Multiscale FEM-simulations of cross-linked actin network embedded in cytosol with the focus on the filament orientation

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Abstract

The present contribution focuses on the application of the multiscale finite element method to the modeling of actin networks which are embedded in the cytosol. These cell components are of particular importance with regard to the cell response to external stimuli. The homogenization strategy chosen uses the Hill-Mandel macro-homogeneity condition for bridging two scales: the macroscopic scale which is related to the cell level, and the microscopic scale related to the representative volume element. For the modeling of filaments, the Holzapfel–Ogden β-model is applied. It provides a relationship between the tensile force and the caused stretches, serves as the basis for the derivation of the stress and elasticity tensors and enables a novel finite element implementation. The elements with the neo-Hookean constitutive law are applied for the simulation of the cytosol. The results presented corroborate the main advantage of the concept, namely its flexibility with regard to the choice of the representative volume element as well as of macroscopic tests. The focus is particularly placed on the study of the filament orientation and of its influence on the effective behavior.

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1. Introduction

The study of cell activity is certainly necessary to more efficiently find out why some diseases such as cancer, atherosclerosis or glaucoma occur. However, this is a challenging task requiring profound knowledge on cell morphology. The present contribution focuses on the study of mechanical stability of eucariotic cells which is mainly regulated by the activity of the cytosol and the cytoskeleton. The cytosol is the gel-like material that surrounds the organelles and inclusions. It constitutes more than half of the total cell volume and consists primarily of water, dissolved molecules and proteins, salts and enzymes. Much of the water and several proteins within the cytosol are bound to the cytoskeleton. The cytoskeleton gives the cell its form, endows the cell with structural integrity and the ability to move within the extracellular space, aids in cell division, helps to anchor the cell to its neighbors and the substrate, and speeds up the transport of materials within certain types of cells. It consists of three major types of filaments: cable-like actin filaments, pipe-like microtubules and rope-like intermediate filaments.

Microfilaments, also called actin filaments, are the major constituents of the cytoskeletal network found in virtually all eukaryotic cells. In non-muscle cells, actin makes up 1-5% of the total weight of the cell protein, while in muscle cells, actin makes up 10% of the cellular protein. The elementary building block of actin is the globular, monomeric protein called G-actin, which may polymerize and transform to long filamentous double-stranded helical polymers. The long filamentous polymers are then known as F-actin filaments. Actin filaments can be organized differently depending on where they are located in the cell. Actin filaments located in the cell cortex are mainly organized in a lattice-like network and are also referred to as actin gel.

Microtubules are long, straight and hollow tube-like cylindrical structures that are located with one end close to the center of the cell called centrosome, and with the other end pointing out from the center of the cell. Microtubules are highly dynamic, even more than actin, and undergo constant polymerization and depolymerization with short intervals. They are constructed from
monomers of α and β-tubulin. The microtubule is the thickest one among the cytoskeletal filaments with an outer diameter of \(~30\text{ nm}\) and an inner diameter of \(~18\text{ nm}\) Gittes et al. (1993a), while Pampaloni et al. Pampaloni et al. (1993) reported \(~25\) and \(~15\text{ nm}\), respectively. The hollow cylinder exhibits a high bending stiffness, greater than that of an actin filament.

Intermediate filaments are tough and durable filaments made from fibrous proteins. They can increase in density in response to increased mechanical stress. The intermediate filaments are currently sub-categorized into few different types such as keratin, vimentin and neurofilament Alberts et al. (2008). Keratin is the most widely distributed intermediate filament protein. An intermediate filament has a diameter of 8-10 nm and a length that varies depending on the type of intermediate filament.

As the previous overviews show, the rapid development of microscopy has already made a significant contribution to the investigation of the cell morphology. However, it can be expected that the high level computer technology can also significantly contribute to this topic. The pioneering works in this area were based on the continuum hypothesis and did not explicitly consider the effect of inhomogeneity inside the cell (Fung, 1967). The more precise models incorporating data of the microstructure are more recent. For example, tensegrity models were first proposed by Ingber and co-workers Ingber et al. (1981); Ingber (1993). This concept has furthermore been developed by Coughlin and Stamenović Coughlin and Stamenović (1997) who proposed a model simulating filaments as linear elastic cables bearing tension. On the contrary, microtubules have been simulated as struts able to bear both tension and compression. Additionally, struts have been allowed to buckle under compression. Later on, Wendling et al. Wendling et al. (1999) used large-scale tensegrity models to show the stiffening behavior of microscale living cells under deformation. The postbuckling behavior of the microtubules has been studied by Volokh et al. Volokh et al. (2000). The possible softening response of the cell due to buckling of microtubules has also been investigated in this work. A detailed description on tensegrity models simulating the mechanical behavior of living cells is available in, e.g., Volokh Volokh (2011).

Apart from the tensegrity models, there are several alternative approaches worth mentioning. For example, Wu and co-workers (Wu et al., 1999; Wu and Herzog, 2000) proposed a large strain macro-micro model in which the effective macroscopic behavior is determined by a linear homogenization of a microstructure with a single spherical cell inclusion, according to the Es-
helby’s principle (Eshelby, 1956; Christensen, 1991). On the other hand, the group of Reddy and co-workers (Unnikrishnan et al., 2006; Reddy et al., 2009) applied the Mori-Tanaka homogenization scheme (Mori and Tanaka, 1973) for that purpose. This method enables the simulation of a more complex microstructure but is not appropriate for large deformations. Furthermore, mathematical models based on the biphasic theory (Barocas and Tranquillo, 1997b,a; Lai et al., 2013) and on a combination of continuum and statistical approaches (Zahalak et al., 2000) have been proposed to account for the active behavior of cells on the macroscopic tissue behavior. Another strategy certainly worth mentioning is the rule-of-mixtures model proposed by the group of Humphrey (Humphrey, 2002; Na et al., 2004). Surprisingly, the multiscale finite element method (FEM) has been rarely applied in this field. The first step in this direction was made in the work by Breuls et al. (Breuls et al., 2002), however, their contribution deals with the modeling of soft tissue consisting of a matrix material and single cells, and it does not consider the cell microstructure in detail.

Nowadays, the multiscale FEM (Miehe et al., 1999, 2002; Ilic and Hackl, 2009; Klinge and Hackl, 2012) is one of the most widely used numerical homogenization schemes. The reason is its applicability to different macroscopic and microscopic problems even with the highly nonlinear material behavior. The authors have already successfully used this strategy for the modeling of reinforced polymers Klinge et al. (2012b,a), for the simulation of diffusive processes Klinge and Hackl (2017) and for the investigation of bone tissue Ilic et al. (2010, 2011).

The application of the multiscale FEM requires suitable continuum mechanical models for component materials. In the present case, this relates to the actin network and the cytosol. Actin filaments build the cross-linked network known as actin cortex. The latter is connected to the cell membrane and plays an important role as regards the cell reaction to external stimuli Alenghat and Ingber (2002). Typical solutions suitable for simulating actin networks are the one-filament approach of Palmer and Boyce Palmer and Boyce (2008), the single filament model of MacKintosh et al. MacKintosh et al. (1995) for inextensible fibers, the eight-chain model of Arruda and Boyce Arruda and Boyce (1993) and the β-model for extensible fibers proposed by Ogden and Holzapfel Holzapfel and Ogden (2011). The β-model has already been implemented in a microsphere approach Unterberger et al. (2013) characteristic of the homogenization of polymers Miehe et al. (2004), however, the intention here is to apply the multiscale FEM to this end. The
choice of the multiscale FEM is substantiated by the fact that this approach is more flexible with regard to the modeling of the representative volume element (RVE) depicting the material microstructure. The present work focuses on the computer modeling of two components influencing the mechanical cell behavior, namely the interaction of the actin network and cytosol. In the future, the developed model should serve as a basis for extended models taking into account the influence of the remaining cytoskeleton components.

