reorientation response. For higher frequencies, the preferential orientation becomes visible and the reorientation time decreases. (b): Average speed of reorientation v_m , computed in a suitable interval where the evolution curve is approximately linear, as a function of the imposed angular frequency in logarithmic scale. Recalling that we used $\lambda = \lambda_\theta = 6.6$ s, a transition occurs when $\lambda\omega = 1$, i.e., the inflection point in $\omega \approx 0.15$ rad/s. Then, there is a second threshold of about 2π rad/s, above which a further increase does not induce a significantly faster response [30]. (c): Plot of the model characteristic time $\tau := (\theta_{eq} - \theta_0)/v_m$ as a function of the angular frequency in logarithmic scale, together with experimental results for rat embryonic fibroblasts (REF cells) taken from [30].

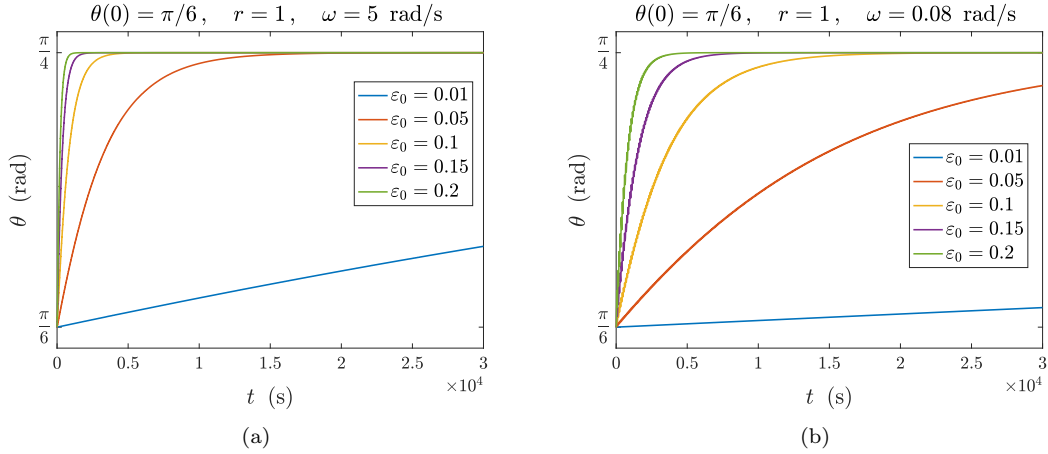


Figure 5: Evolution of θ according to (35) in (a) the high frequency and (b) the low frequency cases for different values of the stretch amplitude ε_0 and fixed biaxiality ratio $r = 1$.

- 1 the system (35) rewrites in dimensionless terms formally substituting $\widehat{K}_{\parallel}\lambda_{\theta}$ with $\widetilde{\Lambda}_{\theta} = \lambda_{\theta}\omega/\varepsilon_0^2$
- 2 and λ with $\widetilde{\Lambda} = \lambda\omega$. In formulas, defining $t = \widetilde{t}/\omega$, $\mathbb{T} = \widetilde{\mathbb{T}}\widehat{K}_{\parallel}\varepsilon_0$, $\mathbb{E} = \widetilde{\mathbb{E}}\varepsilon_0$ and $\mathbb{C}_0 = \widetilde{\mathbb{C}}_0\widehat{K}_{\parallel}\lambda$ yields

$$\left\{ \begin{array}{l} \frac{d\theta}{d\widetilde{t}} = -\frac{1}{\widetilde{\Lambda}_{\theta}} \frac{\partial \widetilde{\mathbb{T}}}{\partial \theta} : \widetilde{\mathbb{E}}, \\ \frac{d\widetilde{\mathbb{T}}}{d\widetilde{t}} + \frac{1}{\widetilde{\Lambda}} \widetilde{\mathbb{T}} = \widetilde{\mathbb{C}}_0 \frac{d\widetilde{\mathbb{E}}}{d\widetilde{t}}. \end{array} \right. \quad (37a)$$

$$\left\{ \begin{array}{l} \frac{d\theta}{d\widetilde{t}} = -\frac{1}{\widetilde{\Lambda}_{\theta}} \frac{\partial \widetilde{\mathbb{T}}}{\partial \theta} : \widetilde{\mathbb{E}}, \\ \frac{d\widetilde{\mathbb{T}}}{d\widetilde{t}} + \frac{1}{\widetilde{\Lambda}} \widetilde{\mathbb{T}} = \widetilde{\mathbb{C}}_0 \frac{d\widetilde{\mathbb{E}}}{d\widetilde{t}}. \end{array} \right. \quad (37b)$$

As already discussed in Sections 2.1 and 2.2, the former dimensionless group $\widetilde{\Lambda}_{\theta}$ is related to the time needed by the cell to re-orientate in terms of the oscillation frequency and amplitude, while the latter $\widetilde{\Lambda}$ identifies the relative role of viscoelasticity. In particular, focusing on $\widetilde{\Lambda}_{\theta}$, if the amplitude of oscillation increases (e.g., doubles) the evolution of the orientation angle θ remains the same provided that the reorganization time λ_{θ} is suitably increased (e.g., quadruples). On the other hand, if λ_{θ} is kept constant, as done in Fig. 5, cells re-orient faster, and the re-orientation time scales like the square of the oscillation amplitude. However, to simplify the direct comparison with experimental results, we decided to perform all simulations using dimensional quantities.

Finally, in Figure 6 we report the evolution of the Cauchy stress components T_{xx} and T_{yy} , both normalized with respect to \widehat{K}_{\parallel} . In particular, Figs. 6(a) and 6(b) show the stresses in the high frequency case, for a fixed biaxiality ratio $r = 0.3$. It can be observed that, starting from a stress-free configuration, there is a first increase in both stress values up to a peak, after which relaxation begins and completes in about 100 seconds. Once the transient is passed, the stress components start to oscillate around zero, meaning that the system is behaving purely elastically. In the low frequency case, plotted in Fig. 6(c) and 6(d), the response of the system is much slower, since the viscous component emerges visibly. Concerning the stress magnitude when the equilibrium orientation is reached, as predicted by the model, we observe that the stress components in the low frequency case differ from the ones in the high frequency case by a factor $\lambda\omega_{low} \approx 0.53$.

