

# Free Energy Analysis of Cell Spreading

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## ABSTRACT

In this study we present a steady-state adaptation of the thermodynamically motivated stress fiber (SF) model of Vigliotti *et al.* (2015). We implement this steady-state formulation in a non-local finite element setting where we also consider global conservation of the total number of cytoskeletal proteins within the cell, global conservation of the number of binding integrins on the cell membrane, and adhesion limiting ligand density on the substrate surface. We present a number of simulations of cell spreading in which we consider a limited subset of the possible deformed spread-states assumed by the cell in order to examine the hypothesis that free energy minimization drives the process of cell spreading. Simulations suggest that cell spreading can be viewed as a competition between (i) decreasing cytoskeletal free energy due to strain induced assembly of cytoskeletal proteins into contractile SF, and (ii) increasing elastic free energy due to stretching of the mechanically passive components of the cell. The computed minimum free energy spread area is shown to be lower for a cell on a compliant substrate than on a rigid substrate. Furthermore, a low substrate ligand density is found to limit cell spreading. The predicted dependence of cell spread area on substrate stiffness and ligand density is in agreement with the experiments of Engler *et al.* (2003). We also simulate the experiments of Théry *et al.* (2006), whereby initially circular cells deform and adhere to “V-shaped” and “Y-shaped” ligand patches. Analysis of a number of different spread states reveals that deformed configurations with the lowest free energy exhibit a SF distribution that corresponds to experimental observations, i.e. a high concentration of highly aligned SFs occurs along free edges, with lower SF concentrations in the interior of the cell. In summary, the results of this study suggest that cell spreading is driven by free energy minimization based on a competition between decreasing cytoskeletal free energy and increasing passive elastic free energy.

## KEYWORDS

Cell Spreading, Thermodynamically Consistent Active Model, Cytoskeletal Free Energy, Cell Adhesion, Finite Element

## ABBREVIATIONS

Stress fiber – SF; Focal adhesion – FA; Representative volume element – RVE

## 1. INTRODUCTION

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Several experimental studies demonstrate that control of cell spreading using substrate micro-  
patterning has a significant impact on cell behavior. A study by McBeath *et al.* (2004) reveals  
that stem cell differentiation can be controlled by limiting cell spread area. It has also been  
shown that the contractility of smooth muscle cells increases with increasing cell area Tan *et*  
*al.* (2003). Lamers *et al.* (2010) show the spread geometry and stress fiber (SF) distribution of  
osteoblasts on grooved surfaces is highly dependent on groove spacing. Wide grooves result  
in polarized cells with SFs aligned along the grooves. Narrow groove spacing leads to  
randomly oriented cells and SFs. Finally, a study by Théry *et al.* (2006) has shown that when  
a cells spread on a “V-shaped” or “Y-shaped” ligand patch SFs align predominantly along the  
free edge of the cell and focal adhesions assemble along the perimeter of the ligand patch.

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The bio-chemo-mechanical model proposed by Deshpande, *et al.* (2006) was used by  
McGarry *et al.* (2009) to analyze the aforementioned micro-post experiments of Tan *et al.*  
Simulations reveal that as cells spread the increasing number of adhered posts provide  
increasing support for SF tension and therefore reduce SF dissociation. Simulations also  
correctly predict that SFs are highly aligned along the free edges of the cell where the stress  
state is uniaxial. Using the same framework Pathak *et al.* (2008) analyzed the experiments of  
Théry *et al.* and, similar to McGarry *et al.*, highly aligned stress fibers are predicted along  
the free edge of the cell. While these studies demonstrate the importance of tension support  
for stress fiber formation, they reveal a number of shortcomings of the phenomenological  
framework of Deshpande *et al.* (2006). Firstly, a high level of isotropic SF formation is  
incorrectly predicted to occur in regions of biaxial stress in the center of the cell. Experiments  
reveal that limited SF formation occurs in such regions. Secondly, the spread-state of the cell  
is assumed as the undeformed reference configuration. Clearly the cell deforms significantly  
from its spherical suspended state to reach the final spread-state.

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In the current study we attempt to address these shortcomings by developing a steady-state finite element implementation of the recent thermodynamically motivated stress fiber model of Vigliotti *et al.* (2015). Our simulations of cells on micro-patterned substrates incorporate the following significant improvements on previous approaches: (i) The spread-state of the cell is not assumed as the strain free reference configuration. Rather, the cell deforms from a suspended geometry to reach its final spread configuration. The strain state of the deformed configuration is a key determinant of SF distribution in the cell. (ii) The number of cytoskeletal proteins in the cell is a finite and conserved quantity, requiring the development of a non-local numerical implementation. In contrast, McGarry *et al.* and Pathak *et al.* do not impose a global limit on SF formation. (iii) In addition to the advances presented in terms of our SF finite element model, we also propose a further development of the thermodynamically motivated focal adhesion assembly model of Deshpande *et al.* (2008) so that focal adhesion formation may be limited by a prescribed ligand density on the substrate to which a cell adheres.

An important consequence of the modelling approach is that there is not a unique final spread-state for the cell. Even in experiments such as those of Théry *et al.* and Tan *et al.* where the outline of the final spread shape is prescribed by micro-patterning ligand patches on the substrate, there is still an infinite number of ways in which the cell can spread across the patch geometry. Each final spread-state would have a different strain distribution and resultant SF distribution. Despite the infinite ways in which a cell can spread, the experimental heat maps of SF distribution in the study of Théry *et al.* reveal a strong trend of SF formation along free edges for a large number of cells. This suggests that the final spread state of a cell is not randomly generated. **In this study we use our modelling framework to determine the free energy of the cell for a number of spread states and we hypothesize that cell spreading is driven by free energy minimization. Furthermore we ask if predicted SF and**

1 focal adhesion distributions for minimum free energy spread states are in agreement with  
2 experimentally observed distributions.  
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5 This paper is structured as follows: In Section 2 we present our steady-state non-local stress  
6 fiber formation and cell spreading framework, followed by our model for ligand dependent  
7 focal adhesion assembly. We also introduce the factors contributing to the cell free energy. In  
8 Section 3 we consider a simplified example of axisymmetric spreading of a round cell on a  
9 flat substrate in order to demonstrate the key features of the computational framework and  
10 predict experimental trends observed by Engler *et al.* (2003). Finally, in Section 4 we  
11 simulate the experiments of Théry *et al.* by analyzing a number of spread-states for cells  
12 adhered to “V-shaped” and “Y-shaped” ligand patches.  
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## 27 **2. MODELLING FRAMEWORK**

### 28 **2.1. Framework for Stress Fiber Remodeling and Contractility**

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31 The cytoskeleton is composed of actin-myosin stress fibers (SFs), which actively generate  
32 tension through cross-bridge cycling between the actin and myosin filaments. The  
33 thermodynamically consistent model from Vigliotti *et al.* (2015) captures key features of SF  
34 dynamics, including (i) The kinetics of stress fiber formation and dissociation as motivated  
35 by thermodynamic considerations, (ii) the stress, strain, and strain-rate dependence of SF  
36 remodeling, and (iii) global conservation of the cytoskeletal proteins. Here we implement a  
37 steady-state form of this continuum model in a two-dimensional finite element setting.  
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51 We envisage a two-dimensional (2D) cell of thickness  $b$  lying in the  $x_1 - x_2$  plane (Figure  
52 1a). A representative volume element (RVE) in the undeformed state is defined as a disk of  
53 radius  $n^R l_0/2$ . Stress fibers emanate from the center of this disk, each comprised of  $n^R$   
54 functional units (of length  $l_0$ ) in their initial ground state. In 2D plane stress SFs can form in  
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1 a large number of directions, with each direction defined by an angle  $\phi$  with respect to the  
 2  $x_1$ -axis. At steady state, we consider that the (normalized) number of actin-myosin contractile  
 3 units within a SF in direction  $\phi$  in the RVE is given by:  
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$$5 \hat{n}(\phi) = \frac{n(\phi)}{n^R} = (1 + \varepsilon_n(\phi)) / (1 + \tilde{\varepsilon}_{ss}) \quad \#(1)$$

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 8 where  $\varepsilon_n(\phi)$  is the nominal strain in the direction  $\phi$ . When a SF is extended, contractile units  
 9 are added, with the effect that the internal strain in the SF is reduced until a steady state value  
 10  $\tilde{\varepsilon}_{ss}$  is achieved (Figure 1b).  $\tilde{\varepsilon}_{ss}$  is given by the positive root of the relation:  
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$$13 (p - 1)\tilde{\varepsilon}_{ss}^p + p\tilde{\varepsilon}_{ss}^{p-1} - \frac{1}{\beta} = 0 \quad \#(2)$$

14 where  $\beta$  and  $p$  are non-dimensional constants that govern the internal energy  $\psi$  of  $n^R$   
 15 functional units within a SF. Conversely, when a SF shortens, functional units are removed.  
 16 In both cases, the internal fiber steady state strain  $\tilde{\varepsilon}_{ss}$  is fixed and in general different from  
 17 the axial material strain in the direction of the fiber,  $\varepsilon_n(\phi)$ .  
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### 20 2.1.1. Mass Conservation of Cytoskeletal Proteins