The present contribution is structured as follows. The main properties of a single actin filament are summarized in Section 2, whereas Sections 3 and 4 recapitulate the basic principles of the $\beta$-model and of the homogenization theory. After this introductory part, Section 5 explains the relations necessary for the development of an element based on the $\beta$-model, and Section 6 provides details on the macroscopic and microscopic formulations and on the differences between the singlescale and the multiscale concept. Finally, representative numerical examples pertaining to the different microstructures and types of load demonstrate the application of the strategy (Section 7). Attention is particularly paid to the influence of the filament orientation on the effective behavior. The paper finishes with conclusions and an outlook.

2. Main properties of single actin filaments

Actin filaments belong to the group of semi-flexible biopolymers. These polymers are characterized by being too stiff to form loops but flexible enough to exhibit thermal bending. A typical form of an actin filament is shown in Fig. A.1. Here, $s \in [0, L]$ denotes the arc-length coordinate along a chain, $L$ is the total arc-length (contour length), $r$ is the end-to-end distance, $u_H$ is the distance between a point and its projection to the end-to-end distance, while $f$ denotes a tensile force.

The material properties essential for the mechanical modeling of a single filament are the stretch modulus $\mu_0$, the Young’s modulus $E$, the bending stiffness $B_0$ and the persistence length $l_p$. The stretch modulus has an approximate value of 40 nN. Kojima et al. Kojima et al. (1994), e.g., found a stretch modulus of approximately 43.7 nN, whereas the fitting procedure in Unterberger et al. Unterberger et al. (2013) yielded a value of 38.6 nN. The values for the Young’s modulus of actin filaments vary between $1.8 \times 10^8$ Pa Kojima et al. (1994) and $2.5 \times 10^9$ Pa Huxley et al. (1994). The Young’s modulus influences the bending stiffness of the filament which is defined as $B_0 = EI$. Here, $I$ is the area moment of inertia of the filament calculated
as $I = \pi a^4/4$ since actin filaments have a circular cross-section ($a$ denotes the radius of the filament). Their radius amounts to approximately 4 nm Ott et al. (1993). Another important property of the filaments is their persistence length

$$l_p = \frac{B_0}{k_B T}.$$  \hspace{1cm} (1)

In this expression, $k_B = 1.38 \times 10^{-23}$ Nm/K is the Boltzmann constant and $T$ is the absolute temperature (in K). The persistence length is determined when the bending energy equals the thermal energy. It can be defined as the maximum length over which the filament will appear straight in the presence of the constant Brownian forces which it experiences in a medium at finite temperature. There is a variety of experimental methods for the determination of the persistence length. Some of the most important ones are atomic force microscopy, optical and magnetic tweezers, electron microscopy and small-angle X-ray scattering. For more details on this topic the reader is referred to the review article of Strick et al. Strick et al. (2000). Earlier studies measured the persistence length of actin filaments to a value of 16-17.7 \(\mu\)m Gittes et al. (1993b); Ott et al. (1993); Janmey et al. (1994), whereas later works measured it to a smaller value, namely 8.75 \(\mu\)m Liu and Pollack (2002). A collection of persistence lengths of actin filaments in different structural and functional states is documented by Isambert et al. Isambert et al. (1995).

3. Holzapfel–Ogden $\beta$-model

The model proposed in Holzapfel and Ogden (2011) starts by considering the local equilibrium conditions at a point of a polymer chain with the assumption that the tensile force and the bending moment are conservative, i.e. they can be expressed as derivatives of a suitably chosen potential. With this assumption, it has been shown that the effect of thermal fluctuation is equivalent to the effect of transverse body force distribution, and that the bending of a chain can be described by a differential equation of the form

$$B_0 u'''' - f u'' = b,$$ \hspace{1cm} (2)

where $b$ is the body force. Equation (2) reassembles the generally known differential equation corresponding to buckling problems. Two important differences need to be pointed out here: (i) the sign in front of the second term
is negative since a tensile force (and not a compressive force) is considered; (ii) the equation is inhomogeneous which enables non-trivial solutions of the problem.

The solution of Eq. (2) can be expressed in terms of Fourier series. Such an ansatz is advantageous in comparison with the sinusoidal solution proposed by MacKintosh MacKintosh (2006), see Holzapfel and Ogden (2011, 2013). As a result, the model yields a relationship between the relative change \( r/L \) and the external load, i.e.

\[
\frac{r}{L} = 1 + \alpha f^* - \frac{(1 + 2 \alpha f^*) (1 + \alpha f^*)} {1 + f^* + \alpha (f^*)^2} \left( 1 - \frac{r_0}{L} \right) .
\]

(3)

Here, \( r_0 \) is the end-to-end distance at zero force, and \( f^* \) is a dimensionless force, i.e.

\[
f^* = \frac{f}{\alpha \mu_0}.
\]

(4)

It is assumed that no compressive force occurs, which means that \( f \geq 0 \) and consequently \( r_0 \leq r \). The dimensionless parameter

\[
\alpha = \frac{\pi^2 B_0}{\mu_0 L^2}
\]

(5)

includes bending and extensional stiffness. Expressions similar to Eq. (3) have been proposed in some other works MacKintosh et al. (1995); Blundell and Terentjev (2009), however, the value of the exponent \( \beta \) is still a matter of discussion. This exponent is addressed as the effective extensional modulus and it holds that \( \alpha \ll \beta \).

Whereas Eq. (3) is a relation between \( r \) and \( f^* \), some applications require a study of the dependence between the stretch \( \lambda \) and the external influences. In order to enable such an analysis, the properties of a single filament in the initial configuration are investigated. Here, a polymer chain has a pre-stretch \( \lambda_0 \) representing the ratio of the initial end-to-end distance \( \tilde{r} \) and end-to-end distance at zero force \( r_0 \)

\[
\lambda_0 = \frac{\tilde{r}}{r_0}.
\]

(6)

The initial stretch \( \lambda_0 \) has to be determined experimentally and it amounts to 1.027 for actin polymers Unterberger et al. (2013). The total stretch is
subsequently expressed as the ratio between the current and the initial end-to-end distance according to

\[ \lambda = \frac{r}{r_0} = \frac{r}{\lambda_0 r_0}. \] (7)

This equation, together with Eq. (3), gives the sought relationship between the stretch \( \lambda \) and the force \( f^* \).

The present model does not assume any explicit formulation for the potential of the external force \( f \). However, since the relationship (3) is available, the derivatives of the missing potential can be calculated indirectly. In order to show this, force \( f \) is expressed as the derivative of the corresponding potential \( \Psi_f \), namely, it holds \( f = \partial \Psi_f / \partial r \). The previous definition and the chain rule are applied to express the first derivative of the potential with respect to stretch \( \lambda \) as

\[ \Psi'_f = \frac{\partial \Psi_f}{\partial \lambda} = \frac{\partial \Psi_f}{\partial r} \frac{\partial r}{\partial \lambda} = f r_0 \lambda_0, \] (8)

where \( f \) has to be determined by solving Eq. (3). In order to determine the second derivative, Eq. (3) is expressed in terms of \( \lambda \) and \( \Psi'_f \) by using Eqs. (7) and (8). The obtained relationship is thereafter derived with respect to \( \lambda \), which finally yields Holzapfel and Ogden (2011, 2013)

\[ \Psi''_f = \frac{\lambda_0^2 r_0^2 \mu_0}{L + Y \left[ \frac{1 + \alpha f^*}{1 + \frac{f^*}{\alpha}} \right]^\beta (L - r_0)}, \] (9)

\[ Y = \frac{\beta (1 + 2 \alpha f^*)^2}{\alpha (1 + f^* + \alpha (f^*)^2) - \beta \frac{1 + 2 \alpha f^*}{1 + \alpha f^*} - 2}. \] (10)

Equations (8) and (9) are now the basis for the derivation of the stress and elasticity tensors, two quantities essential for the development of a corresponding type of element within an FEM code. These steps are shown in Section 5.

