Remodeling of the annulus fibrosus: orientational ordering in 2D polymeric networks on curved substrates

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Orientational ordering in 2D polymeric networks on curved substrates

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Abstract

The annulus fibrosus is an anisotropic tissue which is located at the outer side of the intervertebral disc. The annulus fibrosus is a highly oriented multi layered tissue made of organic fibers like collagen. Each layer consists of well ordered, parallelly oriented, collagen fibers, which are oriented at a specific angle in relation to the axis of the disc and spine. The ordered network of collagen fibers were created in the embryonic phase, where the fibers reorientate on a swelling cylindrical structure known as the notochord. We study the origin of the oriented order in the network and give special attention to the role of the macroscopic shape of the annulus fibrosus. We consider the annulus fibrosus as a single layered cylindrical network of discrete persistent chains, which is a discretized description of the worm-like chain. The energy of the discrete persistent chain consist of spring- and bending energy. To study this we adapt a C++ simulation program, which was used to simulate the behavior of 2D polymeric network with fixed boundaries, into a simulation software which simulates polymeric networks on the surface of a cylinder. Therefore we replace two opposite fixed boundaries with a periodic boundary, and add an additional term to the Hamiltonian of the system to model the bending of a cylinder. The simulation starts with a network of randomly placed fibers. The results of the simulations show tilted alignment of the chains as a consequence of the bending due to the cylindrical shape. However, the ordering of the chains is bimodal, instead of the single directional order in nature. The influence of the cylindrical shape is confirmed by the effect of increasing the bending modulus. This resulted in higher order and steeper alignment.

Keywords: polymeric network, remodeling, intervertebral disc, annulus fibrosus, collagen reorientation.
Chapter 1

Introduction

In biology there is a huge variety of materials, with a wide variety of mechanical behaviors \(^1\). Some materials, like bone and antler, have a large modulus while others, skin and veins for instance, are highly deformable. Despite of the diversity in the mechanical behavior, the main building component of most biological load-bearing materials is collagen. Collagen is a naturally occurring polymer (biopolymer) of which 28 variants are known. These variants can be categorized into fibrillar and non-fibrillar collagen \(^2\). In this study we are only concerned about the fibrillar collagen. Each collagen fiber consists of many long close-packed fibrils, which have an almost circular cross-section with a diameter in the range of 100-500 nm. These fibrils are bundles of even smaller fibrils, the so-called microfibrils, and these microfibrils are built of tropocollagen molecules. Tropocollagen is a macro molecule, made up of more than 50000 atoms, are about 300 nm long and consists of three polypeptides. These polypeptides (molecules) are arranged in a helical structure and are intertwined to form a triple-helix structure. Therefore, collagen fibers are highly hierarchically organized structures which are spontaneously formed by self-assembly of tropocollagen.

Because of the hierarchical structure of collagen, there is a large range of length scales which play a role in the behavior of collagen. The scale-specific properties are determined from complex interactions, Buehler \(^3\) showed full atomistic calculations of a tropocollagen molecule. However, it is impossible to perform a fully atomistic simulation of a complete fiber. To overcome this problem, there is a need for a coarse-grained model. The Worm-Like Chain (WLC) is one of the most used coarse-grained models in biophysics to describe the behavior of biological fibers. In nature there is a large diversity of materials which are made of fibers. Each individual fiber behaves elastically and can be described by the WLC model, which energetically penalizes the bending of the fiber. These fibers form a network by making cross-links between different fibers and the macroscopic behavior of the (bio)materials is not anchored by just describing the behavior of the fibers, the collaboration of the fibers in the network is essential for the behavior. The network behavior strongly depends on the crosslink
density and the orientation of the fibers. Orientation of the fibers can give the network an anisotropic character. An example of this is the intervertebral disc, as shown in fig. 1.1. The intervertebral disc as shown here consists of a soft gelatinous core, the nucleus pulposus (NP), which is surrounded by the annulus fibrosus (AF). The annulus fibrosus is an anisotropic tissue which is located at the outer side of the intervertebral disc. The AF is a highly oriented multi layered tissue made of organic fibers, mostly collagen. The fibers in each layer are oriented under a specific angle with respect to the anatomic axial direction. The orientational ordering in the network is induced by the reorientation of the fibers in the early phases of fetal development. During this stage of fetal development a passive reordering process occurs which leads to the specific order in the AF [4].

The stress-strain behavior of the AF is described in some constitutive models, as shown in [6, 7], still there is no model which describes the origin and the development of the specific order in the AF. Therefore in this thesis we study the origin of the ordered structure in the extracellular matrix of the intervertebral disc and handle with the following question: What causes the fibers in the annulus fibrosus to order under a certain angle with respect to the body’s spine? In this study we gave special attention to influences of the cylindrical-like shape of the IVD and the aspect of the growth. To study this we adjust a computer simulation for simple 2D rectangular polymeric networks with fixed boundaries into a simulation for polymeric networks living on a cylindrical surface and optimize the energy minimization method used by the software. In this simulation we approximate the crossectional surface of the IVD as a cylinder. Only a single-layer network is taken into account and the interaction between the nucleus pulposus and the annulus fibrosus are neglected. The simulation starts with a completely disordered state, and tries to find an ordered state by minimizing the energy. Our expectation is that the cylindrical shape and size of the AF are crucial for the order in the network and the orientation of the fibers, caused by the curvature of the cylindrical shape.
In the remainder of this introduction we provide a background about the intervertebral disc and give a brief explanation about the formation of the annulus fibrosus. Before we discuss this, an outline of this thesis is given.

Chapter 2 considers two papers with the subject of reordering the internal structure of soft matter. The first one describes an experimental method for ordering a network of collagen by straining the sample. The second paper discusses a method to modify the order in the directorfield of a nematic droplet by changing the droplet’s shape. The simulation model is presented in chapter 3, starting with single chain models and an intuitive model to obtain insight into the relevant parameters of the behavior of those chains on a cylindrical surface. Thereafter these single chain models are extended to a network model and this chapter is concluded by describing the characteristic parameters of a network. The results of the simulations are shown in chapter 4 and discussed in chapter 5. We finish this thesis with the conclusion and recommendations in chapter 6.

**Intervertebral disc**

The main body axis of vertebrates is the vertebral column, which extends from neck to tail. The vertebral column (also called backbone or spine) protects the spinal cord (which is part of the central nervous system) and provides the main support for the body. The vertebral column gives the body the functionality to bend or twist and allows humans to stand upright \[8\]. The vertebral column consists of vertebral bodies (humans have 33 vertebral bodies) and intervertebral discs. The vertebral bodies in the backbone are stacked on top of each other with an intervertebral disc in between. Because the vertebral bodies are robust bones, to which the ribs are attached, the flexibility of the backbone is caused by the intervertebral discs. So, the intervertebral discs have to handle with all kind of complex stress states. As mentioned before these intervertebral discs consist of a soft gelatinous core, the nucleus pulposus, surrounded by the annulus fibrosus. The annulus fibrosus is a highly oriented multi layered tissue made of organic fibers, mostly collagen. The fibers in each layer are oriented under a specific angle with respect to the anatomic axial direction, as shown in fig. 1.3b.

The intervertebral disc is formed in the embryonic phase of vertebrates. To describe the formation of the intervertebral disc, we start with the notochord \[9\], \[10\], \[11\]. The notochord is a tube, formed by disc-shaped cells (chordoblasts) which are located on top of each other, which acts as an axial skeleton of the embryo. These cells divide, what results in longitudinal elongation of the notochord. During this elongation of the notochord vacuoles arise inside the notochord, which are compartments inside a cell enclosed by a membrane and are filled by a liquid. These vacuoles accumulate at the center of the notochord and are surrounded by a single layer of non-vacuolated cells. During this vacuolization process a basal lamina, covering the surface of the notochord, is deposited on the surface of the notochord. This basal lamina is the first extracellular matrix secreted by the chordoblasts\[12\]. This matrix acts as a sheath and encapsulates the notochord. After the vacuolization process and
Figure 1.2: 1) Notochord surrounded with dense anlagen of the intervertebral disc (IV) separated by the cartilage of the vertebral bodies (vb). 2) Notochord is bulged out and forms the nucleus pulposus of the intervertebral disc.

formation of the basal lamina a second, thin layer is formed on top of the basal lamina. This layer consists of elastic material and is called the elastica externa.

Now, in between the basal lamina and the elastica externa arises a thick layer which is an extracellular matrix consisting of aggrecan and collagen II fibers which eventually forms the annulus fibrosus. At the same time cartilage is deposited around the notochord on top of the elastica externa which forms the vertebral bodies. Because of the electrostatic charge of the aggrecan the osmotic pressure in the vacuoles rises. This causes the notochord to bulge out between the vertebrae, see fig. 1.2 ultimately forming the nucleus pulposus of the intervertebral disc. During the growing process of the notochord, rearrangement of the fibers of the annulus fibrosus takes place, forming a multi-layered network of cross-linked fibers. After the reorientation all the fibers in one layer are parallel to each other at a distinct angle with respect to the direction of the notochord. This reorientation is shown in fig. 1.3. This specific fiber orientation gives the sheath a nonlinear and an an-isotropic mechanical behavior.

Figure 1.3: Schematic view of the reorientation process. The intervertebral disc before (Left) and after (Right) the reorientation of the collagen fibers.
Chapter 2

Relation to prior work

We study the alignment of the fibers in the NF due to the growth of the IVD and the curved surface of the network substrate. Before we start describing the model used for the simulation, we discuss two, from literature known, methods to reorder soft matter, starting with the reorientation of fibers under the influence of externally imposed strain. Vader et al. [13] showed this in their experiments. Orientation of fibers due to curved substrates is not discussed in the literature, as far as known. However, reorientation of the director field of liquid crystalline material due to the curved shape of the material is shown in recent work of Fernandez-Nieves et al. [14]. They show that a chiral twisted state exists for toroidal nematic droplets.

2.1 Strain-induced collagen reorientation

One way to create an ordered network of fibers is by applying a strain to a fibrous gel or network. Vader et al. showed this experimentally [13]. Two types of in vitro created pancake-like shaped samples of collagen with a typical thickness of 1 mm and 22 mm in diameter were used: gels and polymerised samples, both with a collagen concentration ranging from 0.5 to 4.0 mg/ml. These samples are fixed by two pins to a substrate and strained by pulling the pins apart. No alignment in the gel is observed at low strains (< 5%), at larger strain regimes the fiber alignment increases resulting in an increase of the (nematic) order parameter, see fig. 2.1. So an aligned collagen network can be formed out of a gel by stretching it. Another aspect of ordering by stretching is the reversibility. There is a high reversibility for gels at low strains, however if the stretch exceeds some threshold value, which is about 10%, the orientation is captured in the network and the gel never recovers its initial state. This is shown in fig. 2.2 A, B and C. The initial network structure of the gel is shown in A, B shows the network after a 15% strain and C is the network after releasing the imposed strain. These plots show qualitatively the irreversibility of the network, the order parameter shows the same effect. The order parameter before stretching
Figure 2.1: Nematic order parameter as function of the strain in a collagen gel. The curve beyond 15% strain is dotted due to a lack of confidence of proper detection of the fibers.\textsuperscript{13}

is about 0.1 and is increased towards 0.5 at 15% strain. The order parameter is about 0.45 after a complete cycle. However, the prepolymerized sample has a completely different behavior. This is shown in D, E and F of fig. 2.2. Here the final state is almost the same as the initial state, indicating the reversibility of this network, up to higher strains. This reversibility is caused by crosslinkers which were in polymerized sample before the sample is strained. Fibers can be aligned by stretching a network or gel, however due the reversibility of the network it is difficult to get an ordering in a zero strain prepolymerized sample.