1 We have to stress that, in all simulations, we kept the characteristic times λ and λ_θ constant,
2 to better identify the effects of the oscillation characteristics in terms of frequency and amplitude.
3 However, the dynamics of adhesion to the substrate is more involved because the application of
4 a stress on them has the consequence of both strengthening the bonds, due to an increased
5 clustering of integrins, and prolonging their lifetime. In particular, two types of bonds are
6 identified in the literature, catch and slip bonds [34, 37, 72, 75, 76]. Increasing the applied
7 deformation has the effect of increasing the applied force acting on the bonds and this causes a
8 decrease (resp., an increase) of the lifetime of slip (resp., catch) bonds. So, the dependence of
9 the adhesion bond lifetime, and therefore of λ , on the deformation is not constant and might
10 actually not even be monotone with a maximum corresponding to an applied force of the order of
11 10 pN. However, as discussed in Sections 2.1 and 2.2, including such a strain-dependence lifetime
12 would not change the equilibrium configuration, but only the temporal behaviour of the system.
13 Conversely, a strengthening of the adhesion bond might change both, though we do not expect
14 them to be relevant.

15 5 Discussion

16 The response of cells to mechanical cues is a relevant biological phenomenon which still needs in-
17 vestigations and efforts to be enlightened. Starting from experimental observations showing that,
18 when a monolayer is subject to a biaxial stretch, cells orient themselves in a well-defined con-
19 figuration, in this paper we employed mechanical instruments to further explore this behaviour,
20 focusing on linear elasticity and viscoelasticity. Previous works [40, 42] suggested that a linear
21 elastic model is able to fit the experimental data for a wide range of strain energies, while the
22 impact of nonlinearities seems slight.

23 To account for viscous effects during the reorientation process, due for instance to formation
24 and breaking of integrin bonds between the cell and the substrate, we developed a viscoelastic
25 model which describes the system as an anisotropic continuum with preferential directions in-
26 duced by the presence of stress fibers and lateral protein network. This allowed us to differentiate
27 the behaviour depending on the period of the applied cyclic stretch: if the latter is much shorter
28 than the cell characteristic response times λ and λ_θ , then the response is elastic with cells having
29 not enough time to reorganize. On the other hand, if the imposed frequency is low, viscous-like
30 behaviours emerge slowing down the reorientation, until the process becomes too slow to be rel-
31 evant. We showed that our viscoelastic model is able to capture these differences, which can be
32 recovered by taking the limit of the general constitutive equation in the high and low frequency
33 cases.

34 Then, we studied in detail the steady orientations with two main objectives: to generalize
35 previous linear elastic models using the most general orthotropic model and to analyze the
36 bifurcations that occur when the biaxiality ratio of the deformation is changed. In particular,
37 we considered a general quadratic strain energy involving a priori six elastic parameters: \widehat{K}_\parallel
38 and \widehat{K}_\perp , related to the stiffness along the cell major axis and along the orthogonal direction,
39 respectively; K_s , modelling the resistance to shear; and $K_{\parallel\perp}$, $K_{\parallel s}$, $K_{\perp s}$ including coupled effects
40 among the previous three. The bifurcation analysis is coherent with previous theoretical and
41 experimental works [23, 40, 42], and a linear relation between the parameter $\frac{1}{1+r}$ and $\cos^2 \theta$,
42 where θ is the nontrivial equilibrium orientation, is recovered. The slope of the line is given by
43 the combination of elastic coefficients

$$\mathcal{K} = \frac{\widehat{K}_\parallel - \widehat{K}_\perp}{\widehat{K}_\parallel + \widehat{K}_\perp - 2K_m},$$

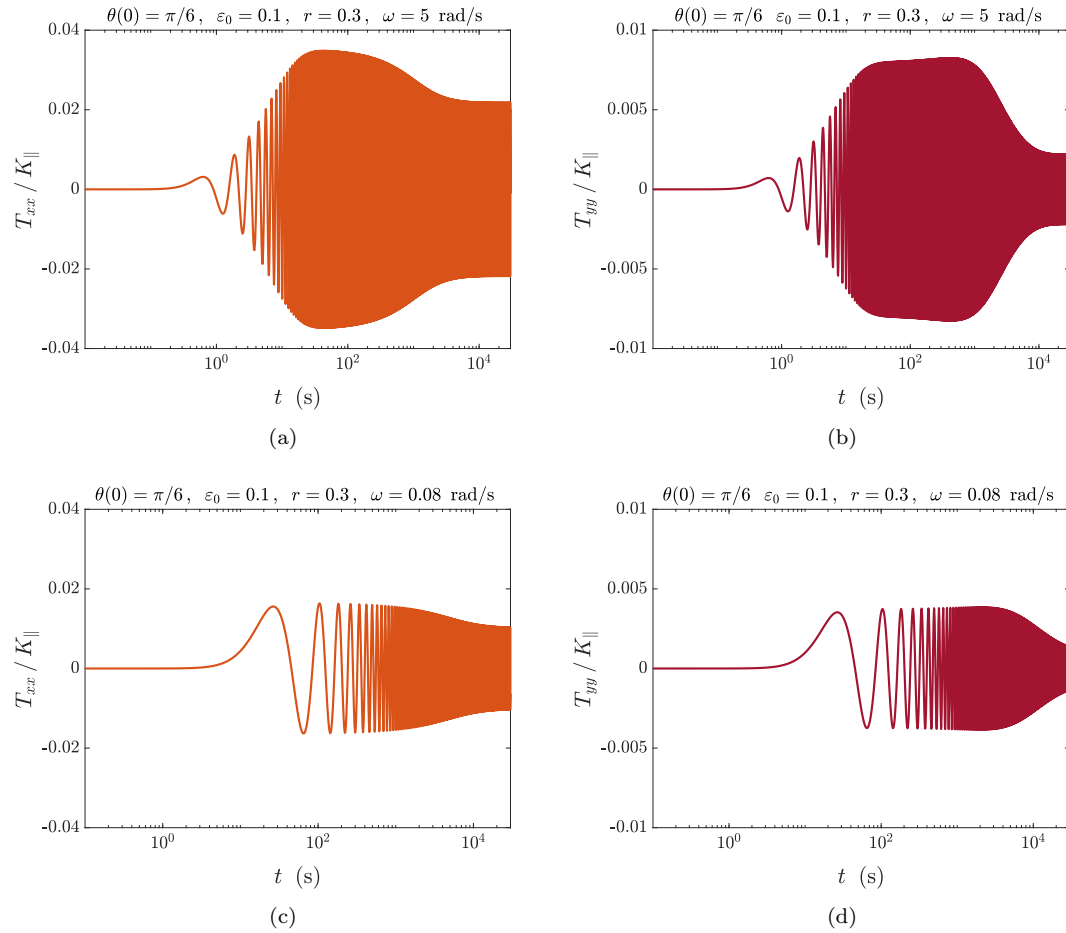


Figure 6: Time evolution of the stress tensor components T_{xx} and T_{yy} (normalized w.r.t. \tilde{K}_{\parallel}), for $r = 0.3$. In the top row the high frequency case is reported, while in the bottom row the plots refer to the low frequency case. The time axis is reported in logarithmic scale to put in evidence the temporal behaviour of stress amplitudes.

