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STUDY OF MICRO-MECHANICS OF BIOPOLYMER NETWORK AT NANO SCALE

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ABSTRACT

A detailed understanding of biopolymer networks can be considered a corner stone for future developments in many fields of modern science and engineering ranging from biophysics and biochemistry to bioengineering, biomedical engineering and material science. The small length scale and high complexity of such networks significantly hamper the application of traditional theoretical and experimental methods. As a consequence, computer simulations have attracted increasing interest in this field over the last years. As molecular dynamics simulations are often too expensive and macromechanical continuum models are not detailed enough, the development of a sufficiently detailed and at the same time efficient micromechanical simulation model has become a major target in biosciences. Biopolymer networks consist of three essential components: filaments, crosslinker molecules connecting them by chemical bonds, and a viscous fluid into which filaments and cross linker molecules are embedded. These three components are modeled as continua, respectively.

Keywords: Nano Tribology, Micro Mechanics, Biopolymer Networks, Linkers, Filaments.

I. INTRODUCTION

Biopolymer networks are formed by three main constituents: polymer filaments, crosslinker molecules (referred to also as 'linkers' in the following) which connect these filaments by transient chemical bonds, and a background fluid into which filaments and linkers are embedded. The background fluid is typically an aqueous solution and therefore nearly transparent. The size of the linkers on the other hand ranges on the nanometer scale. The variety of completely different network architectures which can be formed by one and the same kind of filament just by the application of different linkers is remarkable.

The first important question is how to model the three main constituents of biopolymer networks in view of their small size. It is well-known that the behaviour of atoms as well as of any system constituted by atoms can be predicted in an accurate manner by the application of the laws of quantum mechanics to the complete system. However, the tremendous complexity of the resulting equations renders it impossible to pursue that strategy when trying to simulate systems consisting of more than just some dozens of atoms. To overcome that problem, one often resorts to molecular dynamics (MD) simulations, where effects of quantum physics are not considered

explicitly, and single atoms or small packages of atoms are the smallest units. These are modeled on the basis of Newtonian mechanics, and their equations of motion typically account for potentials due to bonds between neighbouring units, non-bonded potentials as well as potentials originating from coulombic forces.

1.1 Micromechanical Continuum Model of Biopolymer Networks

The mechanics of biopolymer networks is primarily governed by deformations on the length scale of single filaments, which are typically some micrometers long. On this length scale, it is well-known from microfluidics as well as experimental polymer physics that continuum models already apply. Linkers are typically much smaller than filaments so that modeling them accurately by similar techniques may be more difficult. However for linkers, a continuum model is expected to be sufficient when focussing on the mechanics of whole networks rather than of single linkers.

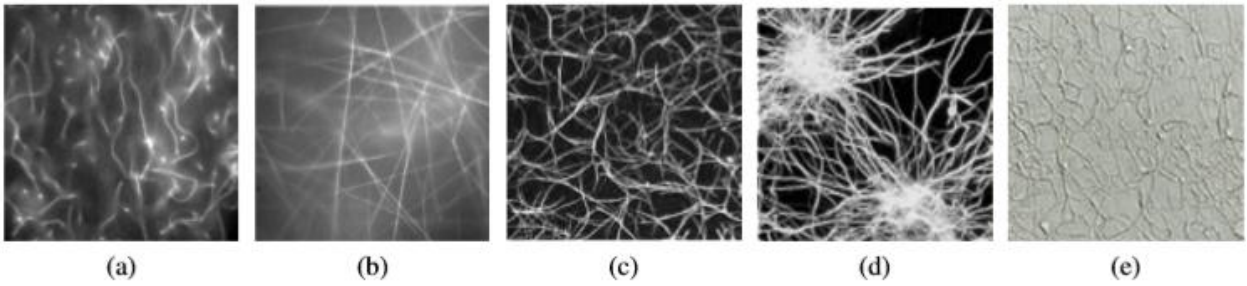

Figure 1.0: Examples for biopolymer networks invitro: (a) network of entangled actin filaments , (b) actin-fascin bundle network, (c) actin-filamin bundle network , (d) actin cluster network, (e) actin layer

1.2Fluids

From microfluidics it is well-known that a continuum model based on the Navier-Stokes equations is suitable for fluid volumes down to the micrometer length scale relevant for biopolymer networks. Modeling the fluid in these networks, one is confronted with two major difficulties: first, on the length scale of biopolymers stochastic thermal fluctuations according to the laws of statistical mechanics affect the fluid velocity field perceptibly; second, the fluid dynamics is affected by complex fluid-structure interactions with all the filaments and linkers in the network. These two effects can be accounted for in detail only at a considerable computational cost. Therefore various simplifications are common in polymer physics. Due to the small length scale, low velocities and high viscosities, Reynolds and Mach numbers are usually small in biopolymer networks. Therefore a linearized version of the incompressible Navier-Stokes equations can be applied [1] so that the fluid velocity field can be separated into a deterministic part and a stochastic part owing to the thermal fluctuations.