2.2 Curvature-induced ordering in nematic liquid crystals

Reorientation of polymeric fibers as a result of the shape of the substrate, in particular cylindrical-like shapes, is not described in literature so far. However, other types of ordered material, liquid crystals for example, showed the tendency to reorder due to the macroscopic shape of the material. Fernandez-Nieves \textit{et al.} \textsuperscript{14} revealed a twisted configuration of the nematic director inside toroidal droplets. Toroidal droplets are particular due to the fact they do not, in contrast to spherical droplets, have topological defects, which are discontinuities in the order of the material.

The free energy of nematics contains bulk and surface components. These bulk components consist of the splay, twist and bend energy. The surface energy is due to saddle-splay deformation. In nematic droplets there is a competition between the energy of tangential anchoring of the boundary and the elastic bulk energy. Fernandez-Nieves \textit{et al.} showed experimental data indicating the axial structure of the director field of a toroidal nematic droplet. The director field is free of splay distortions and tangential to the boundaries of the droplet. The director field is now determined by the twist, bend and saddle-splay
elastic constants in combination with the aspect radius of the torus $\xi$, a dimensionless parameter given by the ratio of the radius of the torus with respect to the tube radius. Fernandez-Nieves et al. present a phase transition between an axial and double twisted director field, as shown in fig. [2.3]. The ratio of the splay-bend constant to the twist elastic constant determines whether the director field is axial or double twisted. So, the macroscopic shape of the droplets can, through its curvature, imprint a special order in the internal structure.

### 2.3 Concluding remarks

Recapitulating, we see that the Vader et al. paper describes a method to order a polymerized network by straining and Fernandez-Nieves et al. influences the order in a nematic droplet
Figure 2.3: Phase diagram of a toroidal nematic droplet, where $K_{24}$ and $K_{2}$ are the splay-bend and twist elastic constant respectively and $\xi$ the aspect radius of the torus. [14].

by modifying the droplet’s shape. The disadvantage of Vader’s et al. method is the loss of the order if the strain is released. The study of Fernandez-Nieves et al. showed the twisted ground state in nematic droplets. An important difference between Fernandez-Nieves work and our study are the cross-links. A nematic droplet is free of cross-links, where these are essential in polymeric networks. In this study we combine the cross-linked structure of polymeric networks with the curvature induced by the macroscopic shape of the network to form a polymeric network with an ordered ground state.
Chapter 3

Model description

In this study we investigate the origin of the specific order of the collagen fibers in the AF due to the macroscopic shape and size of the IVD. In this chapter we present a model which describes the formation of the ordered structure of the collagen network in the AF. Our model consists of a network of fibers and the behavior of the network stems from the collective behavior of all individual fibers. We simulate each fiber individually by the discrete persistent chain model, which is a discrete formulation of the worm-like chain. Therefore we start this chapter by explaining those polymeric chain models and present a intuitive ring model to get insight in the behavior of these chains on a cylindrical surface. This ring model describes the behavior of collagen in the AF if a single fiber forms a complete loop around the cylinder, as shown in fig. [1.3] We conclude this chapter by presenting the network model and describe the characteristic parameters of a network state.

3.1 Individual chain models

3.1.1 Worm-like chain

One of the most used models to describe the behavior of single fibers is the worm-like chain (WLC) model. The WLC is a model which describes a chain by a continuously differential parametric curve \( \hat{r}(s) \), see fig. [3.1]. The WLC can be used for describing both flexible as well as very stiff fibers. A typical characteristic of a chain, and an indication of the stiffness of it, are the persistence length \( l_p \), which is the decay length of the tangent-tangent correlation of the chain, and the contour length of the chain (L). The persistence length is defined by:

\[
\langle \hat{t}(s) \cdot \hat{t}(s + \Delta s) \rangle = \exp \left( -\frac{\Delta s}{l_p} \right) = \exp \left( -\frac{k_b T \Delta s}{k_{bend}} \right),
\]

(3.1)

where \( \hat{t}(s) = \frac{d\hat{r}(s)}{ds} \) and \( k_{bend} \) is the bending modulus.
The persistence length $l_p$ is given by $\frac{k_{bend}}{k_b T}$. In the limiting case where $l_p >> L$ the chain behaves like a stiff rod and in the opposite limit, where $l_p << L$, the chain is highly curved and flexible and it behaves like a random walk. If a force is applied to the chain, the Hamiltonian of the WLC consists of two parts, one part represents the local bending energy and the other part is a force term. The local bending energy is proportional to the square of the local curvature of the chain. The Hamiltonian of the WLC is given by:

$$H[r(s)] = \int_0^L \left[ \frac{k_{bend}}{2} \left( \frac{d^2 r(s)}{ds^2} \right)^2 - \mathbf{f} \cdot \frac{dr(s)}{ds} \right] ds = \int_0^L \left[ \frac{k_{bend}}{2} \left( \frac{dt(s)}{ds} \right)^2 - \mathbf{f} \cdot t(s) \right] ds, \quad (3.2)$$

where $\mathbf{f}$ is a force applied to the chain. The magnitude of the local tangent vector $|t(s)| = \left| \frac{dr(s)}{ds} \right|$ is unity, so $t(s) = \hat{t}(s)$ and this implies local inextensibility of the chain. The Hamiltonian of the SF-WLC is now given by:

$$H = \int_0^L \left[ \frac{k_{bend}}{2} \left( \frac{d\hat{t}(s)}{ds} \right)^2 - \mathbf{f} \cdot \hat{t}(s) \right] ds, \quad (3.3)$$

If the persistence length is large compared to the contour length ($L << l_p$), the chain is almost straight. In the regime where the contour length and the persistence length are of the same order, the model is called the semi-flexible worm-like chain (SF-WLC). To simplify the Hamiltonian of the SF-WLC, we decompose the tangent vector $\hat{t}(s)$ in a component parallel to the applied force $\hat{t}_\parallel(s)$ and a component perpendicular to the applied force $\hat{t}_\perp(s)$, see equation (3.4). Now, for the SF-WLC, we make the assumption that $\hat{t}(s)$ is almost completely in the direction of the applied force, so the perpendicular component of the tangent vector is small.

The tangent vector is given by:

$$\hat{t}(s) = \hat{t}_\perp(s) + \hat{t}_\parallel(s), \quad (3.4)$$

and

$$|\hat{t}(s)| = \sqrt{\hat{t}_\perp^2(s) + \hat{t}_\parallel^2(s)} = 1. \quad (3.5)$$

In the SF-WLC the parallel component $\hat{t}_\parallel(s)$ is given by:

$$\hat{t}_\parallel(s) = \sqrt{1 - \hat{t}_\perp^2(s)} \approx 1 - \frac{1}{2} \hat{t}_\perp^2(s). \quad (3.6)$$
The Hamiltonian of the SF-WLC is:

$$\mathcal{H}[\mathbf{t}_\perp(s)] \approx \int_0^L \left[ \frac{k_{\text{bend}}}{2} \left( \frac{d\mathbf{t}_\perp(s)}{ds} \right)^2 - f \left( 1 - \frac{1}{2} |\mathbf{t}_\perp(s)|^2 \right) \right] ds. \quad (3.7)$$

A fit of the WLC to experimental data of biopolymers is shown in figs. 3.2 to 3.5. Fig 3.2 shows the force-extension curve of a single stranded DNA chain and fig. 3.3 the force extension of DNA during the overstretching transition. The force-extension curve of unfolded...
polypeptide and a type II collagen molecule with WLC fit is shown in fig. 3.4 and fig. 3.5 respectively. Although the different fibers consist of different atoms or monomers, the WLC to describes the behavior of many biopolymers, where the energy of the fiber consists of a bending and a stretching term.

3.1.2 Discrete persistent chain

The discrete persistent chain (DPC), as described in [15], is a discretized representation of a polymer chain, and schematically shown in fig. 3.6. The DPC is due to computational costs more appropriate to simulate a network of polymers compared to the WLC. The energy of the DPC contains, like the WLC, a bending and a stretching part. The bending in the DPC is due to the different orientation of successive segments. In the WLC model, the bending energy is proportional to the square of the local curvature $E_{bend} \propto \int \left( \frac{d^2 r(s)}{ds^2} \right)^2 ds$. In the DPC model, we calculate the bending energy for two successive segments, which is described by three points, following the path of the least bending energy. This path, which actually describes an arc length, has a constant curvature equal to the inverse of the radius of the circular path, see fig. 3.7. The bending energy becomes $E_{bend} \propto \int \frac{1}{R^2} ds$. Now, we calculate the bending energy as a function of the length of the segments $l_1$, $l_2$ and the angle $\theta$. For this we use the fact that the base angles of an isosceles triangle are equal. This gives us:

$$2(\theta + \gamma_1 + \gamma_2) = 2\pi, \quad (3.8)$$

$$\omega_1 + \omega_2 + \gamma_1 + \gamma_2 = \pi, \quad (3.9)$$

and

$$(\gamma_1 - \omega_1) + (\gamma_2 - \omega_2) + \alpha = \pi. \quad (3.10)$$

Now, substitution of (3.8) and (3.9) into (3.10) give the following relation between $\alpha$ and $\theta$:

$$\alpha = 2\theta. \quad (3.11)$$

We can express $c$ by using the cosine rule,

$$c^2 = l_1^2 + l_2^2 + 2l_1l_2 \cos(\theta). \quad (3.12)$$
$c^2$ can also be expressed as

$$c^2 = 4R^2 \sin \left( \frac{\phi}{2} \right) = 4R^2 \sin(\theta). \quad (3.13)$$

So, the curvature is now

$$\frac{1}{R} = \sqrt{\frac{4\sin^2(\theta)}{l_1^2 + l_2^2 + l_1l_2 \cos(\theta)}}. \quad (3.14)$$

We know that the curvature is constant, so the integral $\int ds$ can be replaced by $2|\theta|R$, and $E_{\text{bend}}$ is

$$E_{\text{bend}} = k_{\text{bend}}|\theta| \sqrt{\frac{4\sin^2(\theta)}{l_1^2 + l_2^2 + 2l_1l_2 \cos(\theta)}}. \quad (3.15)$$
Expansion of this expression gives the following equation for the bending energy
\[
E_{\text{bend}} = \frac{2k_{\text{bend}}}{l_1 + l_2} \theta^2 + O((\theta)^4). \tag{3.16}
\]

The Hamiltonian is given by
\[
\mathcal{H}[\{\hat{t}_i\}] = -\sum_{i=1}^{N} \hat{t}_i \cdot f + \sum_{i=1}^{N-1} \frac{2k_{\text{bend}}}{l_i + l_{i+1}} (\theta_{i,i+1})^2, \tag{3.17}
\]
where \(\theta_{i,i+1}\) is the angle between two subsequent links \((i\text{ and } i+1)\) and the summation is over all segments of a chain. The bending energy term is proportional to the square of this angle. In the low force regime, the behavior of the DPC can be described by
\[
\langle r \rangle_L \rightarrow f \kappa_{DPC} + O(f^2),
\]
where \(\langle r \rangle_L\) is the relative extension of the chain due to the force \(f\) and \(\kappa\) is the effective spring constant which is given by \(k_{sp}l\).