1 which in some experimental results was reported to be about 1.26 ± 0.08 [40]. The value of the
2 experimental slope allowed to conclude that the presence of a non negligible response to shear
3 given by K_m is important as well as the major role played by the response to stretch along the
4 direction of cell orientation (given by K_{\parallel}).

5 Our description also allows a continuous variation of \mathcal{K} , which can achieve positive or negative
6 values depending on the parameters appearing in its definition: even if, to our knowledge, there
7 are no experimental data available for situations like $\mathcal{K} < 0$, our elastic model is in principle
8 able to capture them. Moreover, differently from our previous work [42], we included in the
9 energy possible isotropic-anisotropic couplings: as we have seen, their introduction does not
10 qualitatively alter the results and the linear relation between $1/(1+r)$ and $\cos^2 \theta$, but only leads
11 to a redefinition of some parameters.

12 In detail, we observed two supercritical pitchfork bifurcations when $\mathcal{K} > 1$: for low values of
13 the biaxiality ratio r the orthogonal orientation is stable, while two specular equilibria become
14 stable after the first bifurcation. Increasing r even more they finally disappear, leaving the
15 orientation $\theta = 0$ or $\theta = \pi$ as stable. Instead, if $\mathcal{K} < -1$, the two bifurcations become subcritical.
16 The presence of pitchfork bifurcations is coherent with the energy symmetries. In fact, in our
17 framework the orientations $\theta, -\theta, \pi - \theta, \pi + \theta$ are energetically equivalent, because the cell only
18 chooses a direction without distinction between head and tail.

19 Simulations in the case $\mathcal{K} > 0$ confirmed the analytical predictions, showing an evolution
20 towards a steady angle depending on the imposed biaxiality ratio and on the initial condition,
21 but not on the frequency. Although experiments are still needed to deeply investigate such a
22 behaviour of the cells, to our knowledge there is some agreement in the literature on the fact
23 that the preferred orientation angle does not directly depend on the frequency of the cyclic deforma-
24 tion. Instead, the frequency seems to influence the amount of cells found oriented along the
25 preferential direction and the speed of the cytoskeletal response [23, 30, 43, 62]. This latter effect
26 is evident in our simulations where we have shown that, in the low frequency case, convergence
27 is slowed down due to the presence of viscous effects, even if the stationary orientation is still
28 predicted by the bifurcation diagrams. Moreover, in accordance with the results in [28, 30], there
29 is a transition for $\lambda\omega \approx 1$, so that the time required to observe reorientation is of the order of
30 days for small ω 's, saturating to one hour for larger frequencies.

31 Hence, our model seems consistent with previous experimental data and theories describing
32 the behaviour of an ensemble of cells on a stretched substrate, also discussing the case of isotropic-
33 anisotropic couplings in the energy and recovering once more the established linear relation
34 between the squared cosine of the angle and a parameter related to the deformation.

35 However, there are at least three aspects that are not covered yet by the present modelling
36 framework and will be the aim of future works. The first one concerns the influence of the
37 compliance of the substratum, the second one is related to the dependence of the viscoelastic
38 parameters describing the mechanical behaviour of adhesion molecules on the applied deformation
39 or stress, and the third involves the response of cells cultured on monolayer undergoing a static
40 step deformation. This last effect is still debated since some experimental assays showed that,
41 differently from the cyclic case, cells stress fibers or focal adhesions may prefer the parallel
42 orientation [11, 15, 17, 22] also in a pre-stretched condition [39], while other studies reported
43 different results or even no reorientation at all [25, 38]. Further investigation is required in
44 this regard, since experimental conditions are very different from one another. Introducing
45 remodelling of cell cytoskeleton and stress fibers, following an active fiber reorientation approach
46 [12, 13], may be a direction for future research and modelling, on which we are currently focusing.

47 Moreover, in this work we considered a purely homogeneous deformation since we wanted
48 to make analytical considerations and to carry out a detailed bifurcation analysis. However, to
49 realistically reproduce the experimental settings, it would be more accurate to impose the strain

1 only at the boundary of the specimen: this requires a computational effort to solve the elastic
 2 problem, using for instance a finite element scheme. Finally, another possible perspective for
 3 future improvement would be to account for the mechano-chemical response of the cell at the
 4 microscopic scale, considering for instance active mechanotransduction in a multiscale frame-
 5 work. The inclusion of active effects like shape fluctuations, metabolism [20] and cell response
 6 to topographical cues in the substrate, even in absence of external mechanical stimuli [4], could
 7 be of help in understanding the complex interactions between the cell and its environment.

8 A The Asymmetric Case

9 In this Appendix, we turn our attention to a more general case for which $K_{\perp s}, K_{\parallel s} \neq 0$ in the
 10 anisotropic energy (22), to show that their introduction leads to a symmetry breaking bifurcation.
 11 If we do not neglect the contribution of these parameters, the overall strain energy as a function
 12 of θ becomes

$$\begin{aligned}
 U(\theta) = \frac{1}{2}\varepsilon^2 \big\{ & K_{\parallel} [\xi(\theta) - r]^2 + K_{\perp} [1 - \xi(\theta)]^2 + K_s \xi(\theta) [r + 1 - \xi(\theta)] \\
 & + 2K_{\parallel\perp} [\xi(\theta) - r] [1 - \xi(\theta)] - 2K_{\parallel s} [\xi(\theta) - r] (r + 1) \sin \theta \cos \theta \\
 & - 2K_{\perp s} [1 - \xi(\theta)] (r + 1) \sin \theta \cos \theta \big\} \\
 & + 2\varepsilon^2 (1 - r) \left[(K_{14} - K_{16}) \xi(\theta) + (K_{16} - rK_{14}) \right], \tag{A.1}
 \end{aligned}$$