4. The multiscale FEM

For realistic simulations of the cell activity it is necessary to investigate the effective behavior of a network of filaments and their interaction with
other cell compartments. The solution of such a problem requires the application of a homogenization strategy such as the multiscale FEM Miehe et al. (1999, 2002); Ilic and Hackl (2009); Klinge and Hackl (2012). This method is applied for modeling statistically uniform materials, i.e. the group of materials whose microstructure can be represented by a suitably chosen representative volume element (RVE). The latter has to be understood as a material sample whose investigation yields the effective material properties.

In order to define an RVE, attention must be paid to a few limitations: the ratio of the characteristic lengths of the assumed RVE and of a corresponding homogeneous macroscopic body must tend to zero, but simultaneously the RVE must be large enough to contain all necessary information on the material microstructure. A situation typical of the multiscale simulations is shown in Fig. A.2. Here, an RVE is associated to a point of a macroscopic body and includes information on the material microstructure. Accordingly, two boundary value problems (BVPs) are defined: one related to the simulation of the macroscopic body and one to the analysis of the RVE. The first BVP is completely defined except for the constitutive law, which is replaced by the results of the microscopic level. On the other hand, the microscopic BVP is well posed except for the boundary conditions. Their derivation requires an additional condition, however, the multiscale techniques are not unique regarding this issue, as briefly explained in the following passage.

The simplest multiscale model goes back to Voigt (Voigt, 1889) who assumed the effective elasticity tensor to be the volume average of the elasticity tensors at the microlevel. Reuss (Reuss, 1929) met an analogous assumption for the compliance tensor. Nowadays, more modern theories consider energetic aspects of the process, which is especially advantageous in the case of modeling nonlinear materials. This kind of approaches start with the Hill assumption requiring that the macroscopic quantities are expressed dependent on the microscopic quantities acting on the boundary of the RVE (Hill, 1952, 1963, 1972). This can be physically interpreted, if the RVE is understood as a material sample cut from the macroscopic body. The information flow only takes place over the boundary in this case. The macroscopic body does not have any information on the state of variables inside the RVE. Bearing the Hill postulate in mind, the macroscopic deformation gradient $F_{\text{mac}}$ and
the first Piola-Kirchhoff tensor $P_{\text{mac}}$ are defined as follows

$$F_{\text{mac}} := \frac{1}{V} \left( \int_B F \, dV - \int_{\mathcal{L}} x \otimes N \, dA \right) = \frac{1}{V} \int_{\partial B} x \otimes N \, dA, \quad (11)$$

$$P_{\text{mac}} := \frac{1}{V} \int_B P \, dV = \frac{1}{V} \int_{\partial B} T \otimes X \, dA. \quad (12)$$

Here, the notation typical of the theory of finite deformations is used: $X$ is the position vector, $N$ is the normal vector to the surface and $T$ denotes the traction. All these quantities belong to the reference configuration. Their counterparts in the current configurations are typically denoted by the lowercase letters $x$, $n$, $t$, respectively. The averaging is performed over volume $V$ of RVE $B$ with boundary $\partial B$ and the boundary of the voids inside the RVE $\mathcal{L}$. The formulation is general and applies to 2D and 3D cases.

The next step deals with the formulation of a condition regulating the transformation of microscopic quantities into the macroscopic ones. This condition is known as Hill-Mandel macrohomogeneity condition and represents a kind of a balance law (Hill, 1952, 1963, 1972). It postulates that the macroscopic power $P_{\text{mac}} : \dot{F}_{\text{mac}}$ is equal to the volume average of the microscopic power, i.e.

$$P_{\text{mac}} : \dot{P}_{\text{mac}} = \frac{1}{V} \int_B P : \dot{F} \, dV. \quad (13)$$

By using definitions (11) and (12), the Hill-Mandel condition (13) can be rewritten as a boundary integral

$$\frac{1}{V} \int_{\partial B} (T - P_{\text{mac}} \cdot N) \cdot (\dot{x} - \dot{F}_{\text{mac}} \cdot X) \, dA = 0, \quad (14)$$

which directly yields two types of boundary conditions for the BVP at the microscale, i.e.

$$t = P_{\text{mac}} \cdot N \quad \text{on } \partial B \quad \text{- static b.c.} \quad (15)$$

$$x = F_{\text{mac}} \cdot X \quad \text{on } \partial B \quad \text{- kinematic b.c.} \quad (16)$$

The derivation of the periodic boundary conditions, however, is based on another procedure. Here, the microdeformations are assumed in the form dependent on the macrodeformation gradient $F_{\text{mac}}$ and the microfluctuations $\tilde{w}$. Thus,

$$x = F_{\text{mac}} \cdot X + \tilde{w}. \quad (17)$$
Thereafter, the use of the assumption (17) in Eq. (13) yields the conclusion that the microfluctuations $\tilde{w}$ have to be periodic and the tractions $t$ antiperiodic on the periodic boundary of the RVE:

$$\tilde{w}^+ = \tilde{w}^- \quad \text{and} \quad t^+ = -t^- \quad \text{on } \partial B. \quad (18)$$

It is also interesting to note that assumption (17) leads to the additive decomposition of the microdeformation gradient

$$F = \text{Grad } x = F_{\text{mac}} + \tilde{F}, \quad \tilde{F} = \text{Grad } \tilde{w}. \quad (19)$$

Following the concept relying on Eqs. (11)-(19), the multiscale program MSFEAP has been developed Klinge and Hackl (2012); Ilic and Hackl (2009). The flow chart of this program is shown in Fig. A.3 and it consists of two main parts, each of them being a separate FEM program for the solution of one BVP. The FE program FEAP$_{PV}$ developed by the group of Taylor (Zienkiewicz and Taylor, 2000; Taylor, 2013) is used for this purpose. The coupling of both parts is achieved through a bridging subroutine where adjustment parameters are saved and the transfer of data between the scales takes place.

The execution of the program includes the following tasks: Initially, the calculations start at the macrolevel where the preprocessing is performed in a standard manner. However, the evaluations are performed just up to the moment when the constitutive law should be applied. As this law is not available, a bridging subroutine is called from the macroscopic element. At the microlevel, the complete BVP is solved: The macrodeformation gradient is passed from the macrolevel as an input value. The periodic boundary conditions are prescribed for the microfluctuations (alternatively static or kinematic boundary conditions). The primary results are the microfluctuations $\tilde{w}$ and the first Piola-Kirchhoff microstress tensor $P$. The macroscopic stress tensor is finally calculated as the volume average of microscopic stresses (Eq. (12)). Thus, the macroscale calculations can be continued. The microscale subprogram is called for calculating the effective stresses as well as their derivatives. Such calculations are repeated in each Gauss point for each step of the Newton-Raphson iteration (nonlinear materials) and, if necessary, for each step of the time iteration (time dependent problems). In the case of the linear material, the procedure is much simpler. Here, the effective elasticity tensor must be calculated only once as it is independent from deformation.
5. Implementation of the model for actin filaments into the FEM

In order to implement the $\beta$-model (Section 3) into an FE code, a two-node nonlinear truss element is developed. This element, in the reference configuration, has the length $L_e$, the cross-section area $A_e$ and the unit direction vector $G$. The corresponding quantities in the current configuration are $l_e$, $a_e$ and $g = F \cdot G$ (Fig. A.4). The linear shape functions, of nodes $A = 1, 2$, are used to map the physical and the parametric spaces

$$N^1(\xi) = (1 - \xi)/2, \quad N^2(\xi) = (1 + \xi)/2,$$

where $\xi \in [-1, 1]$ is the coordinate in the parametric space. The approximation of coordinates in the reference and current configuration is then written as

$$X^e = \sum_{A=1}^{2} X^{eA} N^A(\xi), \quad x^e = \sum_{A=1}^{2} x^{eA} N^A(\xi). \quad (21)$$