Because of the very small diameter and total volume fraction of filaments and linkers in the whole network (e.g., in a 4μM actin network filament diameter is 5nm and volume fraction 6.5%), the effect of the filament and linker motion on the deterministic part of the fluid velocity field is small. Therefore the deterministic part of the fluid velocity field is computed initially on the basis of the boundary conditions of the simulation volume only, and the drag forces and moments on filaments and linkers due to their relative motion compared to this velocity field are captured by simple friction coefficients assuming a Newtonian fluid. In general, the deterministic part

of the fluid velocity field can be computed by standard methods such as the finite element or finite volume method. In practice, not even this may be necessary as often it is either zero or a simple shear flow which can be calculated analytically without any discretization.

1.3 Filaments

Force transmission in biopolymer networks is usually assumed to occur predominatly via the filaments. These are rod-like structures typically longer than 100nm with a slenderness ratio typically between 10 and far above 1000. From a variety of experiments (e.g. [2, 3, 4, 5]) it is well-known that not only the fluid on that length scale can be modeled as a mechanical continuum,but that also rod-like structure such as biopolymers can be considered as mechanical continua with axial, bending and torsion stiffness so that classical beam theories such as the one of Euler and Bernoulli can be employed [6]. Thus the dynamics of filaments has not to be modeled on the basis of quantum mechanics or molecular dynamics (MD), but rather a coarse grained continuum model can be used instead of an atomistic or molecular one

1.4 Linkers

Filaments in biopolymer networks may be connected mechanically by cross linking molecules, to which we will refer in the following also as linkers. Linkers are typically between 5nm (fascin, [7]) and 100nm (filamin, [8]) long and thus much smaller than the filaments in the networks, which are typically some microns long. They can form up to two chemical bonds with so-called binding sites on the filaments. Hence, we may distinguish between three states of linkers:

- free (i.e., without any chemical bond to any filament),
- singly bound (i.e., with one chemical bond to some filament),
- doubly bound (i.e., with two chemical bonds to filaments).

Free linkers are solved as particles in the fluid and not expected to take part in the force transmission in the network perceptibly. Rather they represent a pool of particles which can form chemical bonds with filaments close by and thereby change to a state of greater mechanical importance. Therefore only the position of these particles matters, because it is the basis for the decision which filaments are close enough to establish a chemical bond. Neglecting their internal structure fluctuations, they are thus modeled as point-shaped particles moving through the network stochastically according to the laws of Newtonian and statistical mechanics.

Linkers which are already bound to one filament are not supposed to perceptibly affect the network mechanics, either. Excessive amounts of such linkers may change the effective stiffness of the filament to which they are attached, but even this effect is expected to be negligible in most cases. Therefore such linkers are still modeled as point-shaped particles, but not in stochastic Brownian motion, but rather attached to a filament at a certain point. If they come close enough to another binding site they may form a second chemical bond and become doubly bound linkers. Linkers having established chemical bonds to two filaments can be modeled as rod-like continua coupling these filaments with an effective stretching, shear, torsion and bending stiffness. As also the filaments themselves, they are subject to viscous damping and stochastic excitation from the fluid.

Altogether, in a computer simulation based on the above linker model, for free and singly bound linkers only the positions have to be tracked, whereas for doubly bound linkers also the force transmission they allow between filaments has to be captured.

1.5 Interactions

Not only fluid, filaments, and linkers have to be modeled, but rather also the interactions between them. As discussed above, the interactions between fluid and filaments can be captured by viscous and stochastic forces and moments in the equation of motion of the filaments. The same is true also for linkers. In experiments so far three main types of such interactions have been observed:

- chemical interactions
- contact interactions
- long-range electrostatic interactions.