As shown by equation (3.5) the WLC is inextensible, this in contrast to the DPC. The length of a segment in the DPC is the length of a straight line between the begin and end point of the segment. This length can differ from the contour-length of the chain, as shown in fig. 3.8. Therefore we can replace the force term in the Hamiltonian by a Hookean spring. The Hamiltonian of the DPC is given by
\[
\mathcal{H}[\{\hat{t}_i\}] = \sum_{i=1}^{N} \frac{1}{2} k_{sp,i} (l_i - l_{0,i})^2 + \sum_{i=1}^{N-1} \frac{2k_{\text{bend}}}{l_i + l_{i+1}} (\theta_{i,i+1})^2 \tag{3.18}
\]

\(l_i\) and \(l_{0,i}\) are the actual and rest length of segment \(i\) respectively, \(k_{\text{bend}}\) and \(k_{sp,i}\) are the bending modulus and spring constant of segment \(i\). The constant \(\kappa\) is equal for all segments, the actual spring constant of a segment \(k_{sp,i}\) depends on the rest length of a segment by \(k_{sp,i} = \frac{\kappa}{l_{0,i}}\). So, short segments are stiffer compared to longer ones. This Hamiltonian describes a chain on a simple 2D surface, however, our chain is placed on a cylindrical surface. We unroll the mantle of this cylinder and make a flat 2D surface of it. In contrast to a flat 2D surface, a cylindrical mantle is a curved area. To compensate for this bending we add an additional term to the system’s Hamiltonian, so the energy of the network consists of three components. This energy term can be calculated by transforming the line of the segment between two nodes.

Figure 3.8: The segment length \(l_0\) of the DPC is the length between two nodes, and is not necessarily the contour length of the chain between two nodes.
crosslinks back to the surface of the cylinder and calculating the bending energy for the segment on the cylinder by using \( E_{\text{bend}} = \frac{k_{\text{bend}}}{2} \int \left( \frac{d^2r(s)}{ds^2} \right)^2 ds \). The transformation for a point \((X,Y)\) on the 2D network to the cylindrical coordinates is given by \((x,y,z) = (R\cos(Y), R\sin(Y), X)\), where \( R \) is the radius of the cylinder, defined by \( R = \frac{Y_{\text{max}}}{2\pi} \). \( Y_{\text{max}} \) is the height of the 2D area. The segments on the 2D network are straight lines, so we know the continuum description of these segments. The parametric representation of a segment between two nodes, \((X_1,Y_1)\) and \((X_2,Y_2)\), is

\[
\mathbf{r}(s) = \begin{pmatrix} u(s) \\ v(s) \end{pmatrix} = \begin{pmatrix} X_1 \\ Y_1 \end{pmatrix} + \frac{s}{\sqrt{(X_2-X_1)^2 + (Y_2-Y_1)^2}} \begin{pmatrix} X_2 - X_1 \\ Y_2 - Y_1 \end{pmatrix},
\]

(3.19)

transforming this to cylindrical coordinates, the segment is described by

\[
\mathbf{R}(s) = \begin{pmatrix} x(s) \\ y(s) \\ z(s) \end{pmatrix} = \begin{pmatrix} R\cos\left(\frac{1}{\pi} \left[Y_1 + \frac{s(Y_2-Y_1)}{\sqrt{(X_2-X_1)^2 + (Y_2-Y_1)^2}}\right]\right) \\ R\sin\left(\frac{1}{\pi} \left[Y_1 + \frac{s(Y_2-Y_1)}{\sqrt{(X_2-X_1)^2 + (Y_2-Y_1)^2}}\right]\right) \\ X_1 + \frac{s(X_2-X_1)}{\sqrt{(X_2-X_1)^2 + (Y_2-Y_1)^2}} \end{pmatrix}.
\]

(3.20)

The bending energy of a segment due to the cylindrical surface is now given by

\[
E_{\text{cyl}} = \frac{k_{\text{bend}}}{2} \int_0^\pi \sqrt{(X_2-X_1)^2 + (Y_2-Y_1)^2} \left( \frac{d^2\mathbf{R}(s)}{ds^2} \right)^2 ds = \frac{k_{\text{bend}}}{2} \frac{(Y_2 - Y_1)^4}{R^2((X_2 - X_1)^2 + (Y_2 - Y_1)^2)^{3/2}}.
\]

(3.21)

To get insight in the "cylindrical bending energy" we rewrite \( Y_1 - Y_2 = L \sin(\phi) \) and \( X_1 - X_2 = L \cos(\phi) \) where \( L \) is the distance between the points \((X_1,Y_1)\) and \((X_2,Y_2)\) in the 2D plane and \( \phi \) the angle to the x-axis. Substituting this gives us

\[
E_{\text{cyl}} = \frac{k_{\text{bend}}}{2} \frac{L \sin^4(\phi)}{R^2}.
\]

(3.22)

This energy term has a maximum if \( \phi = \pm \frac{\pi}{2} \) and a minimum if \( \phi = 0 \) or \( \pi \). Single segments have the tendency to align in the direction of the cylindrical axis, where there is no bending due to the curved surface. A way one might see this cylindrical bending energy is by making the comparison with a "vector field" for a chain. However, in contrast to, for example, the magnetic field, where the spins align in the direction of the magnetic field, the actual direction of the segment to the field is inversion symmetric, so mathematically this can be described by:

\[
-k(\hat{n} \cdot \hat{u})^2 = -k \cos^2(\phi),
\]

(3.23)

where \( \hat{n} \) is the direction of the field (which is the direction of the main axis), \( \hat{u} \) the direction of the segment and \( k \) a positive constant. This \( \cos^2(\phi) \neq \sin^4(\phi) \), so this comparison is not correct.
The total energy of a chain is now given by

\[ E = E_{st} + E_{bend} + E_{cyl} \]

\[ = \sum_{i=1}^{N} \frac{1}{2} k_{sp,i} \cdot (l_i - l_{0,i})^2 + \sum_{i=1}^{N-1} \frac{2k_{bend}}{l_i + l_{i+1}} (\theta_{i,i+1})^2 + \sum_{i=1}^{N} \frac{k_{bend}}{2} \frac{l_i \sin^4(\phi_i)}{R^2} \]  

(3.24)

where N is the number of segments of a chain. The summation in the stretch and cylindrical bending part is over all segments of the chain (N). The summation in the bending energy is from \( i = 1 \) to \( i = N - 1 \), because this energy is determined by the angle \( \theta_{i,i+1} \) between segments \( i \) and \( i + 1 \).

The energy of a chain contains three components: stretch, bending and bending due to the cylindrical shape of the chain. The stretch energy is a second order expansion of the difference between the actual segment lengths and the rest length of the segment. The bending energy is an expansion of the difference between the angle of the direction vectors of two subsequent segments. The cylindrical bending component, caused by the macroscopic shape of the network’s substrate, has a \( \sin^4(\phi) \) term and is not necessarily small because \( \phi \) can be a value between 0 and \( \pi \).

If the chain (or network) forms a closed loop around the cylinder, the cylindrical bending energy is a nonzero term and there is a competition between the (cylindrical) bending energy and the stretching energy in order to minimize the total energy. We can now distinguish two different situations for the fibers around the cylinder. In the first case, each fiber forms a circle or ellipse around the cylinder. This situation will be discussed in the Ring model. In the second situation, not single fibers but a network of fibers encloses the cylinder. This situation will be discussed in paragraph 3.3 and the chapters 4 and 5.

### 3.2 Ring model

To get insight into the behavior of a fiber in the annulus fibrosus we discuss an intuitive "ring model". This model describes the situation where one single fiber encloses the cylinder. In this model the rest length of the fiber \( l_0 \) is the equal to the contour length of the cylinder. The fiber in this simple model is described by a continuous curve and the energy of the fiber consists of two parts: the stretching energy and the bending energy. The Hamiltonian is

\[ \mathcal{H} = \frac{1}{2} k_{sp}(l - l_0)^2 + \frac{1}{2} k_{bend} \int \left| \frac{d\hat{t}(s)}{ds} \right|^2 ds. \]  

(3.25)

The fiber is free to rotate about an angle \( \theta \), as shown in fig. 3.9, forming an ellipse. The minor radius \( R \) of the ellipse is the same as the radius of the cylinder, and the major radius \( R' \) is \( \frac{R}{\cos(\theta)} \). Now, we will investigate the lowest energy configuration of the fiber for a certain combination of energy constants (\( k_{sp} \) and \( k_{bend} \)) and radius of the cylinder. In the limit of \( k_{sp} >> k_{bend} \) the fiber becomes a hookean spring. The energy as function of \( \theta \) is shown in fig. 3.10. The fiber will form a circle at the surface of the cylinder. In the other
Figure 3.9: Ring model used to get insight in the behavior of the fibers located on the cylindrical wall. The energy of the fiber contains a bending and a stretching part, which behaves as a hookean spring. The fiber is free to rotate over an angle \( \theta \) in order to find its lowest energy configuration. The lowest energy configuration depends on the radius of the cylinder \( R \), the rest length of the fiber \( l_0 \) and the spring and bending constants \( k_{sp} \) and \( k_{bend} \) respectively.

Figure 3.10: Typical shape of a stretched dominated regime. a) shows the energy as function of \( \theta \). The energy has a single minimum at energy as a function of \( \theta \). The angle of minimal energy is at \( \theta = 0 \), this is graphically shown in b).
Figure 3.11: Typical shape of a bending dominated regime. a) shows the energy as function of $\theta$. This bending dominated regime is two-fold degenerate, and this results in positive and negative rotated fiber, as illustrated in b) and c).

limit, where $k_{sp} << k_{bend}$, the bending term dominates the fiber’s behavior and the energy becomes proportional to the integral of the square of the local curvature, and the fiber forms an ellipse. Because the fiber forms a circle or an ellipse, the curve of the chain can be described by an 2D parameterization:

$$R(t, \theta) = \begin{pmatrix} x(t) \\ y(t, \theta) \end{pmatrix} = \begin{pmatrix} R \cos(t) \\ R' \sin(t) \end{pmatrix},$$  \hspace{1cm} (3.26)

where $R'(\theta) = \frac{R}{\cos(\theta)}$. With this parametric representation of the fiber, we can calculate the local curvature by $\kappa(t, \theta) = \frac{\frac{dR(t, \theta)}{dt} \times \frac{d^2R(t, \theta)}{dt^2}}{\left| \frac{dR(t, \theta)}{dt} \right|^2}$. The bending energy becomes the line integral of the local curvature along the fiber, and is given by:

$$\mathcal{H} = \frac{1}{2} k_{bend} \int \kappa(t, \theta)^2 \left| \frac{dR(t, \theta)}{dt} \right| dt.$$  \hspace{1cm} (3.27)

This integral can be evaluated numerically. Fig. 3.10 shows the typical shape of the bending energy as a function of $\theta$. This shape and the angle of minimal energy are independent of the radius $R$, however the qualitative values for the minimal energy are not. The graph of the bending energy is symmetric around $\theta = 0$, implying two angles of minimal energy, one at $\theta \approx 0.71$ and one at $\theta \approx -0.71$.