21 We assume spreading takes place during the interphase period of the cell cycle when the cell  
 22 is in a homeostatic state (i.e. the concentration of all proteins within the cell is constant)  
 23 (Weiss, 1996). Therefore, in the finite element framework developed in this study a global  
 24 conservation of the total number of SF proteins  $N_0$  within the entire cell is enforced.  
 25 Cytoskeletal proteins are considered to exist in two states: a bound state and an unbound  
 26 state. The bound proteins make up the functional units of the stress fibers within the RVE  
 27 and thus are not mobile. The unbound proteins are mobile and can diffuse throughout the cell  
 28 cytoplasm. The global conservation of cytoskeletal proteins may be expressed as  
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$$31 N_0 = N_u^{tot} + N_b^{tot} \quad \#(3)$$

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where  $N_u^{tot}$  and  $N_b^{tot}$  are the total numbers of unbound and bound cytoskeletal proteins in the entire cell. We next introduce the local normalized quantities:  $\hat{N}_u = N_u/N_0$ ,  $\hat{N}_b = N_b/N_0$ , and  $\hat{N}_t = N_t/N_0$ , where  $N_u$  and  $N_b$  are the local number of unbound and bound proteins within a given RVE, and the total number of proteins  $N_t$  locally in the RVE is obtained from

$$\hat{N}_t = \hat{N}_u + \hat{N}_b \quad \#(4)$$

Recall that the unbound proteins are mobile. Cytoskeletal proteins can diffuse through the cytoplasm at a rate of  $1.5 \mu\text{m/s}$  (McGrath et al., 1998) which is considered fast relative to the timescales of SF remodeling (Several studies report remodeling takes place over the course of hours (Kaunas et al., 2005; Wang et al., 2001)). Therefore it is reasonable to assume that for time-scales over which SFs remodel the total number of unbound proteins in the entire cell,  $N_u^{tot}$ , is uniformly distributed across all RVEs, i.e.  $\hat{N}_u$  is the same in all RVEs. Bound proteins, on the other hand, are not uniformly distributed throughout the cell, and  $\hat{N}_b$  in a given RVE must be computed from:

$$\hat{N}_b = \int_{-\pi/2}^{+\pi/2} \hat{\eta}(\phi) \hat{n}(\phi) d\phi \quad \#(5)$$

where  $\eta(\phi)$  is the angular SF concentration per unit surface area of the RVE, with  $\hat{\eta}(\phi) = \eta(\phi)n^R/N_0$ . The global conservation condition (Equation 3) can therefore be expressed as:

$$\hat{N}_u = 1 - \frac{1}{V_c} \int_{V_c} \hat{N}_b dV \quad \#(6)$$

where  $V_c$  is the total cell volume. In a numerical implementation, the global integral across the cell volume  $V_c$  in Equation 6 requires a non-local summation of  $\hat{N}_b$  across all integration points in the cell, as described in Section 2.1.5.

### 2.1.2. SF Angular Concentration and Active Stress Tensor

We next consider the kinetic equation for SF formation and dissociation proposed by Vigliotti *et al.*:

$$\dot{\hat{n}}(\phi) = \frac{\hat{N}_u}{\pi \hat{n}(\phi)} \omega_n \exp\left[-\hat{n}(\phi) \frac{\mu_a - \mu_u}{kT}\right] - \hat{n}(\phi) \omega_n \exp\left[-\hat{n}(\phi) \frac{\mu_a - \mu_b(\phi)}{kT}\right] \#(7)$$

The first term on the right is the forward reaction rate for the formation of SFs, where  $\omega_n$  is the molecular collision frequency of the SF proteins,  $k$  is the Boltzmann constant,  $T$  the absolute temperature, and  $\mu_a$  is the activation enthalpy that must be surpassed for  $n^R$  proteins to form a SF. Here  $\mu_u$  is the standard enthalpy of  $n^R$  unbound SF proteins, with  $\mu_u = \mu_{u0} + \Delta\mu_{u0}C$ . The unbound proteins are affected by an activation signal  $C$  and form more readily into their bound states as the signal (e.g. concentration of unfolded ROCK) increases, with  $\mu_{u0}$  is the standard enthalpy of the unbound SF proteins in the absence of a signal ( $C = 0$ ) and  $\Delta\mu_{u0}$  the increase in the enthalpy of the unbound molecules at full signal activation ( $C = 1$ ). At steady state we assume a continuous fully activated signal, i.e.  $C = 1$ . The second term on the right is the backward reaction rate for SF dissociation, with  $\mu_b$  the standard enthalpy of  $n^R$  bound SF proteins, given as:

$$\mu_b \equiv \psi - \sigma_f(\phi)[1 + \varepsilon_n(\phi)]\Omega \#(8)$$

where  $\Omega$  is the volume of  $n^R$  functional units in a SF in an undeformed RVE, and  $\psi$  is the internal energy of  $n^R$  functional units within a SF, given by:

$$\psi \equiv \mu_{b0} + \beta \mu_{b0} |\tilde{\varepsilon}_{ss}|^p \#(9)$$

where  $\mu_{b0}$  is the internal energy of  $n^R$  functional units within a SF in their ground state, and  $\sigma_f(\phi)$  is the tensile stress actively generated by a SF. **In this paper we develop a steady state solution, hence Hill tension-velocity relationship does not need to be considered as  $\sigma_f(\phi)$  is**

necessarily equal to the maximum isometric tension  $\sigma_{max}$ . Here we consider steady state

conditions so that  $\dot{\hat{\eta}}(\phi) = 0$ , therefore Equation 7 reduces to:

$$\hat{\eta}(\phi) = \frac{\hat{N}_u}{\pi \hat{n}(\phi)} \frac{\exp\left[-\hat{n}(\phi) \frac{\mu_a - \mu_u}{kT}\right]}{\exp\left[-\hat{n}(\phi) \frac{\mu_a - \mu_b(\phi)}{kT}\right]} \#(10)$$

and the normalized SF concentration in direction  $\phi$  is given as:

$$\hat{\eta}(\phi) = \frac{\hat{N}_u}{\pi \hat{n}(\phi)} \exp\left[-\hat{n}(\phi) \frac{\mu_b(\phi) - \mu_u}{kT}\right] \#(11)$$

or from Equation 5:

$$\hat{\eta}(\phi) = \frac{\hat{N}_t - \int_{-\pi/2}^{+\pi/2} \hat{\eta}(\phi) \hat{n}(\phi) d\phi}{\pi \hat{n}(\phi)} B(\phi) \#(12)$$

where  $B(\phi) = \exp[-\hat{n}(\phi)(\mu_b(\phi) - \mu_u)/kT]$ ,  $\hat{N}_t$  is the total number of cytoskeletal proteins locally in an RVE, and the integral provides the total number of bound proteins in the RVE.

Finally the 2D active stress tensor follows as:

$$\boldsymbol{\sigma}_{act} = \frac{\sigma_{max} f_0}{J} \int_{-\pi/2}^{\pi/2} \left( \hat{\eta}(\phi) [1 + \varepsilon_n(\phi)] \begin{bmatrix} \cos^2 \phi & \frac{\sin 2\phi}{2} \\ \frac{\sin 2\phi}{2} & \sin^2 \phi \end{bmatrix} \right) d\phi \#(13)$$

where  $f_0$  is the volume fraction of cytoskeletal proteins in the cell, and  $J$  is the determinant of the deformation gradient  $\mathbf{F}$ .

### 2.1.3. Cytoskeletal Free Energy

The cytoskeletal free energy ( $g_{cyto}$ ) is given as follows:

$$g_{cyto} = N_u^{tot} \chi_u + \int_{V_c} N_b \chi_b dV \#(14)$$

Where  $\chi_u$  is the chemical potential of the unbound proteins that form a single SF functional unit, and  $\chi_b$  is the chemical potential of a functional unit with a SF. From Equation 5:

$$g_{cyto} = N_u^{tot} \chi_u + \int_{V_c} \left( \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \eta(\phi) n(\phi) d\phi \right) \chi_b dV \#(15)$$

As previously mentioned we assume infinitely fast diffusion of SF proteins and therefore a homogeneous distribution throughout the cell. Also Equation 10 implies thermodynamic equilibrium with  $\chi_u = \chi_b$  which then simplifies Equation 15 to:

$$g_{cyto} = N_u^{tot} \chi_u + \chi_u \int_{V_c} \left( \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \eta(\phi) n(\phi) d\phi \right) dV \#(16)$$

Here the double integral represents the total number of bound proteins in all RVEs in the cell.

Therefore:

$$g_{cyto} = N_u^{tot} \chi_u + (N_0 - N_u^{tot}) \chi_u = N_0 \chi_u \#(17)$$

The chemical potential of the unbound proteins is  $\chi_u = \mu_u + kT \ln(\hat{N}_u)$  and thus:

$$g_{cyto} = N_0 (\mu_u + kT \ln(\hat{N}_u)) \#(18)$$

with the cytoskeletal energy per unit volume of the cell then given as

$$\bar{G}_{cyto} = \rho (\mu_u + kT \ln(\hat{N}_u)) \#(19)$$

where  $\rho \equiv N_0/V_c$  is the concentration of cytoskeletal proteins.