13 while its first derivative is

$$\begin{aligned}
 U'(\theta) = \varepsilon^2 \big\{ & \left[K_{\parallel} [\xi(\theta) - r] + K_{\perp} [\xi(\theta) - 1] + \left(\frac{1}{2} K_s + K_{\parallel\perp} \right) [r + 1 - 2\xi(\theta)] \right. \\
 & \left. - (K_{\parallel s} - K_{\perp s}) (r + 1) \sin \theta \cos \theta + 2(K_{14} - K_{16})(1 - r) \right] \xi'(\theta) \\
 & \left. - \left[K_{\parallel s} [\xi(\theta) - r] + K_{\perp s} [1 - \xi(\theta)] \right] (r + 1) (\cos^2 \theta - \sin^2 \theta) \right\}. \tag{A.2}
 \end{aligned}$$

14 Before going further, we observe that, in order to have coherence with the experimental
 15 condition $U'(\pi/4) = 0$ for $r = 1$ (which was automatically granted in the case $K_{\parallel s} = K_{\perp s} = 0$),
 16 the following constraint is necessary:

$$K_{\parallel s} = K_{\perp s}.$$

17 Hence, we have that the mixing contributions related to shear must be equal. Under this condi-
 18 tion, the energy derivative rewrites as

$$U'(\theta) = \varepsilon^2 \left\{ [A\xi(\theta) - B(r + 1) + C] \xi'(\theta) + K_{\parallel s} (r^2 - 1) (\cos^2 \theta - \sin^2 \theta) \right\}, \tag{A.3}$$

19 where A , B and C are defined as in (26). Then, differently from Eq. (25), we have an additional
 20 contribution related to $K_{\parallel s}$.

21 In this situation, to derive the equilibrium orientations, we try to write them expliciting r
 22 instead of $\cos^2 \theta$ when imposing that $U'(\theta) = 0$. Therefore, we have that the steady state angles
 23 satisfy

$$2[A(r + 1) \cos^2 \theta - B(r + 1) + C] \sin \theta \cos \theta + (1 - r) K_{\parallel s} (\cos^2 \theta - \sin^2 \theta) = 0,$$

1 that can be readily solved yielding

$$r = \frac{K_{\parallel s}(\cos^2 \theta - \sin^2 \theta) + 2(A \cos^2 \theta - B + C) \sin \theta \cos \theta}{K_{\parallel s}(\cos^2 \theta - \sin^2 \theta) - 2(A \cos^2 \theta - B) \sin \theta \cos \theta}. \quad (\text{A.4})$$

2 Actually, as in the symmetric case $K_{\parallel s} = 0$, a more compact form only depending on a single
3 parameter can be achieved working in terms of $\frac{1}{1+r}$. In fact, with this idea (A.4) rewrites as

$$\frac{1}{1+r} = \frac{C \sin 2\theta - A \sin 2\theta \cos 2\theta + 2K_{\parallel s} \cos 2\theta}{2(2K_{\parallel s} \cos 2\theta + C \sin 2\theta)} = \frac{1}{2} \left[1 - \frac{1}{\mathcal{K}} \frac{\sin 2\theta \cos 2\theta}{\sin 2\theta + 2\gamma \cos 2\theta} \right], \quad (\text{A.5})$$

4 where \mathcal{K} is defined in (29) and

$$\gamma := \frac{K_{\parallel s}}{\widehat{K}_{\parallel} - \widehat{K}_{\perp}}.$$

5 Then, the introduction of the parameter $K_{\parallel s}$, related to the mixed contribution of stretch
6 along the cell axis and shear, brings a new parameter γ into the equation for nontrivial equilibrium
7 orientations. As expected, for $\gamma = 0$ we recover the symmetric situation described in Section 3.2.

8 In order to make some theoretical considerations about stability and bifurcations, the first
9 thing to notice is that if $\gamma \neq 0$ the graph in the (θ, r) plane given by (A.5) presents asymptotes
10 when $\sin 2\theta + 2\gamma \cos 2\theta = 0$, namely if

$$\theta = -\frac{1}{2} \arctan 2\gamma + k\frac{\pi}{2}, \quad (\text{A.6})$$

11 and it has stationary points when $\tan^3 2\theta = 2\gamma$, i.e. whenever

$$\theta = \frac{1}{2} \arctan \sqrt[3]{2\gamma} + k\frac{\pi}{2}, \quad (\text{A.7})$$

12 achieving in them a value such that

$$\frac{1}{r+1} = \frac{1}{2} \left(1 \pm \frac{1}{\mathcal{K} \left(1 + \sqrt[3]{4\gamma^2} \right)^{3/2}} \right).$$

13 Now we discuss the stability of the equilibrium orientations obtained by (A.5). In this case,
14 recalling Eq. (27), the second derivative of the elastic energy can be written in general as

$$U''(\theta) = \varepsilon^2 \left\{ A\xi'(\theta)^2 + [A\xi(\theta) - B(r+1) + C]\xi''(\theta) + 4K_{\parallel s}(1-r^2) \sin \theta \cos \theta \right\}.$$

Our goal is to study the sign of this derivative when evaluated in the equilibrium angles: in particular, we can focus on the stability condition given by the inequality

$$A \sin^2 2\theta(1+r) - A(1+r) \cos^2 \theta \cos 2\theta + B(1+r) \cos 2\theta - C \cos 2\theta + 2K_{\parallel s}(1-r) \sin 2\theta > 0.$$

Substituting Eq. (A.5) and dividing by $\cos^2 2\theta$ leads to

$$\frac{1}{\mathcal{K}} \tan^2 2\theta - \frac{1}{\mathcal{K}} + \frac{1}{\mathcal{K}} \frac{\sin 2\theta}{\sin 2\theta + 2\gamma \cos 2\theta} (1 - 2\gamma \tan 2\theta) > 0 \quad (\text{A.8})$$

15 which is equivalent to

$$\frac{1}{\mathcal{K}} \frac{\tan^3 2\theta - 2\gamma}{\tan 2\theta + 2\gamma} > 0. \quad (\text{A.9})$$