We just saw the two extreme cases, where $k_{sp} >> k_{bend}$ and $k_{bend} >> k_{sp}$. In the first limit there was one minimum (at $\theta = 0$) and in the second were two minima (at $\theta \approx \pm 0.71$). The angle with minimal energy as a function of $\frac{k_{sp}}{k_{bend}}$ for a cylinder with radius $R = 1$ is shown in fig. 3.12. There are two angles with the minimal energy for every finite ratio of
However, only the positive one is plotted. We see a smooth transition form $\theta = 0.7$ towards $\theta = 0$. Another important aspect what could cause reorientation is growth. This is shown in fig. 3.13. The stretch and bending constants ($k_{sp}$ and $k_{bend}$) are 1 and 10, respectively. In the initial stage the rest length of the fiber is equal to the contour length of the cylinder $2\pi R$ and the fiber orientates itself along $\theta \approx 0.6$. The fiber becomes stretched when the cylinder grows and the fiber re-orientates itself towards a more circular orientation in order to reduce the stretch of the fiber. This is shown by the decrease of $\theta$. We see a sharp transition form $\theta > 0$ to $\theta = 0$ at $\approx 1.4$. The data is numerically calculated, however at the transition, where $\theta$ is small, it is possible to make a series expansion of equation (3.25). This results in

$$E(R, \theta, k_{sp}, k_{bend}) = \frac{\pi(2\pi R(R - 1)^2k_{sp} + k_{bend})}{R} + \left(\pi^2 k_{sp}(R - 1)R - \frac{\pi k_{bend}}{4R}\right)\theta^2 + O(\theta^4).$$

Whether a zero angle is a stable or unstable configuration is determined by the sign of $\pi^2 k_{sp}(R - 1)R - \frac{\pi k_{bend}}{4R}$. If the sign of this is positive than $\theta = 0$ is a stable energy minimum, whether this minimum is a local or global minimum is determined by the $\theta^4$ component. A negative sign results in a unstable configuration. The transition radius, $\tilde{R}$, from a stable to a unstable $\theta = 0$ configuration is when

$$\pi^2 k_{sp}(\tilde{R} - 1)\tilde{R} - \frac{\pi k_{bend}}{4\tilde{R}} = 0.$$  (3.29)

The transition radius $\tilde{R}$ for $k_{sp} = 1$ and $k_{bend} = 10$ is 1.403, which is in agreement of fig. 3.13.

This ring model describes the situation where one single fiber encloses the cylinder and shows the ability of the fiber to tilt in order to minimize the total energy. The angle of

Figure 3.12: $\theta$ as function of the ratio $k_{sp}/k_{bend}$ for a fiber with a rest length of $2\pi$.

Figure 3.13: $\theta$ as a function of the radius of the cylinder. The rest length of the fiber is $2\pi$ (and therefore the line starts at $R = 1$), the Young’s modulus is 1 and bending modulus 10.
minimal energy depends on the radius and ratio of \( \frac{k_{sp}}{k_{bend}} \). In the remainder of this thesis we study the effect of the cylindrical shape of the network on the orientation of the fibers in the network. We expect that the additional bending term, caused by the cylindrical shape, causes a shift in the fiber orientation of the network. The network model starts with a completely disordered system and we study the influence of the specific shape of the substrate of the network on the order of the network.

### 3.3 Network model

In the beginning of this chapter we discussed two different chain models which describe the behavior of biopolymers: the WLC and the DPC. From a computational point of view we use the DPC model to simulate the network. The DPC describes a chain by discretizing the chain into segments. In the network model all segments are wedged between two consecutive crosslinkers. Crosslinkers (or nodes) are points where two chains intersect and stick together. These crosslinkers divide the chains into segments, so the number of segments per chain depends on the number of crosslinkers in the chain. The energy of the network is the summation of the energy of all individual chains, and this is given by

\[
E = \sum_{i=1}^{N} E_i = \sum_{i=1}^{N} \left[ \sum_{j=1}^{M(N)} \frac{1}{2} k_{sp,ij} \cdot (l_{ij} - l_{0,ij})^2 + \sum_{j=1}^{M(N)-1} \frac{2k_{bend}}{l_{ij} + l_{ij+1}} \left( \theta_{i(j,j+1)} \right)^2 + \sum_{j=1}^{M(N)} \frac{k_{bend}}{2} \frac{l_{ij} \sin^4(\phi_{ij})}{R^2} \right],
\]

where \( N \) is the number of chains in the network, each chain has \( M(N) \) segments and the spring constant of segment \( ij \) (\( k_{sp,ij} \)) is defined by the effective spring constant and the rest length of the segment as \( k_{sp,ij} = \frac{\kappa}{l_{0,ij}} \).

**Creation of the network**

The polymeric network is created using the Mikado method and initially consists of \( N \) chains of equal length. The chains are formed by randomly picking a starting point and a random chosen direction of propagation, which determines the location of the end-point of the chain. If a chain crosses a periodic boundary, it will proceed at the opposite side. The other two boundaries are fixed, i.e. the points on these boundaries are fixed. When all the chains are created, we search for all points where two chains cross each other, these points are the crosslinkers, inside and on the boundary. These points are shown as red dots in fig. 3.14 The black lines are the chains and the boundaries. The main axis of the cylinder is the horizontal direction of the flat network.

All free ends of the chains, this are the parts of the chains before the first and after the last segment and chains with less than two crosslinkers, will be removed because these ends have no influence on the behavior of the network in the model. This gives a 2D cross-linked
network of randomly placed chains, each chain consisting of one or more segments, as shown in fig. 3.15

3.3.1 Discussion of the energy

Before we can calculate the energy of a network, the system needs to be initialized. During this initialization process all rest lengths of the segments, which are used by calculating the stretch energy, are fixed to the actual lengths of the segments. All segments are initially unstretched, caused by fixing the rest lengths to the initial lengths of the segments, so there is zero stretching energy. Because the chains are formed by random placed rods or straight lines, and these chains are divided into segments, all the segments of a single chain are perfectly aligned. Therefore, the angle between the direction of two successive segments, $\theta$, is zero and this leads to a zero bending energy. So, two out of three energy components are zero. The last energy term, the bending due to the cylindrical curvature, is a nonzero energy term. For each segment, this term is given by:

$$E_{cyl,ij} = \frac{k_{bend}}{2} \frac{l_{ij} \sin^4(\phi_{ij})}{R^2}. \quad (3.31)$$

Now the network is made, we can estimate the initial energy of this component. We know that the chains are randomly orientated, so

$$E_{cyl} = N \frac{k_{bend}}{2} \langle l \rangle \langle \sin^4(\phi) \rangle \frac{1}{R^2}. \quad (3.32)$$
where \( N \) is the total number of segments in a network, \( \langle l \rangle \) the average segment length and \( N(l) \) is the total length of all chains in a network: \( L \). Because all chains are randomly oriented, \( \langle \sin^4(\phi) \rangle \) can be calculated by:

\[
\langle \sin^4(\phi) \rangle = \frac{1}{\pi} \int_{0}^{\pi} \sin^4(\phi) d\phi = \frac{3}{8},
\]

and the cylindrical bending energy is given by:

\[
E_{cyl} = \frac{k_{bend}}{2} \frac{3 L}{8 R^2}.
\]

So, this energy component is a nonzero term, which is determined by the total length of the chains in a network for a given bending modulus. The initial energy of the network with a cylindrical bending constant of 1 calculated by equation (3.34) by measuring the total length of the chains in a network together with the initial energy of the simulation is
shown in fig. 3.16. Here it is obvious to see that (3.34) describes the evolution of the initial energy well, however, the small deviation of both methods is a consequence of the boundary conditions. These boundary conditions cause a small offset in the random orientation of the chains.

This initial chain configuration is unlikely to be the network configuration with the lowest energy and we need a method to minimize the total energy. The minimization method used by the simulation is a multi step method, as described in Appendix I. This minimization process starts with a nonlinear conjugate gradient (NCG) minimization of the initial network’s energy. After this process found a (local) minimum, all crosslinkers get a small random displacement and NCG minimizes this configuration. If this new network state has a lower minimum energy than the old configuration, the program accepts the new state as the actual configuration. This process, which is called annealing, will be repeated until the program fails to find a lower minimum for a predetermined times in succession (we used 100).

Before the minimization process the energy of the network is completely determined by the cylindrical bending energy. Therefore the simulation tries to reduce this cylindrical bending energy. However reducing the cylindrical bending energy by reorienting the

![Figure 3.16: Initial energy of the network with a cylindrical bending constant of 1 calculated by the simulation (black) and by equation 3.34 (red). The input parameters for the calculation are the total length of the chains in the network L and the radius of the network’s substrate R.](image)

Figure 3.16: Initial energy of the network with a cylindrical bending constant of 1 calculated by the simulation (black) and by equation 3.34 (red). The input parameters for the calculation are the total length of the chains in the network L and the radius of the network’s substrate R.
chains can not be done independently of the stretch and bending component. Reducing the cylindrical bending energy gives rise to the other energy components so there is a competition between the three energy components and the simulation searches for the lowest energy configuration.

3.3.2 Simulation test

To see what the results of a simulation look like, we run a simulation for the network shown in fig. 3.15. This initial run is needed to introduce the order parameter, which is slightly different from the nematic order parameter. All constants, the effective spring constant and the bending constants, are 1 in this test run. The outcome of this simulation is shown in fig. 3.17. During the minimization of the energy, reorientation of the segments in the network occurs. In the relaxed network one can distinguish two main angles under which the segments are aligned. To make this more clear we plot the angle of each segment in relation to the component perpendicular to the main axis of the cylinder $\theta$, which is the vertical
axis of the network shown in fig. 3.17a, in a histogram, and compare this with the angle distribution for the initial network, see fig. 3.18. The domain of the angles is from $-\frac{\pi}{2}$ to $\frac{\pi}{2}$, because all individual segments are inversion symmetric. These histograms clearly show the random orientation of segments in the initial configuration, and the preferred angles in the final network state. The histogram of the relaxed network is symmetric around the point 0, this means that not all segments align into one single direction but some segments rotate clockwise while others rotate counterclockwise. The characteristic parameters of the network are the angle in which the segments align and the order in the network. The average angle to which the segments align is, because of the symmetry of the histogram, approximately 0, independent of the specific network. Therefore we define the average angle $\theta_{av}$ as the average of all angles lying in the domain of 0 to $\frac{\pi}{2}$. To quantify the order in the network we use a modified nematic order parameter.