In order to describe the position of an arbitrary point $X^e$ of the truss element, the position of the starting node $X^{e1}$ and the unit vector $G$ are used, i.e.

$$X^e = X^{e1} + \tilde{X}^e G \Rightarrow \tilde{X}^e = \tilde{X}^e G = X^e - X^{e1}. \quad (22)$$

Now, the distance from the starting node to the arbitrary point $\tilde{X}^e$ can be expressed in terms of the $\xi$, i.e.

$$\tilde{X}^e = \sum_{A=1}^{2} \tilde{X}^{eA} N^A(\xi) = \tilde{X}^{e1}(1 - \xi)/2 + \tilde{X}^{e2}(1 + \xi)/2. \quad (23)$$

A transformation of the previous equation yields the expression for $\xi$

$$\xi(\tilde{X}^e) = \frac{1}{L_e} [2\tilde{X}^e - \left(\tilde{X}^{e1} + \tilde{X}^{e2}\right)], \quad (24)$$

which, together with the derivative $\partial \xi / \partial \tilde{X}^e = 2/L_e$, is the basis for the evaluation of derivatives of the shape functions in both configurations. Thus, it holds

$$\nabla_x N^A = \frac{\partial N^A}{\partial \xi} \frac{2}{L_e} G, \quad \nabla_x N^A = \frac{\partial N^A}{\partial \xi} \frac{2}{L_e} g. \quad (25)$$

Equation (24) is also useful for the derivation of the deformation gradient $F$ in terms of the stretch and the direction vectors, i.e.

$$F = \frac{\partial x}{\partial X} = \sum_{A=1}^{2} x^{eA} \otimes \frac{\partial N^A}{\partial \xi} \frac{2}{L_e} G = \frac{l_e}{L_e} g \otimes G = \lambda g \otimes G, \quad (26)$$
where \( \mathbf{x}^e = l_e \mathbf{g} \) holds. For the chosen framework, the first Piola–Kirchhoff stress tensor \( \mathbf{P} \) and Cauchy stress tensor \( \mathbf{\sigma} \) are written as the dyadic products

\[
\mathbf{P} = \tilde{\mathbf{P}} \mathbf{g} \otimes \mathbf{G}, \quad \mathbf{\sigma} = \tilde{\mathbf{\sigma}} \mathbf{g} \otimes \mathbf{g}.
\]  

(27)

Here, \( \tilde{\mathbf{P}} \) and \( \tilde{\mathbf{\sigma}} \) are scalars to be evaluated from the constitutive law. Their equality can be easily shown as

\[
\mathbf{\sigma} = \mathbf{P} \text{cof}(\mathbf{F}^{-1}) = J^{-1} \mathbf{P} : \mathbf{F}^T = \tilde{\mathbf{P}} \mathbf{g} \otimes \mathbf{g} \Rightarrow \tilde{\mathbf{P}} = \tilde{\mathbf{\sigma}},
\]  

(28)

where \( J = \det \mathbf{F} > 0 \) denotes the volume ratio. Bearing in mind the common definition for the virtual internal work

\[
\delta \Pi_{\text{int}} = \int_V \mathbf{P} : \mathbf{\delta F} \, dV
\]

and the corresponding expression for the residual due to the internal forces \( \mathbf{f}_{\text{int}} = \int_V \mathbf{P} : \nabla \mathbf{x} N^A \, dV \), the simple relationship holding for a truss element can be derived, i.e.

\[
\mathbf{f}_{\text{int}} = 2 \tilde{\mathbf{P}} A_e \mathbf{g} \frac{\partial N^A}{\partial \xi} = 2 \tilde{\mathbf{\sigma}} A_e \mathbf{g} \frac{\partial N^A}{\partial \xi}.
\]  

(29)

The stiffness matrix is then defined as the derivative \( \mathbf{K}^A = \partial \mathbf{f}_{\text{int}} / \partial \mathbf{x}^B \), which finally yields

\[
\mathbf{K}^A = 4 A_e \frac{\partial \tilde{\mathbf{P}}}{\partial \lambda} \frac{\partial N^A}{\partial \xi} \mathbf{g} \otimes \mathbf{g} \frac{\partial N^B}{\partial \xi} + 4 A_e \frac{\partial N^A}{\partial \xi} (\mathbf{I} - \mathbf{g} \otimes \mathbf{g}) \frac{\partial N^B}{\partial \xi}.
\]  

(30)

At this place, it should be noted that the \( \beta \)-model (Section 3) does not yield the expressions for the stresses directly. However, \( f \) in Eq. (8) is a force in the current configuration such that the following relationships hold:

\[
f = \Psi^f / \lambda_0 r_0 = A_e \tilde{\mathbf{P}}, \quad \Psi'^f / \lambda_0 r_0 = A_e \partial \tilde{\mathbf{P}} / \partial \lambda.
\]  

(31)

Accordingly, the residual and the stiffness matrix for the \( \beta \)-model have the form

\[
\mathbf{f}_{\text{int}} = 2 \frac{\Psi^f}{\lambda_0 r_0} \mathbf{g} \frac{\partial N^A}{\partial \xi},
\]  

(32)

\[
\mathbf{K}^A = 4 \frac{\Psi^f}{\lambda_0 r_0} \frac{\partial N^A}{\partial \xi} \mathbf{g} \otimes \mathbf{g} \frac{\partial N^B}{\partial \xi} + 4 \frac{\Psi'^f}{\lambda_0 r_0} N^A (\mathbf{I} - \mathbf{g} \otimes \mathbf{g}) \frac{\partial N^B}{\partial \xi},
\]  

(33)

where \( \Psi^f \) and \( \Psi'^f \) are given by Eqs. (8) and (9).
6. Implementation of the model for actin filaments into the multiscale FEM

6.1. Macroscopic level

As shown in Section 4, the multiscale FEM concept relies on the solution of two BVPs: the first one is related to the macroscopic structural level, whereas the second one is related to the simulation of the RVE and provides the missing quantities for the effective constitutive law. The present work considers an RVE consisting of a filament network together with the cytosol. The behavior of both these phases is nonlinear such that the nonlinear effective behavior is expected. This argument, together with the fact that a nearly incompressible behavior is expected, motivates the application of an element which is based on the mixed formulation. The three-field potential typical of this case Simo and Hughes (1997); Holzapfel (2000) has the form

\[ \Pi(\mathbf{u}, \Theta, p) = \int_V \left[ \hat{\Psi}(\Theta) + \overline{\Psi}(\overline{C}(\mathbf{u})) + p(J(\mathbf{u}) - \Theta) \right] dV + \Pi_{\text{ext}}. \] (34)

Apart from the displacements \( \mathbf{u} \), which are primary variables, this formulation also depends on volume change \( \Theta \) and pressure \( p \). The functional is split into a volumetric and a deviatoric part. In this formulation, the volumetric part only depends on volume change \( \Theta \) and the deviatoric part depends on the deviatoric right Cauchy-Green tensor \( \overline{C} = J^{-2/3}C \) Holzapfel (2000). The last term in the integral in Eq. (34) represents the Lagrange term introduced in order to stipulate the equality of the determinant of the Jacobian and the volume change. The hat symbol denotes volumetric quantities, and the bar symbol denotes deviatoric ones. The potential corresponding to the external loads is denoted by \( \Pi_{\text{ext}} \). Note, that all quantities here are related to the macroscopic level such that no additional symbols are introduced to distinguish the macroscopic from the microscopic quantities.