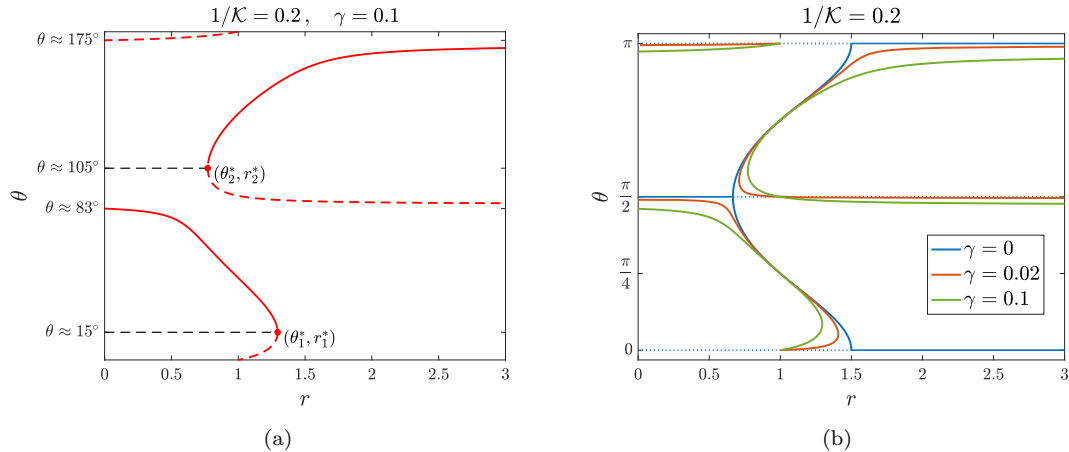


Figure 7: (a) Bifurcation diagram in the general case $K_{s\parallel} \neq 0$ for $1/\mathcal{K} = 0.2$, $\gamma = 0.1$. Differently from the symmetric case, here we have two turning points in (θ_1^*, r_1^*) and (θ_2^*, r_2^*) . (b) The introduction of γ induces a symmetry breaking in the system, switching from pitchfork to saddle-node bifurcations.

1 Therefore, if $\mathcal{K} > 0$, the stable configurations are those with

$$\theta \in \left[\frac{1}{2} \arctan \sqrt[3]{2\gamma}, \frac{\pi}{2} - \frac{1}{2} \arctan 2\gamma \right] \cup \left[\frac{\pi}{2} + \frac{1}{2} \arctan \sqrt[3]{2\gamma}, \pi - \frac{1}{2} \arctan 2\gamma \right]. \quad (\text{A.10})$$

2 Instead, if $\mathcal{K} < 0$, the angles corresponding to stable orientations for the cell are given by

$$\theta \in \left[0, \frac{1}{2} \arctan \sqrt[3]{2\gamma} \right] \cup \left[\frac{\pi}{2} - \frac{1}{2} \arctan 2\gamma, \frac{\pi}{2} + \frac{1}{2} \arctan \sqrt[3]{2\gamma} \right] \cup \left[\pi - \frac{1}{2} \arctan 2\gamma, \pi \right]. \quad (\text{A.11})$$

3 Then, putting together the information given by the second derivative and the equation of
4 the bifurcation curves, we can draw the bifurcation diagram of the system, shown in Figure 7(a)
5 for the case $\mathcal{K} > 0$. In particular, we observe that for $r < r_2^*$ there are two equilibria, one stable
6 and one unstable; however, when r crosses the critical value r_2^* , two new equilibria appear,
7 of which one is stable and another one is unstable. Finally, we have another visible bifurcation for
8 $r = r_1^*$, when the first two equilibria collide and annihilate each other. In order to give an idea
9 of some numerical values, we reported in the plot in Fig. 7(a) some notable values of θ : more
10 specifically, concerning the peculiar case we considered for \mathcal{K} and γ , i.e. $\gamma = 0.1$ and $\mathcal{K} = 5$, for
11 $r < r_2^*$ we have a stable orientation which is less than $\pi/2$. Variations of γ and \mathcal{K} in \mathbb{R}^+ do not
12 alter the qualitative behaviour of the system, but only the shape of the bifurcation diagram (see
13 green and red curve in Fig. 7(b)).

14 The main difference from the symmetric case treated in Section 3.2 lies in the type of bi-
15 furcations involved: here we have two *saddle-node bifurcations*. Then, the introduction of the
16 mixing parameter $K_{\parallel s}$ provokes the disappearance of the pitchfork bifurcations, while two *turn-*
17 *ing points* appear. The biggest consequence of this fact, which can be observed in Figure 7(b),
18 is that a *symmetry breaking* happens, leading for $\gamma \neq 0$ to equilibrium orientations that are not
19 symmetric. This is due to the fact that the introduction of the coefficient $K_{\parallel s}$ brings into the
20 energy a term proportional to $\sin \theta \cos \theta$, which is neither even nor symmetric with respect to $\pi/2$
21 as in the previous case. Consequently, unlike the symmetric case, one has $U(-\theta) \neq U(\theta)$ and

1 $U(\pi - \theta) \neq U(\theta)$, but $U(\pi - \theta) = U(-\theta)$. However, this situation is not biologically meaningful,
2 because there is no reason why one of the two orientations corresponding to $-\theta$ or θ should be
3 energetically preferable for the cell with respect to the other, unless one can envisage an internal
4 (left-right) bias in the cell itself. Therefore, we can conclude that, from the biological point of
5 view, in the problem at hand the assumption $K_{\parallel s} = 0$ made in the paper is justified, since we
6 expect to have symmetries in the system which would be broken if this coefficient is not null.

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13 Declaration of Competing Interests

14 The authors declare that they have no known competing financial interests or personal relation-
15 ships that could have appeared to influence the work reported in this paper.