#### 2.1.4. Passive Elasticity

The formulation is completed by the addition of a non-linear hyperelastic Ogden model (Ogden, 1972) in parallel with the SF model in order to represent the strain stiffening of the

mechanically passive cell components. As we consider the cell volume to remain constant during the analysis, the incompressible formulation is implemented:

$$\boldsymbol{\sigma}_{pas} = \sum_{i=1,2} \frac{2\mu}{\alpha} (\lambda_i^\alpha - (\lambda_1\lambda_2)^{-\alpha}) (\mathbf{m}_i \otimes \mathbf{m}_i) \quad \#(20)$$

where  $\mu$  is the material shear modulus,  $\lambda_{1,2}$  are the principal stretches,  $\mathbf{m}_{1,2}$  are the principal stretch directions, and  $\alpha$  is a material constant. Here the passive elastic free energy per unit volume ( $\bar{G}_{elas}$ ) is given by the Ogden strain energy density function:

$$\bar{G}_{elas} = \frac{2\mu}{\alpha^2} (\lambda_1^\alpha + \lambda_2^\alpha - (\lambda_1\lambda_2)^{-\alpha} - 3) \quad \#(21)$$

The total Cauchy stress tensor at an integration point is obtained by summation of the passive and active contributions:

$$\boldsymbol{\sigma}_{Cauchy} = \boldsymbol{\sigma}_{pas} + \boldsymbol{\sigma}_{act} \quad \#(22)$$

### 2.1.5. Numerical Implementation

In our numerical implementation we consider SF formation in a large number of discrete directions  $M$  ( $M=36$  is found to provide a converged solution) in the 2D plane of each RVE in the cell. Equation 5 is approximated as

$$\hat{N}_b = \frac{\pi}{M} \sum_{i=1}^M \hat{\eta}_i \hat{\eta}_i \quad \#(23)$$

where  $\hat{\eta}(\phi_i) \hat{\eta}(\phi_i)$  is written in shorthand as  $\hat{\eta}_i \hat{\eta}_i$ . Equation 12 is therefore approximated as

$$\hat{\eta}_j \hat{\eta}_j \pi = B_j \hat{N}_t - B_j \left( \frac{\pi}{M} \sum_{i=1}^M \hat{\eta}_i \hat{\eta}_i \right), \quad j = 1, M \quad \#(24)$$

Rearranging, we obtain

$$\hat{\eta}_j \hat{\eta}_j \left( \frac{1}{B_j} \right) - \left( \frac{1}{M} \sum_{i=1}^M \hat{\eta}_i \hat{\eta}_i \right) = \frac{\hat{N}_t}{\pi}, \quad j = 1, M \quad (25)$$

or, in matrix form:

$$\begin{bmatrix} \left( \frac{1}{B_1} + \frac{1}{M} \right) \hat{\eta}_1 & \frac{1}{M} \hat{\eta}_2 & \dots & \frac{1}{M} \hat{\eta}_M \\ \frac{1}{M} \hat{\eta}_2 & \left( \frac{1}{B_2} + \frac{1}{M} \right) \hat{\eta}_2 & \dots & \frac{1}{M} \hat{\eta}_M \\ \dots & \dots & \dots & \dots \\ \frac{1}{M} \hat{\eta}_M & \frac{1}{M} \hat{\eta}_M & \dots & \left( \frac{1}{B_M} + \frac{1}{M} \right) \hat{\eta}_M \end{bmatrix} \begin{Bmatrix} \hat{\eta}_1 \\ \hat{\eta}_2 \\ \dots \\ \hat{\eta}_M \end{Bmatrix} = \frac{\hat{N}_t}{\pi} \begin{Bmatrix} 1 \\ 1 \\ \dots \\ 1 \end{Bmatrix} \quad (26)$$

A solution for  $\hat{\eta}_j$  ( $j=1, M$ ) is obtained by matrix inversion. This steady-state model for SF formation and contractility is implemented via a *user-defined material* (UMAT) subroutine in the commercial finite element (FE) software package Abaqus. Prescribed boundary conditions are applied to the cell at the start of an analysis step, and contact conditions (see Section 2.2) with a substrate are enforced at cell nodes where appropriate. The solution is progressed through the analysis step, with each increment representing an iteration towards the final steady state solution. **At each integration point the axial material nominal strains  $\varepsilon_n(\phi)$  in each of the  $M$  stress fiber directions are determined from the material log strain tensor (STRAN), and number of functional units  $\hat{n}(\phi)$  in each of the  $M$  directions is obtained from Equation 1. In the first increment of the analysis step it is assumed that all cytoskeletal proteins are unbound and uniformly distributed across all integration points in the cell mesh so that  $\hat{N}_t = \hat{N}_u$ . The solution for  $\hat{\eta}(\phi)$  in  $M$  directions is obtained by inversion of the matrix on the left of Equation 26. The local Cauchy stress tensor  $\sigma$  is computed from Equations 13, 20, 22 and **the consistent tangent matrix  $\partial \Delta \sigma / \partial \Delta \varepsilon$  is approximated numerically based on a forward difference perturbation of the deformation gradient matrix (Sun *et al.* 2008), (Nolan *et al.*, 2014), (Reynolds and McGarry, 2015)). At each integration point the local number of bound proteins  $\hat{N}_b$  is calculated at the end of the increment, as per Equation 23. At the end of****

an increment  $i$ , the total number of bound cytoskeletal proteins throughout the entire cell is  
 computed through volume averaged summation of  $\widehat{N}_b|^i$  across every integration point in the  
 mesh in a *user-defined external database* (UEXTERNALDB) file, as outlined in Figure 2. In  
 the subsequent increment the remaining available unbound proteins are redistributed so that a  
 homogeneous distribution of  $\widehat{N}_u|^{i+1}$  unbound proteins is obtained in every RVE. The total  
 number of proteins in the RVE is updated so that  $\widehat{N}_t|^{i+1} = \widehat{N}_u|^{i+1} + \widehat{N}_b|^i$ . Equation 26 is  
 then solved and new values for  $\widehat{\eta}(\phi)|^{i+1}$ , and thus  $\sigma|^{i+1}$  and  $\widehat{N}_b|^{i+1}$  are obtained. Following  
 the final increment of the analysis step the steady-state solution is achieved, and the  
 cytoskeletal free energy  $\bar{G}_{cyto}$  and elastic free energy  $\bar{G}_{elas}$  are computed (Equations 19, 21).

## 2.2. Framework for Focal Adhesion Development

Binding integrins on the cell surface exist in two conformational states: a low affinity (bent)  
 state or an active (straight) state with a high affinity to the appropriate ligand. Only high  
 affinity integrins will bind to the substrate. Here we introduce an extension of the  
 thermodynamic focal adhesion (FA) model from Deshpande *et al.* (2008), whereby we  
 include a dependence of bond formation on ligand availability.

### 2.2.1. Focal Adhesion Model

We first define  $\theta_L = C_L/N_L$  and  $\theta_H = C_H/N_H$ , with  $\theta_L, \theta_H \leq 1$ . Here  $C_L$  and  $C_H$  are the area  
 densities of the unbound low affinity integrins and bound high affinity integrins, respectively,  
 $N_L$  is the area density of the unbound low affinity sites on the cell surface, and  $N_H$  is the area  
 density of ligands on the substrate surface. The chemical potential of low affinity integrins at  
 a density  $C_L$  is dependent on their internal energy and configurational entropy given by:

$$\chi_L = \mu_L + kT \ln \left( \frac{\theta_L}{1 - \theta_L} \right) \#(27)$$

where  $\mu_L$  is the enthalpy of the low affinity integrins, while  $k$  and  $T$  are the Boltzmann constant and absolute temperature. As only high affinity (or straight) integrins interact with substrate ligands, the high affinity chemical potential (at a density  $C_H$ ) includes additional contributions due to the stretching of the bonds:

$$\chi_H = \mu_H + kT \ln \left( \frac{\theta_H}{1 - \theta_H} \right) + \Phi(\Delta_i) - F_i \Delta_i \quad \#(28)$$

where  $\mu_H$  is the enthalpy of the high affinity integrins,  $\Phi(\Delta_i)$  is the strain energy of the integrin-ligand complex, and the  $-F_i \Delta_i$  term is the mechanical work that represents the loss in free energy due to the stretch  $\Delta_i$  of the integrin-ligand (analogous to the pressure-volume term in the thermodynamics of gases), with:

$$F_i = \frac{\partial \Phi}{\partial \Delta_i} \quad \#(29)$$

The stretch energy  $\Phi$  is expressed as a piecewise quadratic potential:

$$\Phi = \begin{cases} \kappa_s \Delta_e^2 & \Delta_e \leq \Delta_n \\ -\kappa_s \Delta_n^2 + 2\kappa_s \Delta_n \Delta_e - \kappa_s \Delta_e^2 & \Delta_n < \Delta_e \leq 2\Delta_n \\ \kappa_s \Delta_n^2 & \Delta_e > 2\Delta_n \end{cases} \quad \#(30)$$

where  $\kappa_s$  is the stiffness of the integrin-ligand bond,  $\Delta_e = \sqrt{\Delta_1 + \Delta_2}$  is the stretch magnitude, and  $\Delta_n$  is the peak bond length. The bond stretch  $\Delta_i$  is related to the displacement  $u_i$  of the cell membrane relative to the substrate as:

$$\Delta_i = \begin{cases} u_i & \Delta_e \leq \Delta_n \text{ or } \left[ \frac{\partial \Phi}{\partial \Delta_e} \Delta_e < 0 \right] \\ 0 & \text{otherwise} \end{cases} \quad \#(31)$$

At thermodynamic equilibrium  $\chi_H = \chi_L$ , so Equations 27, 28 lead to:

$$\left( \frac{1 - \theta_L}{\theta_L} \right) \left( \frac{\theta_H}{1 - \theta_H} \right) = Z^* \quad \#(32)$$

$$\text{with } Z^* = \exp\left(\frac{\mu_L - \mu_H - \Phi(\Delta_i) + F_i \Delta_i}{kT}\right) \#(33)$$

which gives the local area densities of low and high affinity integrins. Similar to the SF model, we implement global conservation of integrins on the cell surface:

$$S_0 C_0 = S_0 (1 - \delta) N_L^0 \theta_L + \int_{S_a} \left[ N_L \theta_L + \frac{N_H Z^* \theta_L}{(1 - \theta_L) + Z^* \theta_L} \right] dS \#(34)$$

where  $S_0$  is the undeformed reference surface area of the cell, and  $C_0$  is the initial density of integrins on the cell surface. The term on the left  $S_0 C_0$  is a conserved value, giving the total number of integrins on the cell surface.  $S_a$  is the surface area in contact with the substrate,  $\delta$  is the fraction of the cell adhered to the substrate, and  $N_L^0$  is the initial undeformed area density of low affinity binding sites on the cell surface. The first term on the right gives the total number of low affinity integrins on the unadhered cell surface, while the second term gives the total number of integrins (high and low affinity) on the adhered cell surface. The local tractions on the cell surface are depend on the concentration of bound high affinity integrins and the force on each ligand-integrin complex, and are balanced by the stresses in the cell:

$$T_i = \sigma_{ij} n_{ij} = -C_H F_i \#(35)$$

Where  $\sigma_{ij}$  is the Cauchy stress in the cell, and  $n_{ij}$  is the surface normal.