**Order parameter**

The nematic order parameter is a measure for the order in a (nematic) system. This order parameter describes how the segments are aligned around the director $\hat{n}$. This director is given by

$$\hat{n} = \langle \hat{u}_i \rangle,$$

(3.35)
Figure 3.19: Distribution function for a perfect order, consisting of a normalized combination of two Dirac delta peaks, at \( \pm \sigma \).

where \( \mathbf{\hat{u}} \) is the unit tangent vector of segment \( i \), \( \mathbf{\hat{n}} \) and \( \mathbf{\hat{u}} \) are both inversion symmetric. \( \mathbf{\hat{n}} \) can also be described in terms of the distribution functions \( P(\mathbf{\hat{u}}) \) or \( P(\theta) \):

\[
\mathbf{\hat{n}} = \frac{\int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \mathbf{\hat{u}} P(\mathbf{\hat{u}}) d\mathbf{\hat{u}}}{|\int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \mathbf{\hat{u}} P(\mathbf{\hat{u}}) d\mathbf{\hat{u}}|} = \frac{\int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} (\mathbf{\hat{x}} \cos(\theta) + \mathbf{\hat{y}} \sin(\theta)) P(\theta) d\theta}{|\int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} (\mathbf{\hat{x}} \cos(\theta) + \mathbf{\hat{y}} \sin(\theta)) P(\theta) d\theta|}. \tag{3.36}
\]

All \( \mathbf{\hat{u}} \) are in the domain of \(-\frac{\pi}{2}\) to \(\frac{\pi}{2}\) because of the inversion symmetry of the segments. The nematic order parameter \( S \) in a 2D system is given by

\[
S = \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} P(\phi)(2 \cos^2(\phi) - 1) d\phi, \tag{3.37}
\]

where \( \phi \) is the angle between \( \mathbf{\hat{u}} \) and \( \mathbf{\hat{n}} \):

\[
\cos(\phi) = \mathbf{\hat{n}} \cdot \mathbf{\hat{u}}. \tag{3.38}
\]

In a perfectly ordered state, where all segments are perfectly aligned in one single direction and \( P(\phi) = \delta(\phi) \), the order parameter \( S = 1 \). In the opposite situation where all angles are equally likely, the angle distribution \( P[\theta] = \frac{1}{\pi} \). The order parameter in this situation is 0. The order parameter changes from 0 to 1 if a state transforms from a completely disordered state into a perfectly ordered state. Now, we test this order parameter by calculating the order parameter in a hypothetical case of a perfect bimodal ordered network. Here, the angle distribution function consists of a normalized combination of two Dirac delta peaks, symmetric around \( \theta = 0 \), as shown in fig. [3.19]. The distribution function is now described by:

\[
P(\theta) = \frac{\delta(\sigma) + \delta(-\sigma)}{2} \tag{3.39}
\]
and,

\[ P(\phi) = \frac{\delta(\sigma) + \delta(-\sigma)}{2}. \]  

(3.40)

The order parameter becomes

\[ S = \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \frac{\delta(\sigma) + \delta(-\sigma)}{2} \left(2\cos^2(\phi) - 1\right) d\phi = 2\cos^2(\sigma) - 1. \]  

(3.41)

Here, the order parameter depends on the angle \( \sigma \). This is undesirable, because we use the order parameter to describe the order in the network, independently of the orientation angle. To overcome this problem we use the absolute value of the angles. To see the result of this we calculate the order parameter for a completely disordered state. The distribution function of an isotropic network after the absolute value operator is now given by:

\[ P[\theta] = \begin{cases} 
0, & \text{if } \theta < 0 \\
\frac{2}{\pi}, & \text{if } \theta > 0
\end{cases}. \]  

(3.42)

The order parameter \( S \) for this distribution is \( \frac{1}{2} \). The order parameter for a isotropic phase is not zero anymore. This is caused by using the absolute value of the angles. This absolute value process brings an artificial order into the network, because the domain of the angle distribution function has shrunk by a factor 2. We correct this by multiplying all angles by a factor 2, so the distribution stretches out over the domain \( 0 \leq \theta < \pi \).

Summarizing, the director \( \hat{n} \) is given by

\[
\hat{n} = \frac{\int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} (\hat{x} \cos(2|\theta|) + \hat{y} \sin(2|\theta|)) P(\theta) d\theta}{|\int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} (\hat{x} \cos(2|\theta|) + \hat{y} \sin(2|\theta|)) P(\theta) d\theta|},
\]  

(3.43)

and the angle of the director is

\[ \langle \theta \rangle = \cos^{-1}(n_x), \]  

(3.44)

where \( n_x \) is the x component of the director. The order parameter \( s \) is now:

\[ s = \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} p(\theta)(2\cos^2[2|\theta| - \langle \theta \rangle] - 1) d\theta. \]  

(3.45)

The order parameter \( s \) is zero in an isotropic network and 1 for a perfect bimodal distributed network where all segments are oriented under an angle of \( \pm \sigma \), independent of the value of \( \sigma \).
Chapter 4

Results

The origin of the order in the network and the orientation of the collagen fibers in the AF is the subject of this thesis. In the ring model we saw reorientation of the fiber due to the curved surface. In this chapter we simulate cylindrical shaped networks of fibers and present the results. These networks consist of, in contrast to the ring model, multiple fibers which are initially randomly oriented. The simulation starts with a disordered network. The energy of the network is given by equation (3.30) and the rest length of the segments are equal to their initial lengths. The simulation searches for the ground state of the network with the minimization protocol as described in paragraph 3.3.1 and Appendix I.

We investigate the networks behavior with the following simulations:

- fixed collagen. The cylinder grows during the simulation and the rest length of the fibers are fixed to the initial length of the fibers.

- growing of the collagen. The fibers in the network grow linearly with the radius of the cylinder.

- modulus dependency.

- number of chains.

In all of these simulations we start with creating a network by placing random chains with a length of $0.45 \cdot 2\pi R$ on a rectangular network with dimensions of $10\pi R$ by $2\pi R$. In stead of one single simulation for a given state, which gives a noisy result, we make an ensemble simulation of 100 runs. This total simulation smooths the results as shown in fig. 4.1a) Shows the same histogram as fig. 3.18b) and b) shows the angle probability of the ensemble simulation.
4.1 Fixed collagen

These simulations show the behavior of a network with fixed rest lengths of the segments on a growing cylinder. The rest lengths, in combination with the actual length of the segments and the effective spring constant, determine the stretch energy of the network (see equation (3.30)). The segments become stretched while the cylinder grows. The simulations start with a disordered network on a cylinder with a radius $R_0$. The rest length of each segment is fixed at the initial length of that segment. The simulation starts with relaxation of this initial network to its ground state. When the energy is minimized, the cylinder grows linearly in all directions followed by another minimization step. These grow-minimization steps will be repeated. The growing of a point $(x_i, y_i)$ is mathematically described by

$$
\begin{pmatrix}
  x_{i+1} \\
  y_{i+1}
\end{pmatrix}
= \begin{pmatrix}
  \lambda & 0 \\
  0 & \lambda
\end{pmatrix}
\begin{pmatrix}
  x_i \\
  y_i
\end{pmatrix},
$$

(4.1)

where $\lambda = \frac{R_{i+1}}{R_i}$.

In these simulations the bending modulus and effective spring constants are set to one and the initial radius is chosen as $\frac{a}{2}$, what results in a 2D network with height of 2. We chose 10 as the width of the network and made a network of 240 chains with a 0.9 length. The results of these simulations are shown in fig. 4.2 and fig. 4.3. Fig. 4.2 shows the evolution of a network with increasing radius. The left side of each figure shows a relaxed network at the indicated radius and the angle probability distribution of the ensemble simulation is shown right. The order parameter $s$, as described in paragraph 3.3.2, average angle $\theta_{av}$ and the energy with its different components are shown in fig. 4.3. The energy component "bending cylindrical curvature" is the energy $E_{cyl}$ and the "segmental bending" component is $E_{bend}$. The average angle is 0 for all situations, due to the symmetry of the angle distribution. However, if we calculate the average angle in the range of $0 \leq \theta < \frac{\pi}{2}$, we see an evolution of the average angle, as shown in the figure.

Figure 4.1: a) Histogram of a single simulation run b) Angle probability of the ensemble run.
Figure 4.2: Effect of growth on the structure of the network. Initially, the networks are ordered and the angle probability shows two peaks, however increasing of the radius causes segments to orient in a more circular way.
4.2 Growing collagen

The growing collagen and fixed collagen simulations both study the radius dependency. In contrast to the fixed collagen simulations, where the rest lengths of the segments are fixed, the rest length of the segments scales linearly with the radius in the growing collagen simulations. The growing collagen simulations are about finding the relaxed state of the network on a cylinder for an entire range of radii, all simulations starts with the same (scaled) disordered initial configuration. At each radius, the simulations restarts with disordered network and initializing of the network.

All energy components in the first set of the growing collagen simulations are set to the
same weight, so the bending and the effective spring constant are set to one. Each network is created by 240 chains with a length of $0.45 \cdot 2\pi R$. Some of the results are shown in fig. 4.5. The network size increases from top to bottom in this figure, in a way that the ratio of $\frac{\text{length}}{\text{width}}$ stays constant, namely 5. The left side shows the result of the simulated network with the initial network as shown in fig. 3.15 and the angle probability of the ensemble simulation is shown at the right side. Here we see at small and at large radii relatively disordered networks, but at intermediate radii the angle probability distribution shows two clearly distinguishable peaks, symmetric to $\theta = 0$. The chains in the network show two preferred directions and chains connected in a head to tail fashion show the tendency to form a straight line. The development of the order parameter as function of the radius is shown in fig. 4.4. The radius in this figure is plotted on a logarithmic scale. There is a steep increase of the order parameter in the first part of the figure, after the order parameter remains constant for more than a decade, and it eventually decreases gradually. The energy of the network is shown in fig. 4.6. The energy in the regime of small radius is dominated by the component due to the cylindrical surface. Increase of the radius results in decrease of this energy part which initially results in a higher ordered structure, however at large radius the stretch energy becomes more important and the order decreases. fig. 4.7 shows the energy of the network before and after the simulation.
Figure 4.5: Relaxed networks on cylinders of different sizes, with all same initial configuration with associated angle probability distribution.
In the following simulations we increased the contribution of the curved surface bending energy part by setting this modulus to 5. The effective spring and segmental bending constants are kept at 1. The results are shown in figs. 4.8 to 4.12. The initial configurations are identical to the one used for simulations with equal moduli. In this figure the trend in the behavior of the structure of the network is the same as the previous in results, however, these angle distribution peaks are narrower, in particular fig. 4.10d. The order in the network is quantified by the order parameter as show in fig. 4.8. This figure shows the evolution of the order parameter when all constants are equal (red), when the cylindrical bending modulus is 5 (black) and 0.2 (blue). Note, the simulation with a 0.2 cylindrical bending constant is an average of only 5 simulations. Here it is clearly shown that the order in the latest situation is higher and the range of ordered networks is considerable larger. The energy of the network is shown in fig. 4.11 and the networks energy before and after the simulation is shown in fig. 4.12 and behaves like the old situation. Another characteristic of the network is the average angle, for which the evolution is shown in fig. 4.9. The average angle in situations where the network is in disordered state is $\frac{\pi}{4}$, but as the states become more ordered the average angle increases towards 1.3.
Figure 4.8: Order parameter $s$ for networks on cylinder with radius $R$. The cylindrical bending modulus is 5 (black), 1 (red) and 0.2 (blue) and both other constants are 1.