16 References

- 17 [1] S. Asano, S. Ito, M. Morosawa, K. Furuya, K. Naruse, M. Sokabe, E. Yamaguchi, Y.
18 Hasegawa. Cyclic stretch enhances reorientation and differentiation of 3-D culture model
19 of human airway smooth muscle, *Biochem. Biophys. Rep.* **16**, 32-38 (2018).
- 20 [2] G. Astarita, G. Marrucci. Principles of Non-Newtonian Fluid Mechanics, McGraw-Hill
21 (1974).
- 22 [3] I.B. Bischofs, U.S. Schwarz. Cell organization in soft media due to active mechanosensing,
23 *Proc. Nat. Acad. Sci. U.S.A.* **100**, 9274–9279 (2003).
- 24 [4] A.B.C. Buskermolen, H. Suresh, S.S. Shishvan, A. Vigliotti, A. DeSimone, N.A. Kurniawan,
25 C.V.C. Bouten, V.S. Deshpande. Entropic forces drive cellular contact guidance, *Biophys.*
26 *J.* **116**, 1994-2008 (2019).
- 27 [5] D.T. Butcher, T. Alliston, V.M. Weaver. A tense situation: forcing tumour progression,
28 *Nat. Rev. Cancer* **9**, 108-122 (2009).
- 29 [6] J.P. Butler, I.M.Tolić-Nørrelykke, B. Fabry, J.J. Fredberg. Traction fields, moments, and
30 strain energy that cells exert on their surroundings, *Am. J. Physiol.: Cell Physiol.* **282**,
31 C595-C605 (2002).
- 32 [7] W. Carver, E.C. Goldsmith. Regulation of tissue fibrosis by the biomechanical environment,
33 *Biomed Res. Int.*, 101979 (2013).
- 34 [8] M. Chaplain, L. Graziano, L. Preziosi. Mathematical modelling of the loss of tissue com-
35 pression responsiveness and its role in solid tumour development, *Math. Med. Biol.*, **23**,
36 197-229, (2006).

- 1 [9] B. Chen, R. Kemkemer, M. Deibler, J. Spatz, H. Gao. Cyclic stretch induces cell reorien-
2 tation on substrates by destabilizing catch bonds in focal adhesions, *PLoS ONE* **7**, e48346
3 (2012).
- 4 [10] K. Chen, A. Vigliotti, M. Bacca, R.M. McMeeking, V.S. Deshpande, J.W. Holmes. Role of
5 boundary conditions in determining cell alignment in response to stretch *Proc. Nat. Acad.
6 Sci. U.S.A.* **115**, 986-991 (2018).
- 7 [11] Y. Chen, A.M. Pasapera, A. P. Koretsky, C.M. Waterman, Orientation-specific responses
8 to sustained uniaxial stretching in focal adhesion growth and turnover. *Proc. Nat. Acad. Sci
9 U.S.A.* **110**, E2352-E2361 (2013).
- 10 [12] J. Ciambella, P. Nardinocchi. Torque-induced reorientation in active fibre-reinforced mate-
11 rials, *Soft Matter* **15**, 2081–2091 (2019).
- 12 [13] J. Ciambella, P. Nardinocchi. A structurally frame-indifferent model for anisotropic visco-
13 hyperelastic materials, *J. Mech. Phys. Solids* **147**, 104247 (2021).
- 14 [14] G. Civelekoglu-Scholey, A. Wayne Orr, I. Novak, J.-J. Meister, M.A. Schwartz, A. Mogilner.
15 Model of coupled transient changes of Rac, Rho, adhesions and stress fibers alignment in
16 endothelial cells responding to shear stress, *J. Theor. Biol.* **232**, 569–585 (2005).
- 17 [15] A.M. Collinworth, C.E. Torgan, S.N. Nagda, R.J. Rajalingam, W.E. Kraus, G.A. Truskey.
18 Orientation and length of mammalian skeletal myocytes in response to a unidirectional
19 stretch, *Cell Tissue Res.* **302**, 243–251 (2000).
- 20 [16] P. Costa, F.V.M. Almeida, J. T. Connelly. Biophysical signals controlling cell fate decisions:
21 How do stem cells really feel? *Int. J. Biochem. Cell Biol.* **44**, 2233-2237 (2012).
- 22 [17] R. De. A general model of focal adhesion reorientation dynamics in response to static and
23 cyclic stretch, *Commun. Biol.* **1**, 81 (2018).
- 24 [18] R. De, A. Zemel, S.A. Safran. Dynamics of cell orientation, *Nat. Phys.* **3**, 655–659 (2007).
- 25 [19] R. De, A. Zemel, S.A. Safran. Do cells sense stress or strain? Measurement of cellular
26 orientation can provide a clue, *Biophys. J.* **94**, L29–L31 (2008).
- 27 [20] V. Deshpande, A. DeSimone, R. McMeeking, P. Recho. Chemo-mechanical model of a cell
28 as a stochastic active gel, *J. Mech. Phys. Solids* **151**, 104381 (2021).
- 29 [21] D.E. Discher, D.J. Mooney, P.W. Zandstra. Growth factors, matrices, and forces combine
30 and control stem cells, *Science* **324**, 1673–1677 (2009).
- 31 [22] M. Eastwood, V.C. Mudera, D.A. McGrouther and R.A. Brown. Effect of precise mechan-
32 ical loading on fibroblast populated collagen lattices: Morphological changes, *Cell. Motil.
33 Cytoskeleton* **40**, 13-21 (1998).
- 34 [23] U. Faust, N. Hamp, W. Rubner, N. Kirchgebner, S. Safran, B. Hoffmann, R. Merkel. Cyclic
35 stress at mHz frequencies aligns fibroblasts in direction of zero strain, *PLoS ONE* **6**, e28963
36 (2011).
- 37 [24] E.D. Goley, M.D. Welch. The ARP 2/3 complex: an actin nucleator comes of age, *Nat. Rev.
38 Mol. Cell Biol.* **7**, 713–726 (2006).