### 2.2.2. Focal Adhesion Free Energy

The adhesion free energy is given by:

$$g_{adh} = \int_{S_a} (C_L \chi_L + C_H \chi_H) dS \#(36)$$

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2  
3 However at thermodynamic equilibrium  $\chi_H = \chi_L$ , so:

$$4 \quad g_{adh} = \chi_L S_0 C_0 \#(37)$$

5  
6 with the adhesion energy per unit cell volume given as

$$7 \quad \bar{G}_{adh} = \frac{\chi_L S_0 C_0}{b S_0} \#(38)$$

8  
9 where  $b$  is the cell thickness in its undeformed configuration. Then,  $\bar{G}_{adh}$  follows as:

$$10 \quad \bar{G}_{adh} = \frac{C_0}{b} \left( \mu_L + kT \ln \left( \frac{\theta_L}{1 - \theta_L} \right) \right) \#(39)$$

### 11 12 13 14 15 16 17 18 19 20 21 22 **2.2.3. Numerical Implementation**

23  
24 The focal adhesions between the cell and the micro-patterned substrates are included in the  
25 analysis through a *user-defined interface* (UINTER) subroutine in Abaqus. Adhesions can  
26 develop at any node on the cell surface that comes in contact with the substrate, dependent on  
27 the local tractions and availability of integrins. At each node  $\theta_L$  is recorded at the end of the  
28 increment, as per Equation 32. Recall that the area density of low affinity integrins  $C_L =$   
29  $\theta_L N_L$ . At the end of an increment  $i$ , the global area density of low affinity integrins on the cell  
30 surface is computed through area averaged summation of  $\theta_L|^i$  across every node on the  
31 surface in a *user-defined external database* (UEXTERNALDB) file (Figure 2). Mass  
32 conservation of integrins is enforced by Equation 34. In the subsequent increment the  
33 remaining available unbound low affinity integrins are redistributed so that a homogeneous  
34 distribution of  $\theta_L|^{i+1}$  is obtained across the surface. We assume the time-scales associated  
35 with integrin diffusion are fast relative to the time-scales of focal adhesion assembly.  
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37 Efficient achievement of a converged solution the UINTER requires the specification of an  
38 accurate stiffness matrix. An exact analytical solution is obtained from:  
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$$\frac{\partial T_i^A}{\partial u_j} = - \left[ C_H \frac{\partial F_i}{\partial u_j} + F_i \frac{\partial C_H}{\partial u_j} \right] \#(40)$$

We make the assumption that the substrate is infinitely stiff relative to the cell, and therefore has a negligible free energy.

### 2.3. Material Parameters

All simulations are reported for cells at a temperature  $T = 310K$ . The parameters for the SF framework are fixed at those used in Vigliotti *et al.* (2015) with the volume fraction  $f_0 = 0.032$ ,  $\Omega = 10^{-7.1} \mu m^3$ ,  $\beta = 1.2$ ,  $p = 2$ ,  $\tilde{\epsilon}_{ss} = 0.35$ , and the maximum isometric tension  $\sigma_{max} = 240 kPa$  (Lucas *et al.*, 1987). In keeping with the parameter studies of Vigliotti *et al.* ( $\mu_{u0} + \Delta\mu_{u0}$ ) =  $8 kT$ ,  $\mu_{b0} = 9 kT$ . The density of cytoskeletal proteins in the cell  $\rho$  is  $2 \times 10^6 \mu m^{-3}$ , calibrated such that the cytoskeletal free energy is competitive with the passive free energy. The passive elastic parameters are  $\mu = 1.66 kPa$  and  $\alpha = 8$ , determined through simulation of the Engler *et al.* (2003) experiments for cells spreading on substrates of increasing stiffness. For the FA model, parameters were constrained to lie within commonly accepted ranges as per Deshpande *et al.* (2008). The total area density of integrins  $C_0$  is  $5000 \mu m^{-2}$  (Lauffenburger and Linderman, 1993), the bond stiffness  $\kappa_s = 0.15 nN \mu m^{-1}$ , and the maximum allowable stretch in the bond  $\Delta_n = 50 nm$ , such that the surface energy  $\bar{\gamma} = \kappa_s \Delta_n^2 / kT$  is in the upper end of the range reported by Leckband and Israelachvili (2001). The difference in the reference chemical potentials for the low and high affinity integrins is taken as  $\mu_H - \mu_L = 5kT$  (McCleverty and Liddington 2003). The model was extended to allow for dependence on the number of available ligands and non-local conservation of integrins. A parametric study was performed to determine an appropriate ligand density to ensure sufficient adhesions could form, taken to be  $N_H = 25 \times 10^3 \mu m^{-2}$ . The availability of binding sites should always be greater than the maximum number of bound high affinity

1 integrins, taken here as  $N_L = 50 \times 10^3 \mu m^{-2}$ . A summary of key parameters is provided in  
2 Table 1.  
3

### 4 5 **3. 2D ANALYSIS OF CELL SPREADING ON INFINITE FLAT SUBSTRATES** 6

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8 We illustrate the features of the modelling framework by considering the axisymmetric  
9 spreading of a round cell on flat substrates under plane stress conditions, as shown in Figure  
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11 **3. Material incompressibility is assumed.** Solutions are presented for both a rigid and a  
12 compliant substrate. Additionally the solutions are presented for both a high and low  
13 substrate ligand density. In the undeformed configuration the cell has a radius  $r$  and  
14 thickness  $b$ . Cell spreading is simulated in two analysis steps: (i) Displacement (“pre-  
15 stretch”) boundary conditions are applied to the cell so that its radius is increased to  $\lambda r$  with a  
16 uniform strain state throughout; (ii) Contact is implemented between the deformed cell and  
17 the substrate and the displacement boundary condition is removed. Surface and integrin-  
18 ligand attachments are formed in accordance with Equations 27-35. The active cell stress  
19 tensor is computed from Equation 13 and is added to the passive stress tensor (Equation 22).  
20 In addition to deformation of the cell and integrin-ligand attachments, the substrate will also  
21 deform due to the passive and active cell stress (except in cases where the substrates can be  
22 considered to be infinitely stiff compared to the cell). This finite element scheme determines  
23 the steady state configuration of the cell, adhesions, and substrate. For a given steady state  
24 configuration the total free energy density of the system is computed from  
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$$48 \quad \bar{G}_{tot} = \bar{G}_{cyto} + \bar{G}_{elas} + \bar{G}_{adh} + \bar{G}_{sub} \#(41)$$

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51 Analyses are performed for a range of “pre-stretch” ( $\lambda$ ) values and the free energy density of  
52 the system is plotted as a function of the steady-state spread area of the cell. As stated in  
53 Section 1, we hypothesize that a cell tends towards a spread-state that reduces the free energy  
54 of the system.  
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3 Results: We first consider the case of cell spreading on a rigid substrate. The force generation  
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5 by the actin-myosin machinery lowers the chemical potential of the stress fiber proteins in the  
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7 bound state and thereby favoring the formation of stress fibers. As the cell stretches to its  
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9 spread configuration, functional units are added to the stress fiber chain in order to reduce the  
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11 internal SF strain ( $\tilde{\epsilon}_n$ ) to the ground state (dictated by  $\tilde{\epsilon}_{ss}$ ). Thereby an increase in cell  
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13 spreading results in a decrease in  $\hat{N}_u$  (Figure 4a), and consequently the free energy of the  
14  
15 cytoskeletal proteins ( $\bar{G}_{cyto}$ ) is lowered (Figure 4b). However as shown in Figure 4c, an  
16  
17 increase in spreading also results in an increase in the elastic free energy of the cell due to  
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19 straining of the passive (hyperelastic) non-contractile components of the cell. This framework  
20  
21 therefore presents cell spreading as a competition between a decrease in cytoskeletal free  
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23 energy due to strain induced stress fiber formation and an increase in elastic free energy due  
24  
25 to straining of the passive cell components. As illustrated in Figure 4e, for the limited number  
26  
27 of spread states considered here, a low free energy configuration is computed at an area of  
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29  $A/A_0 \approx 2.75$ . Any further spreading beyond this point will incur a significant elastic penalty  
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31 due to the strain stiffening hyperelastic passive component of the model. Our computed low  
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33 free energy spread area corresponds closely to the experimental observations of Engler et al.  
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35 (2003), where cell spread areas on rigid substrates are approximately three times higher than  
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37 unspread cell areas.