Figure 4.9: Mean angle of the network in the situation where the cylindrical bending modulus is 5 (black), 1 (red) and 0.2 (blue).
Figure 4.10: Relaxed networks on cylinders of different sizes, with all same initial configuration with associated angle probability distribution. The cylindrical bending energy has a weight of 5 compared to the other two energy components.
4.3 Modulus dependency

The arrangement of the fibers in the network is determined by the competition between the elastic and bending energy. One method to influence the ratio between elastic and bending energy is by varying the radius of the cylinder, as shown in the beginning of this chapter. However, since the structure of the network is not anchored for a given radius, the ratio of the bending modulus to the Young’s modulus is another important aspect. To see how the energy constants influence the structure of the network we run several ensemble simulations of a network with a radius of $\frac{2}{2\pi}$ and a bending modulus of 1. The effective stretching constant varies from 0 to 15. The network initially consists of 240 chains with a length of 0.9. The results are shown in figs. 4.13 and 4.14. At the lowest values of $\kappa$ there is almost no resistance for the chains to orientate optimally into the direction of the lowest cylindrical bending energy what results in a highly oriented network. Increasing the stretching constant restricts the reorientation and finally causes the network to remain in its initial configuration.
Figure 4.13: Results of varying the effective elastic modulus ($\kappa$) of the chains, the network used in these simulations has a $\frac{2}{2\pi}$ radius and a bending constant of 1.
Figure 4.14: Relaxed networks with various effective spring constants. At the lowest values of $\kappa$, the network is able to become ordered. The increase of $\kappa$ decreases the order in the network.
4.4 Number of chains

The last set of simulations is about varying the total number of chains. The radius of the cylindrical network is \( \frac{2}{\pi} \). We vary the total number of chains and quantify each network by the average total length (ATL) of the chain in the network. This is because of the network creating procedure. The typical network and appropriate angle densities are shown in fig. 4.16, and the order parameter, mean angle \( \theta_{av} \) and energy with its separate components are shown in fig. 4.15.

![Graphs showing order parameter, mean angle, and energy versus ATL for varying number of chains](image)

Figure 4.15: Results of varying the number of chains in the network, characterized by the ATL (average total length of the chains).
Figure 4.16: Relaxed networks of various number of chains, which results in different average total length (ATL), with radius of $\frac{2}{2\pi}$, stretch modulus of 1 and bending modulus of 5.
Chapter 5

Discussion

5.1 Discussion of the results

The goal of this project is to study the origin of the orientation of the collagen fibers in the AF of the IVD. To do this we simulate the collagen network on a cylindrical surface and consider each individual collagen fiber as a discrete persistent chain, which is a discretized description of the chain and whose energy consists of a bending and a stretching term. Before we simulated the total network, we studied the behavior of one single ring-like fiber on a cylinder in the "ring-model". This ring model shows the competition between the bending and stretching energy, and the fibers tilt to the main axis of the cylinder in the bending dominated regime. This bending dominated regime is at small radii or high bending modulus. This is a degenerate energy level, caused by the symmetry of the model. If the stretching energy dominates the behavior of the chain, the fiber is circular tied around the cylinder. Now, keeping these results in mind, we will discuss the results of the simulations, performed in Chapter 4.

Fixed collagen

The simulations in the paragraph "Fixed collagen" let the cylinder radius grow, but the rest lengths of the segments were fixed. The "Fixed collagen" simulations and the ring model simulates the same type of growing, both using fixed rest lengths, however the Fixed collagen simulations use a network of fibers in stead of on single fiber.

The radius grows in a stepwise way during a simulation, so the network on a cylinder with small radii evolves to a network on a cylinder with larger radii. The growth can be described by equation (4.1). The initial radius of the cylinder is $2\pi$ and all constants (effective constant and bending constant) are 1. This initial state is, after minimizing the energy, an ordered state as shown in figs. 4.2a and fig. [4.3]. The chosen amplification factor ($\lambda$ in equation (4.1)) is 1.1, which means that the radius and the length of the cylinder increase by a factor 1.1 with each step. The rest length of the segments remains
unchanged. Fig. 4.3 shows the evolution of the energy and order of the system during the growth. The energy plots show the energy is stretch dominated. The scaling of the energy components with the radius is given by equation (5.1), (5.2) and (5.3). The rest length \( l_{0,ij} \) is independent of the radius \( R \) and the stretch energy scale quadratically with \( R \). The bending and cylindrical bending energy scales as \( \frac{1}{R} \). This shows that the stretch energy dominates the behavior of the network at larger radii. To decrease its energy, the chains try to minimize the stretch and do this by orienting in a circumferential way, as shown in fig. 4.2, and in particular by the peak in the angle distribution at \( \theta = 0 \) of (f) in fig. 4.2.

This is the same behavior as seen in the Ring model, see fig. 3.13.

\[
E_{st} = \sum_{i=1}^{N} \sum_{j=1}^{M(N)} \frac{1}{2} k_{sp,ij} \cdot (l_{ij} - l_{0,ij})^2 = \sum_{i=1}^{N} \sum_{j=1}^{M(N)} \frac{1}{2} \kappa \cdot (l_{ij} - l_{0,ij})^2 \sim R^2 \tag{5.1}
\]

\[
E_{bend} = \sum_{i=1}^{N} \sum_{j=1}^{M(N)} 2k_{bend} \delta \theta_{ij}^2 \sim \frac{1}{R} \tag{5.2}
\]

\[
E_{cyl} = \sum_{i=1}^{N} \sum_{j=1}^{M(N)} \frac{k_{bend,ij}}{2} \frac{l_{ij} \sin^4(\phi_{ij})}{R^2} \sim \frac{1}{R} \tag{5.3}
\]

**Growing collagen**

These simulations are intended to show the influence of the cylinder radius on the structure of the network. Each simulation has one fixed radius and all simulations started with a disordered network. After initialization of the network, all lengths of the segments are equal to their rest lengths and all segments in a chain are oriented in the same direction. So, the energy of a network is totally determined by the cylindrical bending energy, which can be calculated by 3.34 and is shown in fig. 3.16. However, the energy minimization process is able to let some components of the energy rise to decrease the total energy of the network. This is clearly shown in fig. 4.6 and fig. 4.11. To see the influence of the radius of the cylinder, we first write the different energy components as a scaling of the radius. Note that the (rest)lengths also scale with the radius. The energy components become:

\[
E_{st} = \sum_{i=1}^{N} \sum_{j=1}^{M(N)} \frac{1}{2} k_{sp,ij} \cdot (l_{ij} - l_{0,ij})^2 = \sum_{i=1}^{N} \sum_{j=1}^{M(N)} \frac{1}{2} \kappa \cdot (l_{ij} - l_{0,ij})^2 \sim R \tag{5.4}
\]

\[
E_{bend} = \sum_{i=1}^{N} \sum_{j=1}^{M(N)} 2k_{bend} \delta \theta_{ij}^2 \sim \frac{1}{R} \tag{5.5}
\]

\[
E_{cyl} = \sum_{i=1}^{N} \sum_{j=1}^{M(N)} \frac{k_{bend,ij}}{2} \frac{l_{ij} \sin^4(\phi_{ij})}{R^2} \sim \frac{1}{R} \tag{5.6}
\]

47
Here equation (5.5) and (5.6) are the same as equation (5.2) and (5.3) respectively, but equation (5.1) and (5.4) are not the same. The reason for this is that rest length scales with the radius in equation (5.4) where it does not scale in equation (5.1).

At large radius the network behavior is stretch dominated, as can be seen from equation (5.4 to 5.6). Here, the cylindrical bending energy is low and every small displacement of the crosslinkers cause an increase in (stretch) energy. Therefore the network is almost locked in the initial configuration. As the radius decreases the initial (cylindrical bending) energy increases and the domination of the behavior by the stretch energy decreases. This causes the segments to align into two preferred directions resulting in an ordered network. We can estimate the radius where the network becomes ordered ($R^*$) by comparing equation (5.4) and (5.6):

$$\kappa R^* = \frac{k_{\text{bend}}}{R^*},$$

and this results in

$$R^* = \sqrt{\frac{k_{\text{bend}}}{\kappa}}.$$

For $\kappa = 1$ and $k_{\text{bend}} = 1$ $R^* = 1$, $k_{\text{bend}} = 5$ results in $R^* = \sqrt{5}$. We can compare these values with the graphs of figs. 4.4 and 4.8 and see that the networks with these radii are ordered.

The angle probability distribution shows no peak indicating a disordered network of fibers at the smallest radii. The order has disappeared as a consequence of the network. As shown by (5.5) and (5.6) the bending and cylindrical bending components of the energy scale as $\frac{1}{R}$. However, initially $E_{\text{bend}}$ and $E_{\text{st}}$ are zero while $E_{\text{cyl}} > 0$. The simulation minimizes the energy of the network (as shown in fig. 4.7 and fig. 4.12) by reorientation of the segments, however reorientation of the segments is counteracted by increasing the spring and bending energy. A small shift of the crosslinks causes a large increase of $E_{\text{bend}}$ and the minimum energy is reached before order arises.

The average, or mean angle, calculated by averaging all angles in the interval $0 \leq \theta < \frac{\pi}{2}$, shows a value of $\frac{\pi}{4}$ in a disordered state. This value is in accordance which one would get by calculating the mean angle of a totally disordered angle distribution. However, if the order of the network increases, the average angle increases. This is a result of the ability of the chains to align into the main axis at the expense of stretching or bending. This is also shown by varying the cylindrical bending modulus. The higher the cylindrical bending modulus, and so the contribution of the cylindrical curvature energy, the larger the ability of the chains to reorientate into the direction of the cylindrical main axis resulting in a higher order parameter and lower mean angle.

What we can conclude here is that the structure of the network is radius depended. If we now look back to the introduction of this thesis, and especially to fig. 1.3 we see a layered structure. These layers are tilted towards the main axis of the cylinder, however the outermost layers are less tilted than the innermost. This behavior can be explained by
the growing collagen simulations with an ordered structure at the smallest radius, where increasing of the radius causes a increase in the \( \theta_{av} \).

**Variation of modulus**

The simulations of paragraph 4.3 vary the effective stretching constant of the segments in a range from 0 to 15. The order and the average angle in simulated networks with low stretching constants are high, see fig. 4.13. This is due to little resistance to reorientation caused by stretching. The energy drops nearly to zero as a result of the reorientation and the free stretching at \( \lim \kappa \rightarrow 0 \). Increasing the effective stretching increases the minimal energy and flattens the angle probability distribution, resulting in lower order parameter and smaller \( \theta_{av} \). Eventually the network is locked into its initial configuration, where each little displacement of the crosslinkers increases the stretch energy more compared to the reduction in cylindrical bending energy. These simulations can be compared to the ring model simulations were the ratio of \( \frac{k_{sp}}{k_{bend}} \) is varied. In fig. 3.12 \( \theta \) starts at \( \frac{\pi}{4} \) because, in the ring model, where one single fiber encloses the cylinder, it is energetically unfavorable to tilt in angles larger than \( \frac{\pi}{4} \). Increasing of the ratio \( \frac{k_{sp}}{k_{bend}} \) decreases the tilting angle towards 0 to reduce the stretch of the fiber. This is different from the behavior seen in the network simulations. Here the initial configuration determines the lowest energy configuration at large \( \kappa \). This initial configuration is a disordered network and the average angle of a disordered network is \( \frac{\pi}{4} \).