- 1 [25] Z. Goli-Malekabadi, M. Tafazzoli-Shadpour, M. Rabbani, M. Janmaleki. Effect of uniax-
2 ial stretch on morphology and cytoskeleton of human mesenchymal stem cells: Static vs.
3 dynamic loading, *Biomed. Tech.* **56**, 259-265 (2011).
- 4 [26] F. Guilak, D.M. Cohen, B.T. Estes, J.M. Gimble, W. Liedtke, C.S. Chen. Control of stem
5 cell fate by physical interactions with the extracellular matrix, *Cell Stem Cell* **5**, 17–26 (2009).
- 6 [27] K. Hayakawa, N. Sato, T. Obinata. Dynamic reorientation of cultured cells and stress fibers
7 under mechanical stress from periodic stretching, *Exp. Cell Res.* **268**, 104–114 (2001).
- 8 [28] H.-J. Hsu, C.-F. Lee, R. Kaunas. A dynamic stochastic model of frequency-dependent stress
9 fiber alignment induced by cyclic stretch, *PLoS ONE* **4(3)**, e4853 (2009).
- 10 [29] D. Ingber. Mechanobiology and diseases of mechanotransduction, *Annals Medicine* **35**, 1–14
11 (2009).
- 12 [30] S. Jungbauer, H. Gao, J.P. Spatz, R. Kemkemer. Two characteristic regimes in frequency-
13 dependent dynamic reorientation of fibroblasts on cyclically stretched substrates, *Biophys.*
14 *J.* **95**, 3470–3478 (2008).
- 15 [31] R. Kalluri, R.A. Weinberg. The basics of epithelial-mesenchymal transition, *J. Clin. Invest.*
16 **119**, 1420-1428 (2009).
- 17 [32] J. Kass, M. Erler, M. Dembo, V.M. Weaver. Mammary epithelial cell: Influence of ECM
18 composition and organization during development and tumorigenesis, *Int. J. Biochem. Cell*
19 *Biol.* **39**, 1987–1994 (2007).
- 20 [33] B.-S. Kim, J. Nikolovski, J. Bonadio, D.J. Mooney. Cyclic mechanical strain regulates the
21 development of engineered smooth muscle tissue, *Nat. Biotechnol.* **17**, 979–983 (1999).
- 22 [34] F. Kong, A.J. García, A.P. Mould, M.J. Humphries, C. Zhu, Demonstration of catch bonds
23 between an integrin and its ligand. *J. Cell Biol.* **185**, 1275-1284 (2009)
- 24 [35] D. Kong, B. Ji, L. Dai. Stability of adhesion clusters and cell reorientation under lateral
25 cyclic tension, *Biophys. J.* **95**, 4034–4044 (2008).
- 26 [36] S. Kumar, V.M. Weaver. Mechanics, malignancy, and metastasis: the force journey of a
27 tumor cell, *Cancer Metastasis Rev.* **28**, 113–127 (2009).
- 28 [37] Z. Li, H. Lee, C. Zhu. Molecular mechanisms of mechanotransduction in integrin-mediated
29 cell-matrix adhesion *Exp. Cell Res.* **349**, 85–94 (2016).
- 30 [38] B. Liu, M.-J. Qu, K.-R. Qin, H. Li, Z.-K. Li, B.-R. Shen, Z.-R. Jiang. Role of cyclic strain
31 frequency in regulating the alignment of vascular smooth muscle cells in vitro, *Biophys. J.*
32 **94**, 1497-1507 (2008).
- 33 [39] C. Liu, S. Baek, J. Kim, E. Vasko, R. Pyne, C. Chan. Effect of static pre-stretch induced
34 surface anisotropy on orientation of mesenchymal stem cells, *Cell Mol. Bioeng.* **7**, 106-121
35 (2014).
- 36 [40] A. Livne, E. Bouchbinder, B. Geiger. Cell reorientation under cyclic stretching, *Nat. Comm.*
37 **5**, 3938 (2014).
- 38 [41] L. Lu, S.J. Oswald, H. Ngu, F. C.-P. Yin. Mechanical properties of actin stress fibers in
39 living cells, *Biophys. J.* **95**, 6060–6071 (2008).

- 1 [42] G. Lucci, L. Preziosi. A nonlinear elastic description of cell preferential orientations over a
2 stretched substrate, *Biomech. Model. Mechanobiol.* **20**, 631–649 (2021).
- 3 [43] T. Mao, Y. He, Y. Gu, Y. Yang, Y. Yu, X. Wang, J. Ding. Critical Frequency and Critical
4 Stretching Rate for Reorientation of Cells on a Cyclically Stretched Polymer in a Microfluidic
5 Chip, *ACS Appl. Mater. Interfaces* **13**, 13934–13948 (2021).
- 6 [44] A.B. Mathur, W.M. Reichert, G.A. Truskey. Flow and high affinity binding affect the elastic
7 modulus of the nucleus, cell body and the stress fibers of endothelial cells, *Ann. Biomed.*
8 *Eng.* **35**, 1120–1130 (2007).
- 9 [45] M. Moretti, A. Prina-Mello, A.J. Reid, V. Barron, P. J. Prendergast. Endothelial cell align-
10 ment on cyclically-stretched silicone surfaces, *J. Mater. Sci.: Mater. in Med.* **15**, 1159–1164
11 (2004).
- 12 [46] M. Morioka, H. Parameswaran, K. Naruse, M. Kondo, M. Sokabe, Y. Hasegawa, B. Suki, S.
13 Ito. Microtubule dynamics regulate cyclic stretch-induced cell alignment in human airway
14 smooth muscle cells, *PLoS ONE* **6**, e26384 (2011).
- 15 [47] C. Neidlinger-Wilke, E.S. Grood, J.H.-C. Wang, R.A. Brand, L. Claes. Cell alignment is
16 induced by cyclic changes in cell length: studies of cells grown in cyclically stretched sub-
17 strates, *J. Orthop. Res.* **19**, 286–293 (2001).
- 18 [48] C. Neidlinger-Wilke, E. Grood, L. Claes, R. Brand. Fibroblast orientation to stretch begins
19 within three hours, *J. Orthop. Res.* **20**, 953–956 (2002).
- 20 [49] R.W. Ogden. Nonlinear elasticity, anisotropy, material stability and residual stresses in soft
21 tissue, in **Biomechanics of Soft Tissue in Cardiovascular Systems**, 65–108, G.A.
22 Holzapfel and R.W. Ogden, Eds., Springer (2003).
- 23 [50] A. Palamidessi, E. Frittoli, N. Ducano, N. Offenhauser, S. Sigismund, H. Kajihio, D. Paraz-
24 zoli, A. Oldani, M. Gobbi, G. Serini, P.P. Di Fiore, G. Scita, L. Lanzetti. The GTPase-
25 activating protein RN-3 controls focal adhesion turnover and cell migration, *Current Biol.*
26 **23**, 2355–2364 (2013).
- 27 [51] A.M. Pasapera, I.C. Schneider, E. Rericha, D.D. Schlaepfer, C.M. Waterman. Myosin II
28 activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin
29 phosphorylation, *J. Cell Biol.* **188**, 877–890 (2010).
- 30 [52] H.E. Pettermann, A. DeSimone. An anisotropic linear thermo-viscoelastic constitutive law,
31 *Mech. Time-Depend. Mater.* **22**, 421–433 (2018).
- 32 [53] H.E. Pettermann, C. Cheyrou, A. DeSimone. Modeling and simulation of anisotropic linear
33 viscoelasticity, *Mech. Time-Depend. Mater.* (2020).
- 34 [54] L. Preziosi, G. Vitale. A multiphase model of tumour and tissue growth including cell
35 adhesion and plastic re-organisation, *Math. Models Methods Appl. Sci.* **21**, 1901–1932 (2011).
- 36 [55] J. Qian, H. Liu, Y. Lin, W. Chen, H. Gao. A mechanochemical model of cell reorientation
37 on substrates under cyclic stretch, *PLoS ONE* **8**, e65864 (2013).
- 38 [56] A. Roshanzadeh, T.T. Nguyen, K.D. Nguyen, D.-S. Kim, B.-K. Lee, D.-W. Lee, E.-S.
39 Kim. Mechanoadaptive organization of stress fiber subtypes in epithelial cells under cyclic
40 stretches and stretch release, *Sci. Rep.* **10**, 18684 (2020).

