Universidade de São Paulo
Instituto de Física

Caracterização reológica de redes poliméricas de actina: medição e modelagem numérica

Mariana Oshima Menegon

Orientador: Prof. Dr. Adriano Mesquita Alencar

Dissertação de mestrado apresentada ao Instituto de Física para a obtenção do título de Mestre em Ciências

Banca examinadora:

Prof. Dr. Adriano Mesquita Alencar (IFUSP)

Prof. Dr. Mario José de Oliveira (IFUSP)

Prof. Dr. Oscar Nassif de Mesquita (UFMG)

São Paulo
2015

FICHA CATALOGRÁFICA
Preparada pelo Serviço de Biblioteca e Informação
do Instituto de Física da Universidade de São Paulo

Menegon, Mariana Oshima

Rheological characterization of actin polymeric networks: measurement and numerical modeling / Caracterização reológica de redes poliméricas de actina: medição e modelagem numérica. São Paulo, 2015.

Dissertação (Mestrado) – Universidade de São Paulo. Instituto de Física. Depto. Física Geral.

Orientador: Prof. Dr. Adriano Mesquita Alencar

Área de Concentração: Física.

Unitermos: 1. Biopolímeros; 2. Difusão; 3. Reologia; 4. Simulação.

USP/IF/SBI-077/2015

University of São Paulo
Institute of Physics

Rheological characterization of actin polymeric networks: measurement and numerical modeling

Mariana Oshima Menegon

Adviser: Dr. Adriano Mesquita Alencar

Master Thesis presented to the Institute of Physics in partial fulfillment of the requirements for the degree of Master in Science

Examination board:

Dr. Adriano Mesquita Alencar (IFUSP)

Dr. Mario José de Oliveira (IFUSP)

Dr. Oscar Nassif de Mesquita (UFMG)

São Paulo
2015

Agradecimentos

O desenvolvimento deste trabalho contou com o apoio de diversas pessoas e instituições. São muitos os membros do grupo que me ajudaram, agradeço a todos pelo que aprendi e pelo convívio que tornou mais prazeroso o meu período de mestrado. Em particular, agradeço à Mariana Ferraz que me acompanhou nos experimentos, ensinando-me a utilizar os equipamentos do laboratório, discutindo procedimentos e análises, sendo companheira e amiga. Agradeço ao meu orientador Adriano Alencar por ter me orientado neste trabalho, por seu engajamento com o desenvolvimento científico e pessoal dos seus alunos e por inspirar-me com seu entusiasmo pela pesquisa e ciência. Agradeço ao professor Kees Storm, da Universidade Tecnológica de Eindhoven, que me recebeu e me orientou durante seis meses de estágio nesta universidade, mostrando-me novas abordagens e novos caminhos. Agradeço aos muitos membros do Instituto de Física da Universidade de São Paulo, por manterem a infraestrutura de que usufruí e pela convivência sempre descontraída. Por fim, agradeço às agências de fomento FAPESP e CNPq que financiaram este trabalho, concederam minha bolsa e me proporcionaram muitas outras oportunidades de aprendizado, como a participação em eventos e o estágio no exterior.

Resumo

Um problema proeminente em mecânica celular é relacionar como as modificações do citoesqueleto das células eucariontes resultam em alterações nas suas propriedades viscoelásticas. A proposta deste projeto é entender o comportamento mecânico das fibras de actina, principal componente do esqueleto celular, através de experimentos e do desenvolvimento de um modelo computacional que o descreva, na tentativa de entender as origens microscópicas da viscoelasticidade de redes de actina. Nesse contexto, existe uma extensa bibliografia voltada a modelos de comportamentos específicos das fibras do esqueleto celular, em particular, dos filamentos de actina. Contudo, a questão global relativa ao nível celular permanece em aberto. Experimentos com géis de actina *in vitro* foram o ponto de partida para conhecer os comportamentos mecânicos das fibras. Os dados experimentais obtidos foram a referência principal aos modelos matemáticos desenvolvidos para simular as propriedades viscoelásticas da rede de actina. Assim, foi montado um ambiente de trabalho, experimental e computacional, para estudar as propriedades mecânicas do citoesqueleto por meio de seu componente mais abundante.

Abstract

An outstanding problem of cellular mechanics is how to relate changes on the cytoskeleton which result in modifications of the viscoelastic properties of the cells. The proposal of this work is to understand the mechanical behavior of actin fibers, the major component of cytoskeleton, by means of experiments and development of a computational model that describe it, in an attempt to understand the microscopic origins of the viscoelasticity of F-actin networks. In this context, extensive scientific literature has focused on models of specific behaviors of the cytoskeleton fibers, specifically on actin filaments. However, the global question related to the cellular level remains open. *In vitro* actin solutions experiments were the starting point to study the mechanical behavior of the fibers. The produced experimental data was the gold standard to the numerical models that was developed to simulate the viscoelastic properties of the actin network. Thus, we built an experimental and computational framework to study the mechanical properties of the cytoskeleton, by the means of its most abundant component.