**Number of chains**

The last set of simulations varies the number of chains in a network. The size of the radius used in these simulations is \( \frac{2 \pi}{\sqrt{2}} \), the effective stretching constant and subsequent segment bending are 1 and the cylindrical surface bending is 5. The networks are characterized by the average total length (ATL) of the chains instead of the number of chains. If there are just a few chains on the cylinder, the network does not surround the cylinder and the chains can easily align to the cylinders main axis without cost of stretching or bending energy, see fig. 4.15 and fig. 4.16. The angle distribution in these networks is peaked at \( \theta = -\frac{\pi}{2} \) and \( \theta = \frac{\pi}{2} \) and the energy is zero. If the total length of chains increases, the peaks of the angle distribution shifts and all three energy components increase almost linearly with the ATL. The order parameter decreases but the network is still in an ordered phase.

**5.2 Simulation remarks**

In this project we studied the reorientation of the chains during the simulation. We started with a disordered state and found, in some cases, ordered states where the chains shows two preferred directions. This reorientation is a result of finding the network configuration with lowest energy. Finding the lowest energy configuration is a difficult job for a computer,
because the minimum found by normal non-linear conjugate gradient schemes depends on
the starting position of the algorithm and is not necessarily the global minimum. Therefore
we used a multi step nonlinear conjugate gradient scheme. This multi step helps us finding
many different minima and therefore it is likely that it finds the global minimum.

In the multistep process all crosslinks get, after a minimization step, a small random
displacement. This random displacement gives the simulation the ability to find minima
with lower energy. The random displacement is only accepted as the new network state if
this new minimum is lower in energy than the old minimum. However, to not get stuck
in the first minima, we force the network to always accept the first random displacement.
This method is also called annealing and can be compared with the annealing process of
metals, where metals are heated up cyclical to refine the structure of the metal.

In the regime where the minimal energy state is far from the initial network configuration,
this method works well. This procedure finds lower minima than a procedure without this
feature, see fig. 5.1. However, if the stretching constant is large, compared to the bending
constant, the lowest energy state is almost the same as the initial state. A random step
brings the system far from minimum, and it is difficult to come back to the initial state.
This causes the increase in energy during the minimization process at large radii, see figs. 4.7
and 4.12. Fig. 5.2 shows the minimization process for a network with a $\frac{160}{2\pi}$ radius and an
effective stretching constant and bending constant of 1. The maximum random length is in
a) 3.2, b) 0.4, c) 0.04 and d) 0.004.

![Energy vs IN plot](image)

**Figure 5.1:** Total energy during the minimization process, where IN is the number of the
iteration step (Iteration Number). The peaks indicates the random movements of the
crosslinks. The radius of the cylinder is $\frac{0.4}{2\pi}, \kappa = 1$, the bending constant is 1 and the
modulus of the cylindrical bending is 5.
Figure 5.2: Multistep minimization process for a $\frac{160}{2\pi}$ radius network with effective stretching and bending modulus of 1. The initial energy of the network was 13.65 and the maximal random step length is varied for the four figures.

5.3 Concluding remarks

The exact way of development and starting conditions of the AF are not clearly described in biology. Therefore we study different types of development in this thesis. The first situation which we study, the ring model, is the situation were the development starts with completely circumferentially tied fibers. In this ring model we saw tilting of the fiber due to the cylindrical surface. The tiling angle was radius and modulus dependent. Increasing of the radius causes less tilting.

In the second situation we simulate the AF as a network of fibers. The simulation starts with a random placing of chains, leading to a disordered network of fibers. Minimizing the energy results in a bimodal distribution of the orientation angles of the segments. With this network model, we simulated two different types of growing cylinders: fixed collagen
and growing collagen. In the fixed collagen simulations the rest lengths of the segments are fixed at their initial lengths. At large radii the network minimizes the energy by minimizing the stretch. The fibers in the network form a circumferential configuration at these large radii. In the case of growing collagen, were the rest lengths of the segments scale with the radius of the cylinder, the network is locked into its initial configuration at larger radii, resulting in a zero stretch energy.

Both radius dependency simulations show a decrease in tilting at larger radii, see figs. 4.3 and 4.9 which agrees with fig. 1.3. However, each method can be compared to another way of the deposition of new layers. The fixed collagen is related to the situation were each new layer is deposited at the inner of the cylinder, pushing the other layers outwards. The growing collagen simulations are more appropriate to describe the situation were each new layer is deposited from the outside, on top of the older layers.
Chapter 6

Conclusion & Recommendations

6.1 Conclusion

The goal of this study is to investigate the origin of the order in the polymeric network of the annulus fibrosus, with special attention to the role of the cylindrical shape of the network. Therefore we adapted a C++ simulation program, which was used to simulate the behavior of 2D polymeric network with fixed boundaries, into a simulation software which simulates polymeric networks on the surface of a cylinder. To do this we replaced two opposite fixed boundaries with one periodic boundary condition, and added an additional term to the Hamiltonian of the system to model the bending of a cylinder. The results of the simulations show, in the cylindrical bending determined regimes, the tilted alignment of the chains as a consequence of the bending due to the cylindrical shape. The fibers showed two preferred directions, and this is clearly shown in the angle probability distributions. These angle probability distributions are symmetric around 0. The influence of the cylindrical shape is confirmed by the effect of increased bending modulus. This resulted in higher order and steeper alignment. The macroscopic shape of the annulus fibrosus and the intervertebral disc can arrange the fibers in the network without straining the material.

While studying the development of the fiber orientation in the AF, it is necessary to distinguish two different types of growing: growing collagen and fixed collagen. In the growing collagen case, the network configuration of minimal energy is at small and large radii more or less the same as in the initial configuration. At intermediate radii the network shows the ordered state. This in contrast to the growth of the cylinder with fixed collagen. In this growing type, the network becomes stretched at large radii and to minimize this stretching, the network chains try to lie circularly around the cylinder. This is also shown in the ring model, where we simulate only one single fiber which is tied around the cylinder.
6.2 Recommendations

The model and the results of the simulations predict a tilted alignment of the fibers in a polymeric network on top of a curved substrate. This is a general behavior for all polymeric networks where individual chains can be described by the WLC. This can experimentally be tested by rolling up a thin flat polymeric network and studying the structure of the network. A second experiment which can be performed to verify our model is about varying the radius. Our model predicts a less tilted alignment at larger radii and this can be checked by studying the orientation of the fibers in different layers of the AF.

Another recommendation is about the biological picture of the AF development. In the literature the AF is shown as schematic representations instead of real images and this could lead to misconceptions. To improve the study of the AF development in the IVD it is needed to clear the following issues:

- The first issue is that it is not clear if all chains in one layer tilt in the same direction or that there is a bimodal distribution of the angles, like in our simulations. If the chains in a layer are directed in one single direction, than it is important to know how the alignment varies through the layers. Is the change in the orientation of the chains, from one direction to the opposite, in a gradual or stepwise way through the radial direction. If the orientation changes in a stepwise way, as shown in fig. 1.3, than it is interesting to know how a layer chooses its direction. If a layer randomly chose its direction, not all of the steps are of equal thickness. The thickness of all steps equal if the direction alternates from layer to layer.

- The second biological issue is that it is unclear whether or not collagen is created during the reorientation of the fibers. Both situations are simulated and the behavior of the networks with large radii is different, see paragraph 4.1 and 4.2.

6.3 Technology assessment

The annulus fibrosus is a beautiful example of a structured network of polymers. In contrast to the most common methods of orienting polymer fibers and inducing ordering in a network, which is straining of a sample, the AF uses its macroscopic shape to arrange its microscopic structure. This technique can give the industry an opportunity to regulate the internal structure of synthetic material from outside without straining the material.

Another aspect of the relevance of the study to the formation of the structure of the AF is of a biomedical kind. The study of formation of the ordered network in the AF increased the knowledge about the intervertebral disc. This knowledge can contribute to better treatments of some back injuries[20].

The last thing we would mention here is that the network structure is not only observed in the annulus fibrosus, but that is a general structure in biology for networks on tubular substrates, like blood vessels for instance.
Bibliography


Appendices
Appendix A

Algorithm

In this appendix we describe the algorithm used by the software to simulate a polymeric network on a cylinder. As discussed in chapter 3, energy minimization is one of the crucial steps in the simulation, and therefore we begin this appendix by explaining the minimization algorithm.

A.1 Energy minimization

In chapter 3 we described the energy of the network. Here we describe the algorithm used in the simulation to minimize the energy: Nonlinear conjugate gradient. For explaining the principle of this method we start with an intuitive method and extend this until we get at the desired method. To describe the minimization method used in the simulation we initially approach the expression of the energy up to the second order around a certain network configuration $\mathbf{x}$:

$$E(\mathbf{x}) = c - \mathbf{b} \cdot \mathbf{x} + \frac{1}{2} \mathbf{x} \cdot \mathbf{A} \cdot \mathbf{x},$$

where $A_{mn} \equiv \frac{\partial^2 E}{\partial x_m \partial x_n}$ which is the generalized elasticity modulus of the network, $b_n \equiv \frac{\partial E}{\partial x_n}$ is a force pulling an individual segment out of equilibrium and $c$ is an energy offset. In the following subsections, we provide some minimization algorithms, starting with the method of steepest descent, followed by conjugate directions scheme, conjugate gradient and eventually the nonlinear conjugate gradient.

Steepest descent

One of the simplest minimization routines one could think of is the method of steepest descent. To illustrate the working of the method of steepest descent, we minimize a function
of quadratic form

\[ f(x) = c - b \cdot x + \frac{1}{2} x \cdot A \cdot x. \]  (A.2)

This method begins at an arbitrary point \( x_0 \) and moves into the direction where the function decreased fastest. This direction \( d \) is \(-\nabla f(x)\) and the step size \( \alpha \) should be chosen in such a way that it minimizes \( f \). The next point is \( x_i = x_{i-1} + \alpha d_{i-1} \) with \( \frac{d}{dx} f(x_i) = f'(x_i)^T \frac{d}{dx} x_i = f'(x_i)^T d_{i-1} = 0 \). To minimize the function \( f(x) \), \( \alpha \) should be chosen in such a way that \( d_{i-1} \) and \( f'(x_i) \) are orthogonal. Now we define the error, which is a vector indicating how far we are from the solution, as \( \vec{e}_i = \vec{x}_i - \vec{x} \) where \( \vec{x} \) is the location of the minimum and define the residual as \( \vec{r}_i \) as

\[ \vec{r}_i = \vec{b} - A\vec{x}_i, \]  (A.3)

which is the error transformed into the same space as \( \vec{b} \). This residual is also the direction of the steepest descent. It is clear to see that at the minimum the residual is zero. The algorithm for the steepest descent method becomes

\[ r_i = b - A \cdot x_i \]  (A.4)

\[ \alpha_i = \frac{r_i^T \cdot r_i}{r_i^T \cdot A \cdot r_i} \]  (A.5)

\[ x_{i+1} = x_i + \alpha_i r_i. \]  (A.6)

This algorithm produces a zigzag trajectory because all the consecutive directions are orthogonal. This zigzag pattern is not the most efficient way to approach the minimum, because it searches several times in one direction and this causes a relatively low speed of convergence. The calculation stops when \( ||\vec{r}_i|| \) is smaller than a preset value \( ftol \).