- 1 [57] I. Rouiller, X.-P. Xu, K.J. Amann, C. Egile, S. Nickell, D. Nicastro, R. Li, T.D. Pollard,
2 N. Volkman, D. Hanein. The structural basis of actin filament branching by the Arp2/3
3 complex, *J. Cell Biol.* **180**, 887–895 (2008).
- 4 [58] L.E. Scott, S.H. Weinberg, C.A. Lemmon. Mechanochemical Signaling of the Extracellular
5 Matrix in Epithelial-Mesenchymal Transition, *Front. Cell Dev. Biol.* **7**, 135 (2019).
- 6 [59] Y. Sun, C.S. Chen, J. Fu. Forcing stem cells to behave: A biophysical perspective of cellular
7 microenvironment, *Annu. Rev. Biophys.* **41**, 519–542 (2012).
- 8 [60] L.A. Taber. Biophysical mechanisms of cardiac looping, *Int. J. Dev. Biol.* **50**, 323–332 (2006).
- 9 [61] A. Tondon, R. Kaunas. The direction of stretch-induced cell and stress fiber orientation
10 depends on collagen matrix stress, *PLoS ONE* **9**, e89592 (2014).
- 11 [62] A. Tondon, H.J. Su, R. Kaunas. Dependence of cyclic stretch-induced stress fiber reorien-
12 tation on stretch waveform, *J. Biomech.* **45**, 728–735 (2012).
- 13 [63] L. Tuan, M.J. Fairchild, J. Michael, A.D., Perkins, G. Tanentzapf. Analysis of integrin
14 turnover in fly myotendinous junctions, *J. Cell Sci.* **123**, 939–946, (2010).
- 15 [64] N.L. Tulloch, V. Muskheli, M.V. Razumova, F. Steven Korte, M. Regnier, K.D. Hauch, L.
16 Pabon, H. Reinecke, C.E. Murry. Growth of engineered human myocardium with mechanical
17 loading and vascular coculture, *Circ. Res.* **109**, 47–49 (2011).
- 18 [65] A.-M. Vlaikou, D. Kouroupis, A. Sgourou, G.S. Markopoulos, E. Bagli, M. Markou, Z.
19 Papadopoulou, T. Fotsis, G. Nakos, M.-E. E. Lekka, M. Syrrou. Mechanical stress af-
20 fects methylation pattern of *GNAS* isoforms and osteogenic differentiation of hAT-MSCs,
21 *Biochim. Biophys. Acta-Mol. Cell Res.* **1864**, 1371–1381 (2017).
- 22 [66] J. H.-C. Wang. Substrate deformation determines actin cytoskeleton reorganization: A
23 mathematical modeling and experimental study, *J. Theor. Biol.* **202**, 33–41 (2000).
- 24 [67] J. H.-C. Wang, P. Goldschmidt-Clermont, J. Wille, F. C.-P. Yin. Specificity of endothelial
25 cell reorientation in response to cyclic mechanical stretching, *J. Biomech.* **34**, 1563–1572
26 (2001).
- 27 [68] H. Wang, W. Ip, R. Bossy, E.S. Grood. Cell orientation response to cyclically deformed
28 substrates: Experimental validation of a cell model, *J. Biomech.* **28**, 1543–1552 (1995).
- 29 [69] S. Wang, D. Lu, Z. Zhang, X. Jia, L. Yang. Effects of mechanical stretching on the morphol-
30 ogy of extracellular polymers and the mRNA expression of collagens and small leucine-rich
31 repeat proteoglycans in vaginal fibroblasts from women with pelvic organ prolapse, *PLoS*
32 *ONE* **13**, e0193456 (2018).
- 33 [70] Z. Wei, V.S. Deshpande, R.M. McMeeking, A.G. Evans. Analysis and interpretation of
34 stress fiber organization in cells subject to cyclic stretch, *J. Biomech. Eng.-Trans. ASME*
35 **130**, 031009-1 – 031009-9 (2008).
- 36 [71] G. Xu, B. Li, X. Feng, H. Gao. A tensegrity model of cell reorientation on cyclically stretched
37 substrates, *Biophys. J.* **111**, 1478–1486 (2016).
- 38 [72] F.J. Vernerey, U. Akalp. Role of catch bonds in actomyosin mechanics and cell mechanosen-
39 sitivity *Phys. Rev. E* **94**, 012403 (2016)

- 1 [73] Y. Yan, S. Sart, Y. Li. Differentiation of neural progenitor cells from pluripotent stem cells
2 in artificial niches, *Int. J. Stem Cell Res. Transpl.* **1**, 22–27 (2013).
- 3 [74] J.-K. Yoon, T. Lee, S.H. Bhang, J.-Y. Shin, J.-M. Myoung, B.-S. Kim. Stretchable piezo-
4 electric substrate providing pulsatile mechanoelectric cues for cardiomyogenic differentiation
5 of mesenchymal stem cells, *ACS Appl. Mater. Interfaces* **9**, 22101–22111 (2017).
- 6 [75] C. Zhu. Mechanochemistry: A molecular biomechanics view of mechanosensing, *Ann.*
7 *Biomed. Engng.* **42**, 388-404 (2014).
- 8 [76] C. Zhu, Y. Chen, L.A. Ju. Dynamic bonds and their roles in mechanosensing, *Curr. Opin.*
9 *Chem. Biol.* **53**, 88-97 (2019).