For the formulation of the element, the first and second variation of potential (34) are needed: the first one in order to minimize the potential, and the second one to linearize the problem. Here, only the second variation is presented since it is the basis for the numerical implementation.
\[
\int_V \text{Grad} \delta u : [\text{Grad} \Delta u \cdot (\bar{S} + \hat{S})] \, dV + \int_V (\text{Grad}^T \delta u \cdot \bar{F}) : (\bar{C} + \hat{C}) : (\bar{F}^T \cdot \text{Grad} \Delta u) \, dV \\
+ \int_V (\text{Grad}^T \delta u \cdot \bar{F}) : J \bar{C}^{-1} \, dV \left(\frac{1}{V} \frac{\partial^2 \hat{\Psi}}{\partial \Theta^2}\right) \int V J \bar{C}^{-1} : (\bar{F}^T \cdot \text{Grad} \Delta u) \, dV + \Delta \delta u \Pi_{\text{ext}} = -\delta u \Pi_{\text{res}},
\]

(35)

\[
\delta u \Pi_{\text{res}} = \delta u \Pi_{\text{ext}} + \delta u \Pi_{\text{int}}, \quad \delta u \Pi_{\text{int}} = \int_V (\text{Grad}^T \delta u \cdot \bar{F}) : (\bar{S} + \hat{S}) \, dV,
\]

where \(\delta u \Pi_{\text{int}}\) is the variation of the internal potential energy \(\Pi_{\text{int}}\) (the internal virtual work), \(\delta u \Pi_{\text{ext}}\) related to the external virtual work, while \(\delta u \Pi_{\text{res}}\) is the residual. In the previous expressions, \(\bar{S}\) and \(\hat{S}\) denote the deviatoric and the volumetric second Piola–Kirchhoff stress tensors while \(\bar{C}\) and \(\hat{C}\) denote the macroscopic deviatoric and volumetric elasticity tensors (Holzapfel, 2000). The Neumann boundary conditions are fulfilled in the integral form while the Dirichlet boundary conditions are fulfilled pointwise. The displacements prescribed on the boundary part \(\mathcal{B}_u\) are denoted by \(u_0\). On that boundary part, the variation of the displacements \(\delta u\) and their increments \(\Delta u\) are equal to zero. Unlike the single-scale formulation, terms \(\bar{S}, \hat{S}, \bar{C}, \hat{C}\) and \(\partial^2 \hat{\Psi}/\partial \Theta^2\) cannot be calculated directly since they depend on the effective potential \(\Psi\) which is not available. For the calculation of these terms, the analysis at the microlevel is applied. For more details on the numerical implementation of this model see Klinge and Hackl (2012, 2017); Klinge et al. (2015) and Appendix Appendix A.1.

6.2. Microscopic level

Three different types of elements are applied for the simulations at the microscopic level. The two-node line elements (Section 5) are used for the modeling of filaments, whereas the triangle elements and four-node tetrahedron elements are used for the modeling of the cytosol. The cytosol elements are standard elements of the FE-Program FEAP Taylor (2013) and use the stored energy corresponding to the neo-Hookean material law

\[
\Psi = (K - \frac{2}{3}G)U(J) + \frac{1}{2}G(I_1 - 3 - 2 \ln J),
\]

(36)
where $K$ is the bulk modulus, $G$ is the shear modulus, $I_1 = \text{tr} \, C$ is the first invariant of $C$ and $U(J) = (\ln J)^2/2$ denotes the volumetric part of the energy. Note, that such a stored energy depicts a different behavior under tension and compression. The second variation gives

$$
\int_{V_{\text{cyt}}} \text{Grad} \, \delta \tilde{w} : (\text{Grad} \Delta \tilde{w} \cdot S) \, dV + \int_{V_{\text{cyt}}} (\text{Grad}^T \delta \tilde{w} \cdot F) : C : (F^T \cdot \text{Grad} \Delta \tilde{w}) \, dV = -\delta \tilde{w} \Pi_{\text{res}}(F),
$$

$$
\delta \tilde{w} \Pi_{\text{res}}(F) = \delta \tilde{w} \Pi_{\text{int}}(F), \quad \delta \tilde{w} \Pi_{\text{int}} = \int_{V_{\text{cyt}}} (\text{Grad}^T \delta \tilde{w} \cdot F) : S \, dV. \quad (37)
$$

The problem here depends on microfluctuations $\tilde{w}$ and no longer on macrodeformations $u$. Integration is performed over the cytosol phase of the RVE ($V_{\text{cyt}}$).

The element itemized requires an adaptation for the application at the microscopic level. Two main differences in comparison with the singlescale (original) formulation have to be pointed out. First, no external forces act on the sample, with the consequence that the residual includes just a term due to the internal response. However, the macroscopic deformation gradient is assumed as the initial value of the microscopic deformation gradient (Eq. (19)). Secondly, over the boundary of the RVE, one type of the boundary conditions specified in Eqs. (15), (16) or (18) must be fulfilled. In subsequent examples, periodic boundary conditions are assumed for two reasons: (i) the periodicity of the microstructure can be assumed as good approximation of the realistic microstructure; (ii) the periodic boundary conditions yield the best numerical results Zohdi and Wriggers (2008). The final solutions at the microscopic level are microstresses, the volume average of which yields its macroscopic counterpart expressed by Eq. (12). More details on the numerical implementation of elements at the microscale are provided in Appendices Appendix A.2-Appendix A.4.

7. Representative numerical examples

7.1. Material properties

The following numerical examples deal with the simulation of macroscopic tests if a two-phase RVE depicts the cell microstructure. The material parameters proposed in Unterberger et al. Unterberger et al. (2013) are chosen for the filaments, whereas a low Young’s modulus and a high Poisson’s ratio are assumed to simulate the nearly incompressible fluid, the cytosol. The
Young’s modulus of cytosol has been chosen such that the results of the macroscopic simulations fit to the experimental data presented in Lin et al. Lin et al. (2009). The Poisson’s ratio is taken from the work of Slomka and Gefen (Slomka and Gefen, 2010). An overview of the material parameters used in the simulations is presented in Table 1.

<table>
<thead>
<tr>
<th>Filaments</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stretch modulus</td>
<td>$\mu_0$</td>
<td>38.6 nN</td>
</tr>
<tr>
<td>Ratio of lengths $c = L/r_0$</td>
<td>1.1</td>
<td>-</td>
</tr>
<tr>
<td>Initial stretch $\lambda_0$</td>
<td>1.027</td>
<td>-</td>
</tr>
<tr>
<td>Extensional modulus $\beta$</td>
<td>0.438</td>
<td>-</td>
</tr>
<tr>
<td>$\alpha$-Parameter $\alpha$</td>
<td>$10^{-7}$</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cytosol</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Young’s modulus $E_c$</td>
<td>20 kPa</td>
<td></td>
</tr>
<tr>
<td>Poisson’s ratio $\nu_c$</td>
<td>0.45</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1: Material parameters used in the simulations.

Note, that the end-to-end distance $r_0$ is not listed in the table but is calculated as the length of two-node elements corresponding to each single filament. Thereafter, the contour length is calculated according to the relationship $L = cr_0$ and the bending stiffness as $B_0 = \alpha \mu_0 L^2/\pi^2$.

7.2. Numerical example studying the effective behavior for different loading cases

This section presents numerical results for different loading cases and includes an analysis of the main properties of the model. The set-up corresponding to the first example is shown in Fig. A.5. The macroscopic problem (Fig. A.5(a)) pertains to the tensile test of a square sample with the dimensions $100 \times 100 \mu$m. The applied tension load is 5 kPa. The vertical displacements are suppressed at the lower boundary and horizontal displacements at the left vertical boundary. The element based on the mixed formulation (Section 6.1 and Appendix Appendix A.1) is applied for the macroscopic simulations. An RVE shown in Fig. A.5 (b) is assigned to each Gauss point of the macroscopic example. The orientation of the RVE is defined by a rotation angle $\varphi$. In this section, the rotation angle is constant for all Gauss points. Two types of simulations are performed: (i) $\varphi = 0^\circ$, (ii) $\varphi = 90^\circ$.  