Chemical interactions lead to the formation of chemical bonds between linkers and filaments and turn thereby free linkers into singly bound ones and singly bound linkers into doubly bound ones. These bonds are in general only temporary and disaggregate after a while stochastically owing to thermal fluctuations. Such unbinding events turn doubly bound linkers into a singly bound ones and a singly bound linkers into a free ones. Contact interactions play an important role when filaments and linkers come close to each other and prevent them from overlapping. Physically, they are short-range electrostatic interactions. In addition to that, in principle also long-range electrostatic interactions may result from the fact that filaments and linkers are in general electrically charged. It is often doubted that long-range electrostatic interactions play an important role in biopolymer networks, because the charge of filaments and linkers is supposed to be shielded on long distances quite effectively by ions in the surrounding fluid. Therefore altogether three types of interactions are in general to be modeled in simulations of biopolymer networks.

II. NUMERICAL MODEL OF LINKERS

For setting up a numerical model of linkers, some initial definitions are helpful. First of all, the distance between the two binding domains of a linker is assumed to range in the interval $[Rcl - \Delta Rcl; Rcl + \Delta Rcl]$. Here Rcl is the characteristic distance between the two binding domains and ΔRcl a tolerance accounting for the fact that the actual distance between the binding domains may vary over time, e.g., owing to minor thermal fluctuations of the linker position, orientation and configuration. Values of Rcl are presented in Table 4.1 for several linkers common in biopolymer networks.

Cross linker type	HMM	α – actinin	filamin	fascin	espin
Rcl	40nm	40nm	98nm	5nm	5nm
Reference	[13]	[14]	[8]	[7]	[7]

Table 1.0: Biologically especially relevant crosslinker molecules, the characteristic distance Rcl they bridge

The position of the point in the middle between the two binding domains is denoted by $x_{cl,c}$. In general, the lower index $\square_{cl}$ is reserved in this thesis to mark properties of linkers. Then linkers can be modeled in computer simulations depending on their current state as follows.

2.1 Free linkers

for a free linker it is enough to keep track of its position in space so that it can be modeled as a point-like particle with position xcl,c and friction coefficient ζcl. In general its equation of motion is then given by

$$f_{visc,cl}(x_{cl,c}, \square_{cl,c}, t) = f_{ext,cl}(x_{cl,c}, t) + f_{stoch,cl}(x_{cl,c}, t) \quad \text{----------(1)}$$

where fvisc,cl is the viscous force the linker experiences moving through the background fluid, fext,cl is the sum of external deterministic forces the linker is subject to, e.g., as a consequence of force fields, and fstoch,cl is the stochastic thermal force exerted by the thermal bath into which it is embedded. In a Newton-fluid, the viscous force is simply given by

$$f_{visc,cl} = \zeta_{cl} \, \square_{cl,c} \quad \text{----------(2)}$$

And the stochastic force is then according to the fluctuation-dissipation theorem

$$f_{stoch,cl} = \sqrt{2kBT\zeta_{cl}} \, \dot{W}_{cl}(t) \quad \text{----------(3)}$$

With the standard wiener process $\dot{W}_{cl}(t)$. For the computation of the position $x_{cl,c}$ a simple backward Euler scheme can be applied to (1) in general.

2.2 Singly bound linkers

The contribution of singly bound linkers to the polymer network is usually negligible so that it is enough keeping track of their position. Their position, however, is in opposition to the one of free linkers not governend by stochastic, thermal motion, but rather by the motion of the filament they are bound to. Thus we do not compute the position of singly bound linkers explicitly, but simply assume that they follow the binding site to which they are bound without affecting the dynamics of the filament on which this binding site is situated.

2.3 Doubly bound linkers

Doubly bound linkers form elastic and often rather stiff connections between two filament binding sites. Anyway, an extensional stiffness has to be attributed to this mechanical connection. Depending on whether experiments suggest either a finite or a negligible bending and torsion stiffness, doubly bound linkers are therefore represented by either finite beam elements or simple truss elements. The reference length of these elements may be set equal to the distance between the two filament binding sites connected by the linker at the point in time when the connection is established. In addition to that, so-called active linkers (motor proteins) may have an additional characteristic property which is the force or moment they can actively exert on the filaments they connect in order to shift or turn them against each other. This property of active linkers is well-known to play a key role in the rearrangement of the cytoskeleton during cell migration so that active linkers in general play an important role in cell biology. A variety of fundamental properties of biopolymer networks,

however, can already be studied only by means of passive linkers. Therefore the focus of this thesis is limited for simplicity on the latter ones, leaving the issue of active linkers for future work.

III. NUMERICAL MODEL OF INTERACTIONS BETWEEN FILAMENTS AND LINKERS

3.1 Chemical interactions

Filaments and linkers may interact with each other by chemical bonds which transmit forces and moments. In the following, we will assume that such bonds arise only between one filament and one linker, respectively. Direct chemical bonds between two filaments or two linkers will not be discussed as in biopolymer networks such bonds are typically assumed either not to arise at all or not to matter. It is emphasized, however, that the quantitative considerations below about bonds between one filament and one linker can be directly applied also to chemical bonds between two filaments or two linkers if this required in some special case in the future.