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47 In the case of cell spreading on a compliant substrate, an increase in cell spread area incurs an  
48  
49 elastic penalty (increasing free energy) from both the passive elastic components of the cell  
50  
51 and the elastically deformed substrate. These elastic penalties are plotted in Figures 5c and 5e  
52  
53 for cell spreading on a compliant neo-Hookean substrate ( $\mu = 8\text{kPa}$ ), and once again are in  
54  
55 direct competition with the reducing cytoskeletal free energy (Figure 5b) as the cell spreads.  
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60 **When  $A/A_0 < 1$  the cell has contracted below the reference area due to substrate**  
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1 deformation. In such cases  $\bar{G}_{elas}$  increases due to compression of the passive cell  
2 components. The lowest free energy configuration on this compliant substrate is computed at  
3 a spread area of  $A/A_0 \approx 1.8$  (Figure 5f). This spread area is 30% lower than the low energy  
4 spread area on a rigid substrate (Figure 4e). Once again, this result corresponds closely to the  
5 experimental study of Engler *et al.* (2003) where the cell spread areas of on 8kPa substrates  
6 are observed to be ~25% lower than on rigid substrates. This further supports our hypothesis  
7 that the cell will tend towards a spread state that reduces its free energy.  
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10 Figure 4d and 5d demonstrate that cell spreading also results in increased focal adhesion  
11 formation, with a consequent reduction in the adhesion free energy. The change in adhesion  
12 free energy over the range of spread configurations is ~3 orders of magnitude lower than the  
13 cell cytoskeletal and elastic free energies. Therefore focal adhesion formation does not  
14 significantly contribute to the energetic competition that governs cell spreading in Figures 4  
15 and 5. However, cell spreading is not possible without a sufficient degree of traction  
16 mediated focal adhesion assembly, as mechanical equilibrium of the spread cell is only  
17 achieved by traction interaction with the substrate. An increase in traction results in an  
18 increase in the density of high affinity integrins ( $C_H$ ). As the cell spreads, the tractions  
19 between the cell and substrate increase (due to both elastic stretching of passive components  
20 and higher contractility due to increased strain induced SF formation), and consequently  $C_H$   
21 increases. The entropy of integrins on the cell surface increases as more integrins are in a  
22 bound state (in accordance with Equation 39). Therefore, an increase in  $C_H$  during spreading  
23 results in a decrease in  $\bar{G}_{adh}$ , as shown in Figure 4d. A higher ligand density will inherently  
24 allow the cell to spread further as higher cellular tractions can be supported by the focal  
25 adhesions. In contrast, Figure 6 considers the case of a rigid substrate with a low ligand  
26 density ( $N_H = 2500 \mu m^{-2}$ ), which limits the cell spreading. The final spread area increases  
27 with the initially applied cell pre-stretch up to a value of  $\lambda \approx 1.5$ . If the cell is initially  
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1 stretched beyond this point, a sufficient number of integrin-ligand bonds cannot be formed to  
2 support the resultant tractions, and the cell shrinks to a steady-state area of  $A/A_0 \approx 1.85$ .  
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4 This is the maximum spread area that the cell can reach for this low ligand density. **Note that**  
5 **if the cell cannot adhere to the substrate (e.g. ligand density of zero), an unadhered cell is**  
6 **predicted to shrink to an area of  $A/A_0=0.735$  and a total free energy density of  $\hat{G}_{tot} = 5.65$  is**  
7 **observed.** As shown in Figure 6b, the total free energy reduces with increasing spread area,  
8  
9 but spread states with  $A/A_0 \geq 1.85$  cannot occur due to the low ligand density. Recall from  
10 Figure 4e that a high ligand density  $N_H = 25000 \mu m^{-2}$  results in a low free energy spread  
11 area of  $A/A_0 = 2.75$  (also shown in Figure 6a for comparison). Our predicted ~33%  
12 reduction in cell spread area for a 10-fold decrease in ligand density is again supported by the  
13 experimental results of Engler *et al.* (2003).  
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#### 31 **4. 2D ANALYSIS OF CIRCULAR CELL SPREADING ON MICRO-PATTERNED** 32 **SUBSTRATES** 33

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36 **We next attempt to simulate the experiments of Théry *et al.* (2006) whereby cells are spread**  
37 **on micro-patterned ligand patches under plane stress conditions.** Two patch geometries are  
38 considered: “V-shaped” patches, and “Y-shaped” patches, as shown in Figure 7. For  
39 simplicity we assume that **the cell is initially circular with radius  $r_c$**  when in suspension. It is  
40 important to note that there is an infinite number of spread states (strain distributions) that  
41 can be assumed by the cell in order to spread on the ligand patch. Here we attempt to  
42 parameterize the spreading process by considering a subset of possible spread states. **In the**  
43 **case of the “V-shaped” patch the cell is stretched so that proportion of the cell perimeter  $\omega r_c$**   
44 **can adhere to the outer edge of the “V”.** The stretch is assumed to be uniform along the patch  
45 and is given as  $\lambda_c = L_s/\omega r_c$ , where  $L_s$  is the fixed patch length. Therefore, by considering a  
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1 range of values of  $\lambda_c$  (or  $\omega$ ) we can simulate a number of spread states and determine which  
2 of these states produces the lowest total free energy. The cell radius in the initial  
3 configuration is  $r_c = 17 \mu m$  and the thickness  $b = 1 \mu m$ . The substrate dimensions are based  
4 on the experiments of Théry *et al.* (2006), i.e.  $L_s = 46 \mu m$  and the substrate letter width was  
5 determined to be  $7 \mu m$ . Once again the total steady state free energy density is computed from  
6 Equation 41.  
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14 Results: Similar to the simplified axisymmetric example presented in Section 4, the cell free  
15 energy during spreading can be interpreted as a competition between the increasing elastic  
16 free energy ( $\bar{G}_{elas}$ ) and the decreasing cytoskeletal free energy ( $\bar{G}_{cyto}$ ). Simulations of cell  
17 spread on a V-shaped substrate reveal that  $\bar{G}_{tot}$  is minimized at a cell perimeter stretch of  
18  $\lambda_c = 1.3$  (Figure 8a). Examination of the strain distribution in this lowest free energy (LFE)  
19 configuration (Figure 8b) reveals that the maximum tensile strain occurs close to the free  
20 unadhered edge of the spread cell. A spread state characterized by a lower stretch ( $\lambda_c = 1.1$ )  
21 results in an elevated total free energy  $\bar{G}_{tot}$ , despite a high concentration of straight SFs  
22 directly along the free edge. Such a configuration results in extremely high strains along the  
23 free unadhered edge, causing a very high elastic free energy penalty. A spread state  
24 characterized by a higher stretch ( $\lambda_c = 1.6$ ) results in a high strain in the region of the  
25 adhered edges. Although this allows more a similar level of SF formation on all three edges  
26 of the spread cell (Figure 8c), the high elastic penalty due to stretching along the adhered  
27 edges is too large to be compensated for by the reduction in  $\bar{G}_{cyto}$  due to SF formation along  
28 all three edges. The density of bound SF proteins is characterized by  $\zeta^{SF}(\phi) = \hat{n}(\phi) * \hat{\eta}(\phi)$ ,  
29 with Figure 8c showing the dominant SF orientation at each material point. The focal  
30 adhesion distribution in these highlighted configurations (Figure 8c) show evident clustering  
31 in the direction of traction, denoted by  $\hat{C}_H = C_H/N_H$ . However, the variance in  $\bar{G}_{adh}$  between  
32 these configurations was negligible.  
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3 Figure 9 shows the dominant SF alignment (d) and FA distribution (c) in the LFE  
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5 configuration for cells spread on the V-shaped substrate. We see that the highest SF  
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7 concentration and actin density ( $\zeta^{\text{SF}}$ ) is in the region of the free edge where an arc of SFs  
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9 curve towards the center of the cell. A similar distribution is reported in the experimentally  
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11 determined heat maps of SF distribution reported by Théry *et al.* (Figure 9b). At the center of  
12  
13 the cell, where the strain is lowest in both cases, Equation 12 dictates that the SF  
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15 concentration in any direction will be lower than that along the free edge. Focal adhesions  
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17 ( $\hat{C}_H$ ) are predicted to form along the perimeter of the ligand patch due to a shear-lag type  
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19 distribution of traction between the cell and the patch. Such a FA distribution is also reported  
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21 in the experimental study of Théry *et al.* (2006) (Figure 9a).  
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28 Simulations of cell spread on a Y-shaped substrate reveal that  $\bar{G}_{tot}$  is minimized at a  
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30 perimeter stretch of  $\lambda_c = 1.34$  (Figure 10a) on two of the free edges, with a slightly higher  
31  
32 strain and SF concentration on the third (top) edge. Once again a spread state characterized  
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34 by a higher or lower value of  $\lambda_c$  results in an elevated  $\bar{G}_{tot}$  due to an extremely high elastic  
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36 free energy.  
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41 The experimental SF heat maps for the Y-patterned substrate from Théry *et al.* (2006) exhibit  
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43 an expected symmetry, with similar SF patterns on all three free edges. However, in the  
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45 computed LFE configuration (Figure 10) one edge has a higher strain and SF concentration.  
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47 In order to compare our computational results to an experimental heat map (constructed using  
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49 data from several observations) we should acknowledge that there are three LFE  
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51 configurations due to symmetry of the Y-shape. Therefore we rotate the distributions shown  
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53 in Figure 10c-d through  $120^\circ$  and  $240^\circ$  we then construct a “computational heat map” by  
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55 taking the average of these three LFE distributions. The “computational heat map” is shown  
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1 in Figure 11c-d and exhibits an identical SF distribution on all three free edges and can be  
2 directly compared to the experimental heat maps. Notably the “computational heat map” free  
3 edge SF concentration is lower than that along the free edge of the V-shape (Figure 9d) (“heat  
4 map averaging” is not necessary for the V-shape as it has only one free edge). This  
5 prediction is supported by experimental results (Figures 9b and 11b).  
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## 16 5. DISCUSSION