17
The RVE (Fig. A.5(b)) has the size of $10 \times 10 \, \mu m$ and the displacements are constrained at the corners. The two-node element is used for modeling the filaments (Section 5 and Appendix A.3), and a triangle neo-Hookean element from FEAP Taylor (2013) is used for simulating the cytosol (Section 6.2 and Appendix A.2). The average distance between the filament contacts is $0.7 \, \mu m$, which is an assumption based on the experimental results presented by Niederman et al. Niederman et al. (1983). The dominant filament orientation is taken to be different for the $x$ and $y$-directions in order to study the influence of this factor on the effective behavior. The distribution of filaments is chosen arbitrarily and an alternative RVE can be easily generated according to the experimental observations.

The numerical results for the tension test are presented in Fig. A.6 and enhance the view that the filament network in the tension mode behaves as a reinforcement of the cytosol. In other words, the results show that the deformations are much higher for a homogeneous cytosol (Fig. A.6(a)) than for a cytosol and filament network (Figs. A.6(b) and (c)). Simulations are performed for two different orientations of the RVE with the rotation angles $\phi = 0^\circ$ and $\phi = 90^\circ$. The orientation of filaments in the second case is almost parallel to the load direction such that these filaments are fully activated and provide a high resistance to the deformation.

In addition to the tensile test, a pressure test is conducted for the same geometry of the macroscopic sample and the RVE. The results show that the filaments in this case are hardly activated and that their influence is much lower than if a tensile force is applied (Fig. A.7). The numerical values do not differ much depending on the orientation of the RVE. Figure A.7 shows the displacements for the RVE with $\phi = 0^\circ$, however, these values are close to the ones which are calculated for $\phi = 90^\circ$.

The results for the pressure test are in excellent agreement with the experimental values which are presented in Lin et al. Lin et al. (2009). These authors, among others, show that a stress of 5 kPa corresponds to a strain of approximately 15% (see Lin et al. (2009) and Fig. 2c therein). The same values have been achieved in the simulations presented in this paper.

The applicability of the model will be furthermore illustrated by simulating a shear test (Fig. A.8). Here, a macroscopic sample with the dimensions $100 \times 30 \, \mu m$ is loaded by a horizontal load acting on the upper boundary. The intensity of the load is 2 kPa. The deformation of the lower boundary is constrained in both directions. The simulations are performed for the RVE without rotation ($\phi = 0^\circ$) and results show a difference of approximately 10%
in comparison with the homogeneous cytosol. The influence of filaments is here less pronounced than in the tension mode, and of the same order as in the pressure mode.

The next example was set up to study the influence of the cytosol on the effective behavior of the cytoplasm. For this purpose, the same RVE as before is used \((\varphi = 0^\circ)\), however, the Young’s modulus \(E_c\) of the cytosol was varied within the range 15-30 kPa. The tensile test is performed as shown in Fig. A.6. The results (Fig. A.9) suggest that the deformations significantly change with the change of parameters for the cytosol. Furthermore, this indicates that the influence of the cytosol cannot be neglected as is frequently done in numerical simulations. In contrast, the effective behavior of the cytoplasm is a result of the interaction of all its components, and this must be reliably depicted in the mechanical models.

The dependence of the effective deformation on the filament network density certainly is another interesting issue. In order to study this topic, the already described macroscopic test (Fig. A.5(a)) is simulated again, however, different RVEs are proposed this time. The number of line elements in the RVEs is varied within the range of 444-1218, whereas the size of the RVE is kept constant at \(10 \times 10 \, \mu m\). Two examples of the RVE are presented in Fig. A.10(a) and (b). The results (Fig. A.10(c)) show that the number of elements significantly influences the effective behavior. As expected, deformations decrease with the increasing number of filaments.

The final example in this section simulates a 3D test (Fig. A.11). The macroscopic sample is a hemisphere with a diameter of 20 \(\mu m\) loaded by a single tension force of 5 nN. Here, 1925 tetrahedron elements are used for the discretization. The vertical displacements are suppressed at the lower boundary. The RVE (Fig. A.11(c)) has the dimensions \(2.1 \times 2.1 \times 2.1 \, \mu m\) and contains 361 filament elements and 232 tetrahedron cytosol elements. The results (Figs. A.11(a) and (b)) once more show that the deformations are higher for the homogeneous cytosol than for the cytosol combined with filament network. Moreover, they indicate that the gradients of displacements are different. In the case of the homogeneous material, the displacements are rather high close to the acting point of the force, but diminish rapidly with the increasing distance from that point. In the case of the heterogeneous material, the filament network acts as a reinforcement: deformations are smaller and spread out over a larger volume.
7.3. Numerical example studying the influence of the filament orientation

The examples presented in the previous section assume that the orientation of the RVE is constant over the complete macroscopic body. Such a regular structure might be met in some synthetic man-made materials, but, in the case of a cell cytoplasm, the orientation of filaments is rather randomly distributed and the notion of dominant orientation can be introduced (Barocas and Tranquillo, 1997b,a; Lai et al., 2013). In order to study this effect, an additional example is provided such that the orientation of the RVE is generated from the Gauss distribution defined by the expression

\[ f(\varphi, \varphi_0, \sigma) = \frac{1}{\sigma \sqrt{2\pi}} \exp \left( -\frac{(\varphi - \varphi_0)^2}{2\sigma^2} \right), \]

where \( \varphi \) is a variable to be generated, i.e. the orientation angle of the RVE in each Gauss point, \( \varphi_0 \) is the average value and \( \sigma \) is the deviation. Within this example, two groups of tension tests are performed. In the first case the average value of the RVE orientation is \( \varphi_0 = 0^\circ \) in the second case the average orientation is \( \varphi_0 = 90^\circ \). In both cases the deviation takes values from the range \([0, 30]\) with step size 2.

The calculated displacements at the middle point of the upper boundary are shown in Fig. A.12(b). As expected, the results in the first group of tests increase and in the second group of tests decrease. The values change gradually. The starting points of both curves in the diagram correspond to the values shown in Fig. A.6(b) and (c). By the deviation in the range \([25, 30]\), the displacements obtained in the different tests become nearly equal. The end values obtained in both simulations correspond to the case of a completely random microstructure without any dominant direction. Of course, a similar study can be carried out for other parameters such as the density or the length of filaments.

8. Conclusions and outlook

The present study focuses on the application of the multiscale FEM to the modeling of the actin network embedded in the cytosol. The actin filaments are simulated by the newly developed two-node element which is based on the Holzapfel–Ogden \( \beta \)-model, whereas the triangle element and the four-node tetrahedron element with neo-Hookean material are used for modeling the cytosol. The numerical results meet the expectation that the actin network
behaves as the reinforcement of the cytosol. Another observation is that it has a strong influence on the material response shown in tensile tests, whereas the influence by the shear and pressure tests are less pronounced. This is explained by the fact that the actin filaments are only active in the tension regime. The examples chosen demonstrate that the procedure can be applied for the simulation of various types of microstructures and for different macroscopic tests. An important observation is the strong influence of the filament orientation on the effective response. This effect is studied by performing simulations with the RVE orientation generated from the Gauss distribution.

The developed approach and the achieved results certainly give rise to several open issues. In a first step, the models for both phases can be improved by considering viscous effects which are typically present in actin networks. This is also important to be considered for the gel-like cytosol which does not bear any shear except in the case of a dynamic loading. In this context, an extended form of free energy is necessary. A more realistic RVE can also be built by including additional components such as intermediate filaments, microtubules and organelles. The first step in this direction is the formation of a more complex framework including different types of line elements that correspond to the microtubules and the microfilaments. Such an RVE can be understood as a tensegrity model which is additionally embedded in the cytosol. In a further step, line elements corresponding to intermediate filaments should be introduced. The implementation of new element types, however, requires the application of appropriate material models. These can be developed on the basis of experimental data and of existing material models.