To form a chemical bond, a linker L and a free filament binding site F have to be sufficiently close to each other, i.e., the linker has to be within the so-called reaction volume Vreact of the binding site. Once this is the case, both molecules can form a bond if they reach a proper relative position and orientation. In a very short time interval Δt, where the probability of multiple binding and unbinding events is negligible, this can be modeled by a Poisson process with a so-called on-rate kreact,on, and the probability for binding can be computed by

$$p_{on} = 1-e^{(-k_{react,on}\,\Delta t)} \qquad \text{----------(4)}$$

Similarly, unbinding of a linker already bound to a filament happens with an off-rate kreact,off and the probability

$$p_{off} = 1-e^{(-k_{react,off}\Delta t)} \qquad \text{----------(5)}$$

In computer simulations based on the method introduced in the preceeding sections, filament and linker positions are known at each point in time. Thus for a numerical model of chemical interactions between filaments and linkers, one just has to define the position of the binding sites on the filaments and their respective reaction volume.

In principle, a binding site may be any point on the filament. In reality, binding sites are usually periodically distributed over filaments with a characteristic distance hbind. If filaments are discretized with finite elements, the simplest way of modeling binding sites is setting the finite element discretization length h equal to hbind and defining the finite element nodes as the binding sites of the filament. This allows for modeling doubly bound linkers just as finite beam or truss elements connecting two already existing nodes in the filament discretization.

Modeling the reaction volume is possible in various ways. Assuming that free linkers can react with binding sites, if the distance between the linker center xcl,c and the binding site ranges in the interval $[(Rcl - \Delta Rcl)/2;(Rcl + \Delta Rcl)/2]$. Singly bound linkers on the other hand can bind to another binding site only if the distance of this binding site and the one they are already attached to ranges in the interval $[Rcl-\Delta Rcl;Rcl+\Delta Rcl]$. By means of these two distance criteria one can decide whether a linker is in the reaction volume of a binding site. If so, the calculation of the binding probability between both is simply possible by (4) for a simulation time step of length Δt.

3.2 Contact interactions

Mechanical contact between filaments and linkers poses kinematic constraints to filament and linker motion which can be accounted for as usual in finite element simulations [10]. In the equations of motion, contact forces can be accounted for by deterministic external forces. As linkers are typically much smaller than filaments, the volume of free and singly bound linkers may either be neglected completely in contact computations or modeled as ball around the linker center or binding site to which the linker is attached. Doubly bound linkers are represented by beam elements and their volume can be accounted for in contact computations accordingly. If free and singly bound linkers are neglected, contact can be modeled exclusively as what is referred to in finite element textbooks and articles as beam contact. In principle, the methods described there can be directly applied to biopolymer networks.

In studies of single polymers, contact interactions play a role only if the polymer is flexible enough for self-contact. This is expected to be the case only for so-called flexible polymers whose length L is much larger than their persistence length Lp, whereas for semi-flexible polymers with $L \approx Lp$ and stiff polymers with $L << Lp$ self-contact is not expected.

3.3 Long-range electrostatic interactions

Both filaments and linkers in biopolymer networks may exhibit an electric charge. For actin filaments, e.g., the linear charge density is 4e/nm [10]. This charge may on the one hand affect the effective stiffness of filaments. In more complex cases, it may on the other hand cause long-range interactions between different filaments and linkers or just different segments of one and the same filament or linker. Additionally, the electric charge may entail complex ionic patterns in the surrounding fluid shielding it partially. For biopolymer networks, the situation is more complicated. So far, no evidence has been presented that electrostatic forces play a major role for their mechanics if self. However, diffusion of charged particles [11] through networks or the network architecture in the presence of certain linker types [12] may be significantly affected by electrostatic effects beyond just an altered filament stiffness.

IV. CONCLUSION

Three main constituents of biopolymer networks – filaments, linkers and fluid – are distinguished and modeled as continua, respectively. The mechanics of filaments is assumed to dominate the mechanics of the networks, and thus only coarse models are employed for linkers and fluid. The fluid is modeled as a continuum on the basis of the incompressible linearized Navier Stokes equations. The fluid velocity field is computed in the absence of filaments and linkers, and the friction which these experience is then computed by certain friction coefficients and their velocity relative to the fluid.

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