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18 In this paper we present a steady-state adaptation of the thermodynamically motivated  
19 **continuum** SF model of Vigliotti *et al.* (2015). We implement this formulation in a non-local  
20 finite element setting where we consider global conservation of the total number of  
21 cytoskeletal proteins within the cell, global conservation of the number of binding integrins  
22 on the cell membrane, and a finite ligand density on the substrate surface.  
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31 When a number of cytoskeletal proteins assemble to form contractile SFs, the free energy of  
32 the proteins bound within the SF is lower than the total free energy of the same proteins when  
33 they are unbound (not assembled in a SF). During spreading the strain in a cell increases.  
34 This results in assembly of cytoskeletal proteins into contractile SFs, and a consequent  
35 lowering of the total cytoskeletal free energy in the cell. Of course an increase in cell strain  
36 during spreading also results in an increase in the elastic free energy of the mechanically  
37 passive components of the cell (e.g. the membrane, intermediate filaments etc.). Therefore  
38 cell spreading can be viewed as a competition between the reducing cytoskeletal free energy  
39 and the increasing elastic free energy. **Our analyses suggest that the driver of cell spreading**  
40 **is a lowering (or perhaps a minimization) of the total free energy of the system.**  
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57 To simulate cell-substrate contact we present an extension of the Deshpande *et al.* (2008)  
58 model for FA kinematics, whereby we account for a dependence on the substrate ligand  
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1 density. Variance of the substrate ligand density has significant impact on cell behaviour.  
2 Combined with the mass conservation of integrins, this affects the maximum cellular  
3 tractions the FAs can withstand, and therefore the spread shape and area.  
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6  
7 In Section 3 the key features of the model are examined through a series of simplified  
8 simulations of axisymmetric cell spreading. By considering a number of parameterized cell  
9 spread states on a rigid substrate our analyses suggest that the lowest free energy spread-state  
10 has an area that is 2.75 times higher than an unspread cell area. This prediction is in  
11 agreement with the experimental measurements of Engler et al. (2003). Furthermore, when  
12 cells spread on compliant substrates ( $\mu = 8\text{kPa}$ ) we predict that the lowest free energy spread  
13 area is ~30% lower than the corresponding area on a rigid substrate. Once again this finding  
14 is supported by the experimental trends reported in Engler et al. (2003). Finally, we predict  
15 that a low substrate ligand density will limit the spread area of a cell, with a 10-fold decrease  
16 in ligand density on a rigid substrate resulting in a ~33% reduction in spread area on a rigid  
17 substrate. Again, this prediction is in broad agreement with the experimental measurements  
18 of Engler et al. (2003) and Gaudet et al. (2003). Our hypothesis that cell spreading is driven  
19 by a lowering of free energy appears to provide an explanation for the broad trends observed  
20 by Engler et al. (2003).  
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42 A recent study by Shenoy *et al.* (2016) suggests that the cellular free energy decreases with  
43 increasing substrate stiffness, which provides an energetic basis for durotaxis. The results  
44 from Section 3 of the current study also provides insight to this phenomenon. In the lowest  
45 energy spread configuration on a compliant substrate, the cell has a predicted free energy of  
46  $\widehat{G}_{\text{tot}} = 4.85$ . However, on a rigid substrate the lowest free energy configuration is observed at  
47  $\widehat{G}_{\text{tot}} = 4.2$ . Therefore, we suggest that durotaxis is the result of a cell attempting to lower its  
48 free energy by migrating towards a stiffer substrate. Similarly, chemotaxis may be explained  
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by the inability of a cell to attain a minimum free energy configuration if the concentration of ligands is very low, thus inducing the cell to migrate to a region of higher ligand density in order to reduce the free energy.

In Section 4 a number of parameterized spread-states are simulated, whereby a circular cell adheres to “V-shaped” and “Y-shaped” ligand patches based on the experiments of Théry *et al.* (2006). The free energy associated with each spread state is computed, and we demonstrate that the spread-state with the lower free energy exhibits a SF distribution that corresponds to experimental observations, i.e. a high concentration of highly aligned SFs occurs along free edges, with lower SF concentrations at the interior of the cell. The simulation of the complex SF and FA distributions observed experimentally in cells spread on the V- and Y- shaped ligand patterns demonstrates the predictive power of the model. Future implementations will also consider cell spreading on grooves (Lamers *et al.* 2010) and micro-posts (McGarry *et al.* 2009; Ronan *et al.* 2013). The current analysis presents a movement away from traditional deterministic approaches to computational cell biomechanics in which the experimentally observed spread state is incorrectly assumed to be the reference undeformed state. Such approaches neglect cell strain as a driver of SF assembly. Also, global conservation of a finite number of cytoskeletal proteins within the cell has been neglected. The model of Pathak *et al.* (2008) simulates the experiments of Théry *et al.* (2006) using such assumptions. The degree of SF alignment (characterized by a variance parameter) is correctly predicted, with uniaxial SFs being predicted in a region of uniaxial stress along the cell free edge (in accordance with the model of Deshpande *et al.* (2006) SFs orthogonal to the free edge dissociate due to the stress-free condition). However, the framework incorrectly predicts full SF formation in all directions (isotropic distribution) in areas of biaxial stress and in regions where the cell is bonded to the ligand patch. The current

1 study corrects such shortcomings by considering strain associated with cell spreading, in  
2 addition to implementing a global conservation of cytoskeletal proteins.  
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5 In this study we consider a very small subset\* of the possible spread-states of a cell on a  
6 micro-patterned substrate, in order to examine the dependence of the SF distribution on the  
7 manner in which a cell spreads (\*our subset is primarily chosen based on ease of  
8 parameterization, as illustrated in Figure 7, rather than on any consideration of the actual cell  
9 spreading process). Our analysis of a number of spread states allows us to examine the  
10 hypothesis that the final spread state is driven by minimization of the free energy of the  
11 system. In reality however, there are an infinite number of spread configurations that the cell  
12 can assume. A rigorous treatment of the stochastic problem of cell spreading requires the  
13 development of a statistical mechanics framework that allows for the analysis of an **extremely**  
14 **large number of spread states**. The finite element framework developed here is prohibitively  
15 computationally expensive for such an approach.  
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32 The underlying premise in this work is that minimum/low free-energy configurations are the  
33 most likely states to be observed. In statistical thermodynamics a closed system in a constant  
34 temperature and pressure environment attains equilibrium at minimum Gibbs free-energy.  
35 However, a cell is not a closed system and in fact never attains an equilibrium state in this  
36 sense while alive. The approach taken here of searching for low free-energy states rests on  
37 the “homeostatic ensemble” developed by Shishvan et al. (2017) who show that in their  
38 homeostatic state cells attain a fluctuating equilibrium where low free-energy states are more  
39 probable. The results presented here should be viewed in this light, in the sense that the  
40 minimum free-energy configurations predicted in our analyses have the highest probability of  
41 being observed in experiments.  
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## 58 **6. CONCLUDING REMARKS**

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1 We combine the thermodynamically consistent model for the stress fiber cytoskeleton  
2 developed by Vigliotti *et al.* (2015) with a focal adhesion model (again motivated by  
3 thermodynamic considerations) to analyze two problems: (i) spreading of cells on elastic  
4 substrates and (ii) spreading of cells on substrates with specific geometrical ligand patterns.  
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10 Spreading of cells is shown to be a competition mainly between the elastic energy and  
11 cytoskeletal energy of the cell, as well as the elastic energy of the substrate. With increasing  
12 cell spreading the elastic energy of the cell and substrate typically increases, but the  
13 cytoskeletal energy decreases as a larger fraction of the cytoskeletal proteins form stress  
14 fibers. The equilibrium configuration is assumed to be that corresponding to the lowest free  
15 energy. In agreement with the experiments of Engler *et al.* (2003) we show that the spread  
16 area of the cell increases with increasing substrate stiffness. When the spreading of cells is  
17 constrained by specific geometric patterns of ligands, we show that, in the lowest free-energy  
18 configuration, stress fibers preferentially form along the un-adhered edges of the cell, in line  
19 with the observations of Théry *et al.* (2006). **This framework presents a potential  
20 computational tool to design substrates and scaffolds that will yield a desired cell spread  
21 state.**  
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40 The simulations presented here suggest that computed low (or minimum) free-energy spread  
41 cell configurations are broadly consistent with experimentally observed spread cell  
42 configurations. However, it is worth emphasizing that cells do not attain an equilibrium  
43 minimum free-energy configuration in the traditional sense, as observations clearly show that  
44 spread cells are in a perpetually fluctuating state. Thus, **the minimum free-energy  
45 configuration is best viewed as the most probable state to be observed**, rather than a unique  
46 equilibrium state.  
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## APPENDIX A

The influence of an applied steady-state nominal strain  $\varepsilon_n$  on the steady-state active and passive Cauchy stress is illustrated in Figure A1(a) (material parameters as per Section 2.3) for a cell subjected to series of uniaxial stretches. The dependence of stress fiber formation (Equation 12) on steady-state strain (Figure A1(b)) is reflected in the strain dependence of the active stress (through Equation 13). It must be noted that the active stress curve in Figure A1(a) is not representative of a stress-strain constitutive law. Rather, it is a plot of the steady-state active stress computed for an applied steady-state strain. In contrast, previous modelling approaches (e.g. Deshpande *et al* 2006) do not include a dependence of stress fiber formation on applied strain, so that the computed stress fiber activation-level (SFA) and, consequently, the active stress are independent of the applied steady state strain in Figure A1.