Microtubules, e.g., are commonly modeled as a continuous elastic structure such as an elastic beam or elastic shell. Standard works following this type of approach treat a microtubule as a free-standing element Wang et al. (2006); Tounsi et al. (2010); Xiang and Liew (2012) and deal with the effects such as buckling and vibrations. However, many investigations have shown that microtubules can bear a compressive force much higher than the theoretical critical buckling force of a free-standing element Li (2008). For this reason, newer contributions simulate the surrounding biopolymers by treating them as a homogeneous elastic foundation Brangwynne et al. (2006); Jiang and Zhang (2008); Ghavanloo et al. (2010); Shen (2011); Zeverdejani and Beni (2013). The RVE proposed in the present contribution is advantageous from this point of view since it does not require any artificial foundation.
The simulation of crosslinkers and increased stability of the cytoskeleton is achieved by introducing the cytosol as an additional phase. Whereas a considerable number of contributions that simulate microtubules is already available, the modeling and the investigation of intermediate filaments are at their early stage. This topic is, e.g., investigated by Buehler and co-workers Qin et al. (2009, 2010), which uses the principles of molecular dynamics, cryo-electron microscopy and atomic-force microscopy. An alternative approach was proposed in a study by Wang and Stamenović Wang and Stamenovic (2002) which treats vimentin as a line element of a tensegrity structure. This model along with the up-to-date experimental results Block et al. (2015) might be an appropriate basis for a multiscale implementation.

The previous overview clearly shows that the multiscale FEM is a promising method with regard to a reliable simulation of the mechanical behavior of cells. The method is especially advantageous since the RVE can be easily redefined and the new components of the microstructure can easily be introduced. For this reason, not only mechanics, but also the simulation of the cell metabolism can be envisaged as an exciting and challenging objective of future work.

ACKNOWLEDGEMENT

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Appendix A. Numerical implementation

Appendix A.1. Macroscopic level: Element based on the mixed formulation (Section 6.1)

The element used at the macroscale relies on the mixed potential proposed by Simo, Taylor and Pister Simo and Hughes (1997); Holzapfel (2000), recall Eq. (34). Since the potential provided in Eq. (34) defines a nonlinear problem, the corresponding BVP includes the second variation of Eq. (34) together with the Dirichlet boundary conditions, i.e. Eq. (35). Note, that in Eq. (35), terms that depend on the constitutive law ($\mathbf{S}$, $\mathbf{\dot{S}}$, $\mathbf{C}$, $\dot{\mathbf{C}}$, $\partial^2\Psi/\partial\Theta^2$) cannot be calculated directly, but can be determined by using the data from the microscale.
The numerical approximation of Eq. (35) is performed in a standard manner, by replacing the original functions with expressions depending on the nodal values and shape functions, i.e.

\[
\begin{align*}
\mathbf{u}^e & = \mathbf{N} \cdot \hat{\mathbf{u}}^e, \quad \mathbf{B} = \text{Grad} \mathbf{N}, \quad \text{Grad} \Delta \mathbf{u}^e = \mathbf{B} \cdot \Delta \hat{\mathbf{u}}^e, \quad \text{Grad} \delta \mathbf{u}^e = \mathbf{B} \cdot \delta \hat{\mathbf{u}}^e. \\
\end{align*}
\]

(A.1)

Here, \( \mathbf{N} \) is a matrix containing the shape functions and \( \mathbf{B} \) is a matrix containing the derivatives of the shape functions. The check symbol denotes the nodal values and "e" indicates that all the DOFs of an element are considered. The implementation of approximations (A.1) into Eq. (35) leads to the stiffness matrix \( \mathbf{K}^e \), i.e.

\[
\begin{align*}
\mathbf{K}^e &= \int_{V^e} \mathbf{G}^T \cdot (\bar{\mathbf{S}} + \hat{\mathbf{S}}) \cdot \mathbf{G} \, dV + \int_{V^e} (\mathbf{B}^T \cdot \mathbf{F}) : (\bar{\mathbf{C}} + \hat{\mathbf{C}}) : (\mathbf{F}^T \cdot \mathbf{B}) \, dV \\
& \quad + \int_{V^e} (\mathbf{B}^T \cdot \mathbf{F}) : \mathbf{J} \mathbf{C}^{-1} \, dV \left( \frac{1}{V} \frac{\partial^2 \Psi_{\text{vol}}}{\partial \Theta^2} \right) \int_{V^e} \mathbf{J} \mathbf{C}^{-1} : (\mathbf{F}^T \cdot \mathbf{B}) \, dV,
\end{align*}
\]

with matrix \( \mathbf{G} \) defined in the following way

\[
\int_{V^e} \text{Grad} \delta \mathbf{u}^e : [\text{Grad} \Delta \mathbf{u} \cdot (\bar{\mathbf{S}} + \hat{\mathbf{S}})] \, dV = \delta \hat{\mathbf{u}}^e \cdot \int_{V^e} [\mathbf{G}^T \cdot (\bar{\mathbf{S}} + \hat{\mathbf{S}}) \cdot \mathbf{G}] \, dV \cdot \Delta \hat{\mathbf{u}}^e.
\]

By using the same approximation (A.1), the residual, as provided in Eq. (35), turns into

\[
\begin{align*}
\delta \mathbf{u}^e_{\text{res}} &= \delta \mathbf{u}^e_{\text{ext}} + \delta \mathbf{u}^e_{\text{int}} = -\delta \hat{\mathbf{u}}^e \cdot (\mathbf{f}^e - \mathbf{f}^e_{\text{int}}(\mathbf{u}_i)), \\
\delta \mathbf{u}^e_{\text{int}} &= \int_{V^e} (\text{Grad}^T \delta \mathbf{u}^e \cdot \mathbf{F}) : (\bar{\mathbf{S}} + \hat{\mathbf{S}}) \, dV = \delta \hat{\mathbf{u}}^e \cdot \int_{V^e} (\mathbf{B}^T \cdot \mathbf{F}) : (\bar{\mathbf{S}} + \hat{\mathbf{S}}) \, dV = \delta \hat{\mathbf{u}}^e \cdot \mathbf{f}^e_{\text{int}}(\mathbf{u}_i),
\end{align*}
\]

where \( \mathbf{f}^e \) is the vector of the external nodal forces, and \( \mathbf{f}^e_{\text{int}}(\mathbf{u}_i) \) the vector of the internal nodal forces due to the deformations from the previous step of the Newton-Raphson iteration. Finally, the assembling process yields the global system of algebraic equations

\[
\mathbf{K}(\hat{\mathbf{u}}_i) \cdot \Delta \hat{\mathbf{u}}_{i+1} = \mathbf{f} - \mathbf{f}^e_{\text{int}}(\hat{\mathbf{u}}_i),
\]

(A.2)

where the indexes \( i \) and \( i + 1 \) denote two succeeding steps of the Newton-Raphson iteration.
Appendix A.2. Microscopic level: Elements simulating cytosol (Section 6.2)

The simulation of cytosol is performed by applying three-node elements for 2D tests and four-node tetrahedron elements for 3D tests. The formulation of these elements uses the neo-Hookean potential (36).