**Conjugate directions**

The speed of convergence can be increased by using the method of conjugated directions. The search direction in this method are A-orthogonal to all the search directions in previous steps. Two vectors \( \vec{d}_i \) and \( \vec{d}_j \) are A-orthogonal if \( \vec{d}_i A \vec{d}_j = 0 \). Now, we define the error as \( \vec{e}_i = \vec{x}_i - \vec{x} \) and call the direction of the line search \( \vec{d}_i \). After each step we find a point

\[ x_{i+1} = x_i + \alpha_i \vec{d}_i. \]  (A.7)

Minimizing the function \( f(\vec{x}) \) shows the functionality of the conjugate gradient method:

\[ \frac{d}{d\alpha} f(\vec{x}_{i+1}) = f'(\vec{x}_{i+1})^T \frac{d}{d\alpha} \vec{x}_{i+1} = f'(\vec{x}_{i+1})^T \frac{d}{d\alpha} \vec{d}_i = -\vec{r}_{i+1}^T \vec{d}_i = 0 \]  (A.8)
\[ \vec{d}_i^T A \vec{e}_{i+1} = 0. \]  
(A.9)

This means that the direction \( \vec{d}_i \) is \( A \)-orthogonal to the error \( \vec{e}_{i+1} \). The initial error term \( \vec{e}_0 \) can be expressed as a linear combination of the search directions:

\[ \vec{e}_0 = \sum_{j=0}^{n-1} a_j \vec{d}_j. \]  
(A.10)

This method differs the error step by step and computes \( \vec{e} \) in \( n \) steps. All that is needed for using this method is a set of \( n \) \( A \)-orthogonal search directions. These search directions can be generated by the conjugate Gram-Schmidt process of \( n \) linearly independent vectors \( \vec{u}_0, \vec{u}_1, ..., \vec{u}_{n-1} \). The search direction \( \vec{d}_i \) is constructed by taking \( \vec{u}_i \) and subtracting any component which is not \( A \)-orthogonal to all the previous search directions.

\[ \vec{d}_i = \vec{u}_i + \sum_{k=0}^{i-1} \beta_{ik} \vec{d}_k \]

, where \( \beta_{i,k} \) are defined for \( i > k \). We use the \( A \)-orthogonality of \( \vec{d}_i \) and \( \vec{d}_j \) to find the values of \( \beta_{i,k} \):

\[ \vec{d}_i^T A \vec{d}_j = \vec{u}_i^T A \vec{d}_j + \sum_{k=0}^{i-1} \beta_{ik} \vec{d}_k^T A \vec{d}_k \]  
(A.11)

\[ 0 = \vec{u}_i^T A \vec{d}_j + \beta_{ij} \vec{d}_k^T A \vec{d}_k, i > j \]  
(A.12)

\[ \beta_{ij} = -\frac{\vec{u}_i^T A \vec{d}_j}{\vec{d}_k^T A \vec{d}_k}. \]  
(A.13)

The step \( \alpha_i \) can be calculated by substituting equation (A.10) into (A.9), this gives

\[ \vec{d}_i^T A (\vec{e}_i + \alpha_i \vec{d}_i) = 0 \]  
(A.14)

so,

\[ \alpha_i = \frac{\vec{d}_i^T A \vec{e}_i}{\vec{d}_i^T A \vec{d}_i} = \frac{\vec{d}_i^T \vec{r}_i}{\vec{d}_i^T A \vec{d}_i}. \]  
(A.15)

The difficulty with this method of Conjugate Directions is the fact that constructing the search directions requires \( O(n^3) \) operations, see [21]. To overcome this problem, we use the conjugate gradient method.
Conjugate gradient

This method is similar to the conjugate directions method in the way that it searches just one time in each direction, but differs in the way it chooses its search directions. The search directions in the conjugate gradient method are constructed out of the residuals, by setting $\vec{u}_i = \vec{r}_i$. So, after each step, the residual becomes

$$\vec{r}_{j+1}^T = \vec{r}_j - \alpha_j A\vec{d}_j.$$  \hfill (A.16)

Taking the inner product of this equation and $\vec{r}_i$ and rearranging this gives

$$\vec{r}_i^T A\vec{d}_j = \frac{1}{\alpha_i} (\vec{r}_i^T \vec{r}_j - \vec{r}_i^T \vec{r}_{j+1}).$$  \hfill (A.17)

So,

$$\vec{r}_i^T A\vec{d}_j = \begin{cases} \frac{1}{\alpha_i} \vec{r}_i^T \vec{r}_i & i = j \\ -\frac{1}{\alpha_{i-1}} \vec{r}_i^T \vec{r}_i & i = j + 1 \\ 0 & \text{otherwise} \end{cases}.$$  \hfill (A.18)

The Gram-Schmidt constants can now be constructed. Most of the constants disappeared, only the $\beta_{i,i-1}$ constants are nonzero, henceforth calling $\beta_i$. These constants are

$$\beta_i = \frac{\vec{r}_i^T \vec{r}_i}{\vec{r}_{i-1}^T \vec{r}_{i-1}}.$$  \hfill (A.19)

Now, we put all together and the algorithm of the conjugate gradient method becomes

$$\begin{align*}
\vec{d}_0 &= \vec{r}_0 = \vec{b} - A\vec{x} \\
\vec{x}_i+1 &= \vec{x}_i + \alpha_i \vec{d}_i \\
\vec{r}_{i+1} &= \vec{r}_i - \alpha_i A\vec{d}_i \\
\beta_{i+1} &= \frac{\vec{r}_{i+1}^T \vec{r}_{i+1}}{\vec{r}_i^T \vec{r}_i} \\
\vec{d}_{i+1} &= \vec{r}_{i+1} + \beta_{i+1} \vec{d}_i
\end{align*}$$

This method is suitable for finding minima for linear quadratic equations. However, our energy equation is not of a quadratic form, so we need the nonlinear conjugate gradient method which is described in the next subsection.
Figure A.1: Initial minimization of the network’s energy using the Nonlinear conjugate gradient scheme. In this the energy of the network after each iteration line search is plotted, and one can see the convergence towards the (local) minima. The energy consists of three components and although the total energy decreases, some energy components gain in energy to accomplish the minimal energy state.

**Nonlinear conjugate gradient**

The nonlinear conjugate gradient method has three changes compared to the linear method: the recursive equation for the residuals is not correct anymore, it is more complicated to calculate the step size $\alpha_i$ and there are several options for $\beta$. In this method the residual is set to the negation of the gradient: $\vec{r}_i = -f'(\vec{x}_i)$. The search directions are calculated by the conjugated Gram-Schmidt process and two of the choices for calculating $\beta$ are the Fletcher-Reeves formula, this is used in the linear conjugate gradient method, and the Polak-Ribière formula:

\[
\beta_{FR}^{i+1} = \frac{\vec{r}_i^T \vec{r}_{i+1}}{\vec{r}_i^T \vec{r}_i} \\
\beta_{PR}^{i+1} = \frac{\vec{r}_i^T (\vec{r}_{i+1} - \vec{r}_i)}{\vec{r}_i^T \vec{r}_i}
\]  

(A.20)

(A.21)
Figure A.2: Multistep minimization of the networks energy. Each single minimization step is performed using the nonlinear conjugate gradient scheme, see fig. A.1. After each step, all crosslinks get a random displacement out of equilibrium and then minimizes towards a new minimum energy state. This new state is accepted as the systems state if it has less energy than the old state. This procedure is repeated until the new state is denied for n times in a row, where n is a preset value.

The minimization routine now becomes:

\[
\begin{align*}
\vec{d}_0 &= \nabla f(\vec{x}_0) \\
\vec{\alpha}_i &= \text{argmin}_{\alpha \in \mathbb{R}} \{ f(\vec{x}_i + \alpha \vec{d}_i) \} \\
\vec{x}_{i+1} &= -\nabla f(\vec{x}_{i+1}) \\
\vec{r}_{i+1} &= \vec{r}_i - \alpha_i \vec{d}_i \\
\beta_{i+1} &= \beta_{i+1}^{FR} \text{or} \beta_{i+1}^{PR} \\
\vec{d}_{i+1} &= \vec{r}_{i+1} + \beta_{i+1} \vec{d}_i
\end{align*}
\]

(A.22)

where \( \text{argmin}_{\alpha \in \mathbb{R}} \) is the value of the step size \( \alpha_i \) where \( f \) is minimized. This value can be found with the Newton-Raphson or the Secant method. The function \( f \) is not a quadratic function, so it has more minima. The nonlinear conjugate gradient method is therefore not guaranteed to converge to the global minimum but only to a local minimum. The minimization using this nonlinear conjugated gradient scheme is shown in fig. A.1. There are 4 lines plotted in this graph, three of these lines are the stretch, 2D bending in plane and the cylindrical bending components and the fourth line is the total energy. Before minimization
the energy is, as explained before, totally determined by the cylindrical bending. In order to achieve the lowest energy configuration some energy components gain in energy during the minimization process. It is clear that the networks energy converges towards a (local) minimum. Now, to find the global minimum of the network we gave all the crosslinkers a small random displacement out of equilibrium and minimize this new state with the nonlinear conjugated gradient algorithm. If this new state has a lower energy then the old state, the new state is accepted. Otherwise the new state is denied. This process will be repeated until the new state is denied for \( n \) times in a row, where \( n \) is a preset value. This process is shown in fig. A.2.

A.1.1 Flowchart

To conclude this appendix we give a flowchart of the code which visualizes the structure of the simulation. This flowchart is shown in fig. A.3. The simulation begins with the generation of the chains, afterwards the code searches for the crosslinks between the chains and initializes the network. The next step is minimization of the energy, which is carried out by the nonlinear gradient method. If this minimization step is finished, a loop will start. All crosslinks get a small random displacement, and the configuration relaxes then to a (new) minimized state. If this state is lower in energy than the previous relaxed state, this new state is accepted as the systems state, otherwise it is rejected. The loop stops if the new state is rejected for \( N_{\text{max}} \) times in a row. The simulation is ready if the loop is finished, the only thing the code still has to do is exporting of data. This data includes the coordinates of the networks crosslinks, the total energy and the three different components of the energy.
Figure A.3: Flowchart of the simulation. The simulation starts with the creating of the chains and initialization of the system. After this the energy will be minimized using the nonlinear gradient method as described in section 3.2.4. in combination with a small random displacement of the crosslinkers. The simulation stops if there are $N_{max}$ rejected states in a row.
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