## ACKNOWLEDGMENT

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## FIGURE CAPTIONS

**Figure 1:** a) Schematic of a 2D cell on a ligand-coated substrate with the coordinate system marked. The networks of stress fibers and focal adhesions within the 2D RVE are shown in the inset; b) Remodeling of a SF subjected to a nominal tensile strain  $\varepsilon_n$ : (i) SF in ground state, with functional unit strain  $\tilde{\varepsilon}_n = 0$ ; (ii) SF subjected to tensile strain  $\varepsilon_n$  which reduces the actin-myosin overlap; (iii) Remodeling of SF by addition of functional unit; (iv) Remodeled SF now in low energy state, with functional unit strain  $\tilde{\varepsilon}_n = \tilde{\varepsilon}_{ss}$ . (Vigliotti et al.,2015)

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**Figure 2:** Outline of solution scheme. Total steady state energy density  $\bar{G}_{tot}$  is calculated at the end of the analysis through the use of a UEXTERNALDB subroutine.

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**Figure 3:** Axisymmetric cell spread schematic. A cell of radius  $r$  stretches over an infinite ligand patterned substrate.

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**Figure 4:** For a rigid substrate, the relationship between cellular spread area and (a) the number of available unbound cytoskeletal proteins ( $\hat{N}_u$ ), (b) the cytoskeletal free energy ( $\bar{G}_{cyto}$ ), (c) the elastic free energy ( $\bar{G}_{elas}$ ), (d) the adhesion free energy ( $\bar{G}_{adh}$ ), and (e) the combined total free energy density ( $\bar{G}_{tot}$ ). Free energy densities characterized by normalized quantity  $\hat{G} = \bar{G}/\rho kT$ .

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**Figure 5:** For a compliant neo-Hookean substrate ( $\mu = 8kPa$ ), the relationship between cellular spread area and (a) the number of available unbound cytoskeletal proteins ( $\hat{N}_u$ ), (b) the cytoskeletal free energy ( $\bar{G}_{cyto}$ ), (c) the elastic free energy ( $\bar{G}_{elas}$ ), (d) the adhesion free energy ( $\bar{G}_{adh}$ ), (e) the substrate free energy ( $\bar{G}_{sub}$ ), and (f) the combined total free energy density ( $\bar{G}_{tot}$ ). Free energy densities characterized by normalized quantity  $\hat{G} = \bar{G}/\rho kT$ .

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**Figure 6:** (a) Steady state cell spread area as a function of applied cell “pre-stretch”  $\lambda$  for a low and high ligand density  $N_H$  ( $\mu m^{-2}$ ) on a rigid substrate. The spread area with the lowest free energy (from Figure 4e) is marked by the grey circle. (b) The relationship between cell spread area and the total free energy ( $\bar{G}_{tot}$ ) for a low ligand density ( $N_H = 2500 \mu m^{-2}$ ). Free energy densities characterized by normalized quantity  $\hat{G} = \bar{G}/\rho kT$ .

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**Figure 7:** Parametric study schematic of cell spreading on a) V- and b) Y- shaped substrates. For a cell of radius  $r_c$ , the spreading process is parameterized in terms of the proportion of the cell perimeter  $\omega r_c$  that stretches along the ligand coated patch ( $\lambda_c = L_s/\omega r_c$ ). The shaded patch represents locations focal adhesions may form with the cell surface.

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1 **Figure 8:** Predicted steady-state cell spread on V-shaped ligand pattern in a series of configurations:  
2 a) Free energy of the system ( $\hat{G}_{tot} = \bar{G}_{tot}/\rho kT$ ) for a range of spread states characterized by the  
3 stretch of the cell on the fixed edge ( $\lambda_c$ ). Three states are highlighted: 1. A large stretch on the  
4 unadhered edge ( $\lambda_c = 1.1$ ); 2. The lowest free energy configuration ( $\lambda_c = 1.3$ ); 3. A large stretch on  
5 the adhered edge ( $\lambda_c = 1.6$ ); b) Maximum principal strain ( $\varepsilon_p^{max}$ ) distribution in the spread cell in  
6 the highlighted states; c) Distribution of vinculin or focal adhesions characterized by normalized  
7 quantity  $\hat{C}_H = C_H/N_H$ , and the dominant SF alignment in the highlighted configurations with  
8  $\zeta^{SF}(\phi) = \hat{n}(\phi) * \hat{\eta}(\phi)$ .

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15 **Figure 9:** Cell spread on V-shaped micro-patterned substrates: Experimental images of average (a)  
16 vinculin and (b) actin distributions (Reproduced with some modifications from Théry et al. (2006).  
17 Copyright © John Wiley & Sons, Ltd.); (c) Distribution of vinculin or focal adhesions in the LFE  
18 configuration, characterized by normalized quantity  $\hat{C}_H = C_H/N_H$ ; (d) Dominant SF alignment in the  
19 LFE configuration, with  $\zeta^{SF}(\phi) = \hat{n}(\phi) * \hat{\eta}(\phi)$ . The insets show full SF distribution for all M  
20 discrete directions.

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26 **Figure 10:** Predicted steady-state cell spread on Y-shaped ligand pattern in a low free energy  
27 configuration: a) Free energy of the system ( $\hat{G}_{tot} = \bar{G}_{tot}/\rho kT$ ) for a range of spread states  
28 characterized by the stretch of the cell on the fixed edge ( $\lambda_c$ ). A lowest free energy (LFE)  
29 configuration is observed at  $\lambda_c = 1.34$ ; b) Maximum principal strain distribution in the spread cell;  
30 c) Distribution of vinculin or focal adhesions in the LFE configuration, characterized by normalized  
31 quantity  $\hat{C}_H = C_H/N_H$  d) Dominant SF alignment in the LFE configuration, with  $\zeta^{SF}(\phi) =$   
32  $\hat{n}(\phi)\hat{\eta}(\phi)$ . The insets show full SF distribution for all M discrete directions.

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38 **Figure 11:** Cell spread on Y-shaped micro-patterned substrates: Experimental images of average  
39 vinculin (a) and actin (b) distributions (Reproduced with some modifications from Théry et al.  
40 (2006). Copyright © John Wiley & Sons, Ltd.); (c) Predicted average distribution of vinculin or focal  
41 adhesions, characterized by normalized quantity  $\hat{C}_H = C_H/N_H$ ; (d) Predicted average actin  
42 distribution, with  $\zeta^{SF}(\phi) = \hat{n}(\phi) * \hat{\eta}(\phi)$ .

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49 **For appendix:**

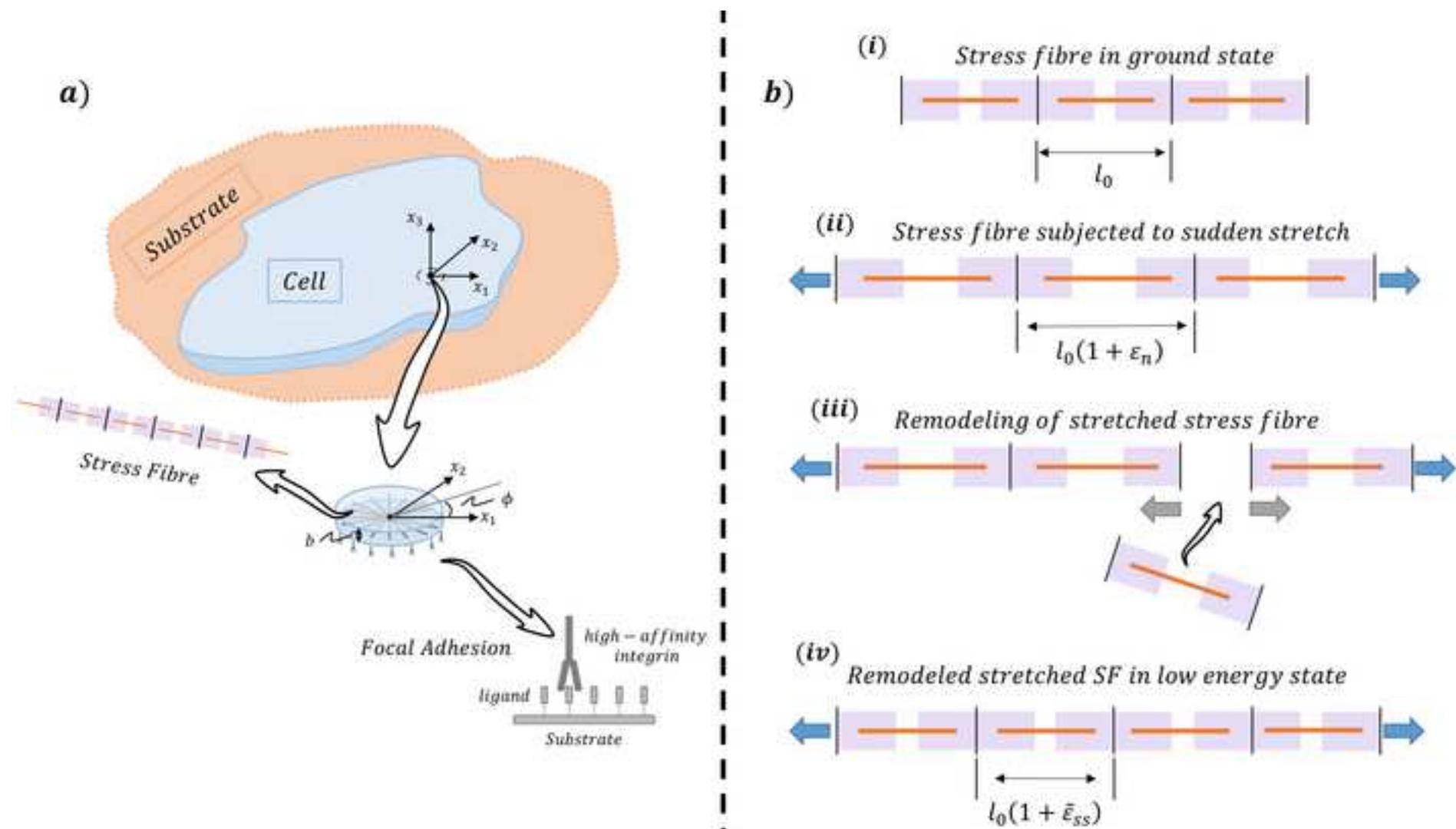
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51 **Figure A1.** (a) Computed active steady state stress as a function of applied steady state nominal  
52 strain  $\varepsilon_n$  for the current model (active(1)). The passive and total stresses are also shown. For  
53 comparison the active stress computed by the Deshpande et al. (2006) model (active(2)) is shown. (b)  
54 Computed values of  $\hat{\eta}$  as a function of applied steady state nominal strain  $\varepsilon_n$  for the current model.  
55 For comparison the stress fiber activation (SFA) level computed by the Deshpande et al. (2006)  
56 model is plotted to highlight the absence of strain dependence on SF remodeling in this previous  
57 model.