The second Piola-Kirchhoff stress tensor $\mathbf{S}$ and the elasticity tensor $\mathbf{C}$ are determined in a standard manner, namely $\mathbf{S} = 2\partial \Psi / \partial \mathbf{C}$ and $\mathbf{C} = 4\partial^2 \Psi / \partial \mathbf{C}^2$, where $\Psi$ is the potential defined by Eq. (36). The dependency of the formulation on the microfluctuations is given through relationship (19), with the variation

$$\delta \mathbf{F} = \delta \tilde{\mathbf{F}} = \text{Grad} \, \delta \tilde{\mathbf{w}}.$$ 

By using the same kind of approximation as in Eq. (A.1), the stiffness matrix $\mathbf{K}^e$ and the residual vector $\mathbf{f}_{\text{int}}^e$ are derived as follows

$$\mathbf{K}^e = \int_{V^e} \mathbf{G}^T \cdot \mathbf{S} \cdot \mathbf{G} \, dV + \int_{V^e} (\mathbf{B}^T \cdot \mathbf{F}) : \mathbf{C} : (\mathbf{F}^T \cdot \mathbf{B}) \, dV,$$

$$\mathbf{f}_{\text{int}}^e = \int_{V^e} (\mathbf{B}^T \cdot \mathbf{F}) : \mathbf{S} \, dV.$$

Appendix A.3. Microscopic level: Line element simulating an actin filament (Sections 5 and 6.2)

In order to explain details on the numerical implementation of this element, the following notation is introduced: Vector $\mathbf{x}^e$ includes coordinates of the nodes $A$ and $B$ in the current configuration and vector $\tilde{\mathbf{w}}^e$ includes the corresponding microfluctuations

$$\mathbf{x}^e = \begin{bmatrix} x^{eA} & x^{eB} \end{bmatrix}^T, \quad \tilde{\mathbf{w}}^e = \begin{bmatrix} \tilde{x}^{eA} & \tilde{x}^{eB} \end{bmatrix}^T.$$

Bearing in mind the definition for the deformation at the microscale (Eq. (17)), it can be easily shown that the following equalities hold for the variations and the increments

$$\delta \mathbf{x}^e = \delta \tilde{\mathbf{w}}^e, \quad \Delta \mathbf{x}^e = \Delta \tilde{\mathbf{w}}^e.$$

Furthermore, the deformation gradient $\mathbf{F}$ for the chosen line element is expressed by

$$\mathbf{F} = \sum_{A=1}^{2} \mathbf{x}^{eA} \otimes \nabla \mathbf{x} \cdot \mathbf{N}^A,$$
as shown in Eqs. (25) and (26).

The special feature of the line elements developed in the present contribution is that the exact formulation of the potential is not available, but that its derivatives can be reconstructed (Section 3 and Eqs. (8) and (9)). For this reason, not the potential, but its first variation is considered as

$$\delta \Pi_{\text{int}} = \int_{V^e} \mathbf{P} : \delta \mathbf{F} \, dV = \int_{V^e} \mathbf{P} : \left( \sum_{A=1}^{2} \delta \mathbf{w}^e \otimes \nabla^e N^A \right) \, dV = \int_{V^e} \mathbf{P} : \left( \sum_{A=1}^{2} \delta \mathbf{\tilde{w}}^e \otimes \nabla^e N^A \right) \, dV$$

$$= \int_{V^e} \sum_{A=1}^{2} \delta \mathbf{w}^e \cdot \mathbf{P} \cdot \nabla^e N^A \, dV = \int_{V^e} \sum_{A=1}^{2} \delta \mathbf{\tilde{w}}^e \cdot \mathbf{P} \cdot \nabla^e N^A \, dV.$$

The last integral in the previous expression represents residual forces corresponding to node $A$, i.e.

$$f_{\text{int}}^A = \int_{V^e} \mathbf{P} \cdot \nabla^e N^A \, dV. \quad (A.3)$$

By using relationships explained in Section 3: $\nabla^e N^A = (\partial N^A / \partial \xi) (2/L_e) \mathbf{G}$; $V^e = A_e L_e$; $\mathbf{P} = \mathbf{\tilde{P}} \mathbf{g} \otimes \mathbf{G}$, expression (A.3) is further transformed into

$$f_{\text{int}}^A = 2 \mathbf{\tilde{P}} A_e \mathbf{g} \frac{\partial N^A}{\partial \xi}.$$

The complete residual vector includes the forces for both nodes $f_{\text{int}} = [f_{\text{int}}^A \ f_{\text{int}}^B]^T$, which yields the short expressions for the first and second variations of the potential

$$\delta \Pi_{\text{int}} = \delta \mathbf{\tilde{w}}^e \cdot f_{\text{int}}, \quad \Delta \delta \Pi_{\text{int}} = \delta \mathbf{\tilde{w}}^e \cdot \frac{\partial f_{\text{int}}}{\partial x^e} \cdot \Delta \mathbf{\tilde{w}}^e.$$

From the last relationship, the computation of the stiffness matrix is straightforward. Hence,

$$K^e = \frac{\partial f_{\text{int}}}{\partial x^e},$$

and it can be written in the block form

$$K^e = \begin{bmatrix} K^{eAA} & K^{eAB} \\ K^{eBA} & K^{eBB} \end{bmatrix},$$

where the single parts are defined as follows

$$K^{eAB} = \frac{\partial f_{\text{int}}^A}{\partial x^e B} = 4A_e \frac{\partial \mathbf{\tilde{P}}}{\partial \lambda} \frac{\partial N^A}{\partial \xi} \otimes \mathbf{g} \frac{\partial N^B}{\partial \xi} + 4A_e \frac{l_e}{L_e} \hat{N}^A (\mathbf{I} - \mathbf{g} \otimes \mathbf{g}) \frac{\partial N^B}{\partial \xi}.$$
Appendix A.4. Microscopic level: Assembling process

The global system of equations at the microscale is formed by incorporating stiffness matrices and residual vectors of elements simulating cytosol and filament network. Contrary to the macroscopic problem (Eq. (A.2)), the solution of this system of equations yields the increment of the microfluctuations, i.e.

\[
K(F_{\text{mac}}, \tilde{w}_i) \cdot \Delta \tilde{w}_{i+1} = - f_{\text{int}}(F_{\text{mac}}, \tilde{w}_i).
\]

The BVP is completed by the periodic boundary conditions as required by the homogenization scheme (Section 4)

\[
\tilde{w}^+ = \tilde{w}^-, \quad \text{on} \quad \partial B.
\]


Figure A.1: Geometry and main properties of a single actin filament.

Figure A.2: Multiscale scheme for the modeling of heterogeneous materials.
Figure A.3: The structure of the multiscale finite element program MSFEAP.

Figure A.4: Reference and current configurations of a filament with the unit direction vector $\mathbf{G}$ and its related quantity $\mathbf{g}$ mapped by the deformation gradient $\mathbf{F}$. 
Figure A.5: Geometry and discretization of the macroscopic sample (a), and of the RVE (b). The rotation angle $\varphi$ of the RVE is constant for all Gauss points.

Figure A.6: Results of a tensile test: (a) homogeneous material; (b) heterogeneous material without a rotation of the RVE ($\varphi = 0^{\circ}$); (c) heterogeneous material with a rotated RVE ($\varphi = 90^{\circ}$). Displacement 2 represents a vertical displacement in $\mu$m. Applied tension is 5 kPa.
Figure A.7: Results of a pressure test: (a) homogeneous material; (b) heterogeneous material. Displacement 2 represents a vertical displacement in \(\mu m\). Applied tension is 5 kPa.

Figure A.8: Shear test: (a) macroscopic sample; (b) numerical results for the cytosol with the filament network (without rotation, \(\varphi = 0^\circ\)). Displacement 1 represents a horizontal displacement in \(\mu m\). Applied tension is 2 kPa.

Figure A.9: Comparison of the results for different Young’s moduli \(E_c\) of the cytosol: (a) 15 kPa; (b) 20 kPa; (c) 30 kPa. Displacement 2 represents a vertical displacement in \(\mu m\). Applied tension is 5 kPa.
Figure A.10: (a) RVE with 444 line elements; (b) RVE with 1218 line elements; (c) vertical displacement at the middle of the upper boundary of the macroscopic sample versus the number of line elements. Applied tension is 5 kPa. The RVE is not rotated ($\varphi = 0^\circ$).

Figure A.11: Results of a three-dimensional test: (a) homogeneous material; (b) heterogeneous material; (c) the RVE (the line elements only). Displacement 3 represents a vertical displacement. Applied tension is 5 nN.

Figure A.12: (a) Tension test for a sample with the orientation of RVEs generated from the Gauss distribution; (b) vertical displacement at the middle of the upper boundary depending on the RVE orientation. Test group 1: $\varphi_0 = 0^\circ$; test group 2: $\varphi_0 = 90^\circ$. 