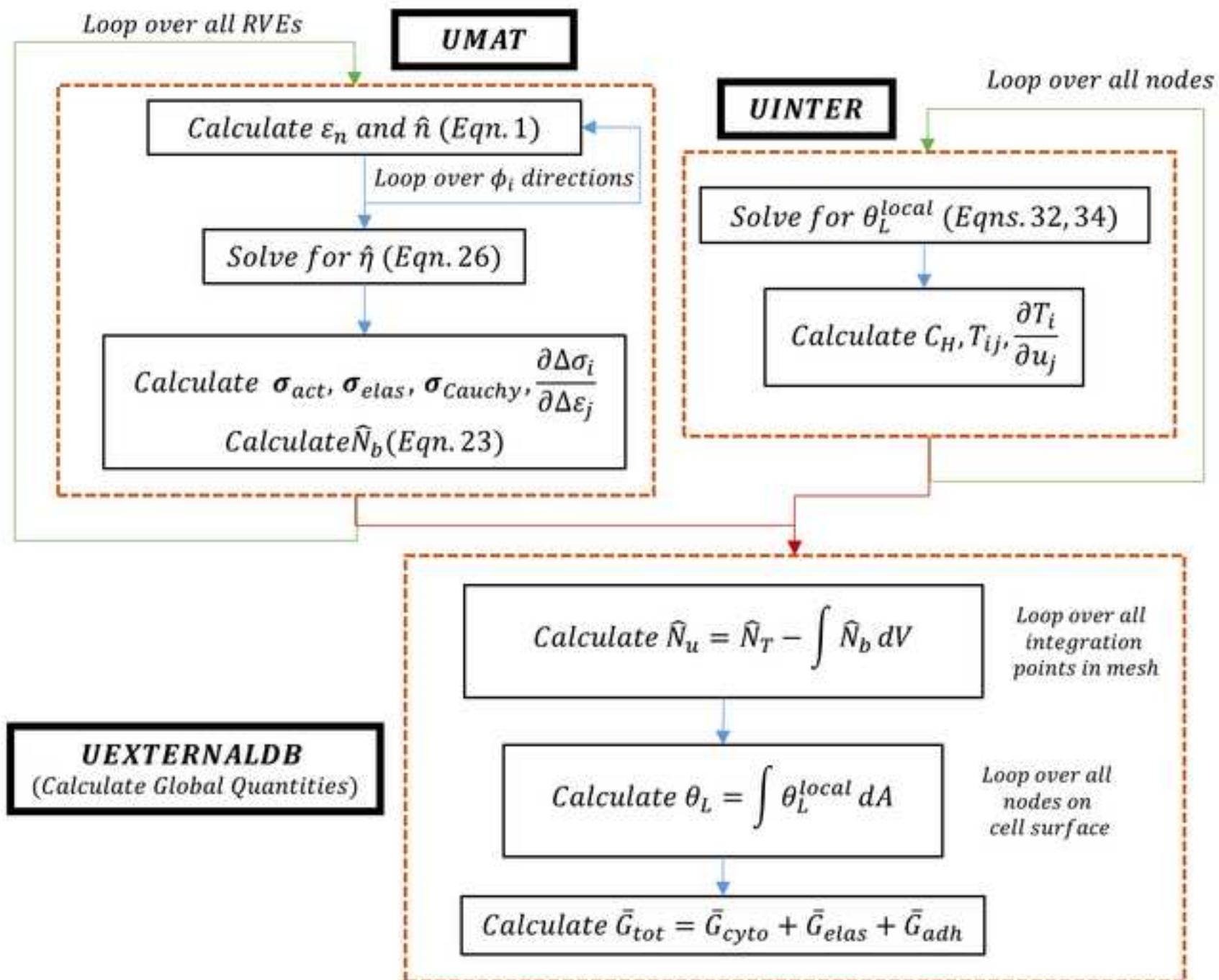
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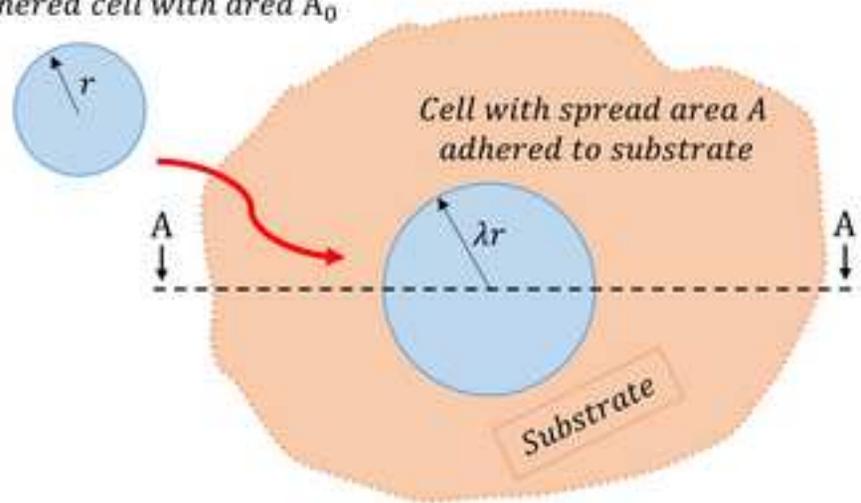
Parameter symbol	Brief description
$n; n^R$	Number of functional units in a stress fiber; reference number of functional units within stress fiber in an undeformed RVE
$\eta$	Angular concentration of stress fibers at orientation ( $\phi$ )
$\Omega$	Volume of $n^R$ functional units of the stress fiber
$l_0$	Undeformed length of a functional unit
$\varepsilon_n; \tilde{\varepsilon}_{ss}$	Nominal strain of a stress fiber; functional unit strain at steady state
$N_b; N_u$	Number of cytoskeletal proteins bound in functional units; number of unbound cytoskeletal proteins
$\mu_a; \mu_u; \mu_b$	Activation enthalpy for $n^R$ cytoskeletal proteins; enthalpy of $n^R$ cytoskeletal proteins in the unbound state; enthalpy of $n^R$ cytoskeletal proteins in bound state
$\mu_{u0}; \mu_{b0}$	Standard enthalpy of $n^R$ functional units in the unbound and bound states
$\psi$	Internal energy of $n^R$ functional units within a stress-fiber
$\sigma_f; \sigma_{max}$	Stress fiber stress; maximum tensile stress of a stress-fiber
$f_0; \rho$	Volume fraction of cytoskeletal proteins in the cell; concentration of cytoskeletal proteins
$\chi_u; \chi_b$	Chemical potential of the unbound cytoskeletal proteins that form a single functional unit; chemical potential of a functional unit within a stress fiber
$C_0; C_L; C_H$	Initial area density of integrins on the cell surface; area densities of the unbound low affinity integrins and bound high affinity integrins
$S_0; S_a$	Undeformed reference surface area of the cell; surface area in contact with substrate
$N_L; N_H$	Area density of the unbound low affinity sites on the cell surface; Area density of ligands on the substrate surface
$\theta_L; \theta_H$	$C_L / N_L; C_H / N_H$
$\mu_L; \mu_H$	Enthalpy of the low affinity integrins; enthalpy of the high affinity integrins
$\Phi$	Strain energy of the integrin-ligand complex
$\Delta_i; \Delta_n$	Stretch of the integrin-ligand complex; peak bond length
$\kappa_s$	Stiffness of the integrin-ligand complex
$\chi_L; \chi_H$	Chemical potential of low affinity integrins; chemical potential of high affinity integrins
$\bar{G}_{tot}; \bar{G}_{cyto}; \bar{G}_{elas}; \bar{G}_{adh}; \bar{G}_{sub}$	Total, cytoskeletal, elastic, adhesion, and substrate free energy densities

**Table 1:** A summary of key parameters of the model





Unadhered cell with area  $A_0$



Section A – A