Contents

1	Introduction	1
1.1	Motivation	2
1.2	Aim and outline of this Master thesis	6
1.3	Actin: from the molecular to the cellular level	7
1.4	Physical characteristics of actin filaments and networks	10
1.4.1	Persistence length	11
1.4.2	Mesh size	12
1.5	Rheology and microrheology	13
1.6	Passive microrheology	15
1.6.1	Generalized Stokes-Einstein relation	17
1.6.2	Viscoelastic moduli from the GSE relation	18
1.A	Appendix: Concepts of rheology	19
1.B	Appendix: Brownian dynamics and diffusion	22
2	Experimental Part	25
2.1	Materials and methods	26
2.1.1	Experimental setup	26
2.1.2	Preparation of F-actin and other solutions	27
2.1.3	Sample preparation	29
2.1.4	Analysis of data	31
2.2	Viscoelasticity of F-actin solutions	33
2.2.1	Control	33

2.2.2	Results and discussion	34
2.2.3	Conclusions	35
2.3	Elasticity of gels of collagen type I	36
2.3.1	Sample preparation	37
2.3.2	Results and discussion	37
2.3.3	Conclusions	38
3	Numerical Part	39
3.1	Numerical methods	40
3.1.1	Lattice simulations	40
3.1.2	Random walk simulations	41
3.1.3	Generating Mikado networks	44
3.1.4	Molecular dynamics simulations	46
3.1.5	Numerical estimation of the persistence length	49
3.2	Preliminary studies with the two-dimensional lattice	50
3.2.1	Results and discussion	50
3.2.2	Conclusions	53
3.3	Simulations with three-dimensional models	54
3.3.1	Static approach	54
3.3.2	Nonstatic approach	58
3.3.3	Comparison with experimental data	60
3.3.4	Conclusions	64
3.A	Appendix: The damp parameter	66
4	Final considerations	69
4.1	Conclusion	70
4.2	Outlook	72

Chapter 1

Introduction

In this introductory chapter we present the motivation, the aim and structure, and the background to this Master thesis. The work is in the context of microrheological characterization of entangled networks of filamentous actin (F-actin). F-actin is the major protein of the cytoskeleton and, for this reason, is of great interest to the studies of the cells mechanics. Microrheology, on the other hand, consists in probing the mechanical response of materials sampling at microliter scale. Microrheology can be done by using embedded colloidal particles that have their movement driven either by thermal energy or external forces. In this study we used the former, which is called passive microrheology. Details about the F-actin and the passive microrheology are also provided in this chapter.

1.1 Motivation

Smaller strains and smaller samples: these two advantages of microrheology over bulk rheology have made the technique very convenient to assess the viscoelastic properties of biological materials which are often fragile and available in limited quantities. Microrheology studies of actin networks and other biopolymers became usual during the last few decades and they have been helping scientists to reveal the origin of the cells mechanics.

The relevance for the mechanics and shape of cells made the F-actin an interesting object of study to our group, within the Laboratory of Microrheology and Molecular Physiology (LabM²), that has been working in the field of cells mechanics since the group beginning, in 2010, at the Physics Institute of the University of São Paulo. The main techniques used with this purpose are the Optic Magnetic Torsion Cytometry (OMTC), diffusion in cells, and Traction Force Microscopy (TFM).

Indeed, actin is a biopolymer whose mechanical properties has been extensively investigated by several groups around the world, and there are two main reasons for that. The first one comes from the context of the mechanical studies of cells, as our case, where both cytoskeleton and its constituent biopolymers are related with the complex movements and properties of the eukaryotic cell, see Fig. 1.1.1. For being the most abundant cytoskeletal protein [1], the F-actin is considered to be the main responsible for the mechanical properties of the cytoskeleton. There are several evidences of this importance, with examples of diseases, cell motility and shape, wound healing, and the formation of the *filopodia* and *lamellipodia* protusions, as can be seen in references [2–5].

It is important to highlight that the biopolymers of the cytoskeleton cannot be understood by the polymer theory, as most of the synthetic polymers [6]. The main reason for this is related with their semi-flexible nature. F-actin, for example, has a persistence length of $\sim 17 \mu\text{m}$. Here it comes the second main reason to the extensive study of F-actin: it is the typical model to study the properties of semi-flexible polymers.

For these reasons, accurate data relative to actin and also to other polymeric components of the cell are relevant to our group, specially the quantification of factors influencing viscoelasticity measurements. Experiments investigating changes in the viscoelastic

Figure 1.1.1: Confocal fluorescence microscopy of the cytoskeleton of pulmonary cells (Carlos Lenz César/UNICAMP). Microtubules in green, microfilaments of actin in red, and nucleus in blue.

properties of cells in the presence of a drug or a nanoparticle, for example, can provide additional information about the specific action of the component on its biopolymer constituents. However a theoretical and experimental framework to measure, analyse, and interpret data from microrheology of gels and polymer solutions is needed and the development of this framework was one of our main motivations.

Our initial motivation was to understand the relations between the mechanics of F-actin networks and cells. However, with the development of this work, other interesting aspects arose. They are also presented in the next paragraphs. Some new questions came after the contact with the first experiments and the study of the specific scientific literature. Then we rebuilt the goal of this project to better understand the main factors that determine the mechanical properties of the F-actin solutions.

Main factors Polymerize purified F-actin and study its solutions and gels *in vitro* is simpler and controlled when compared with the same component within the cell. But, besides looking homogeneous at bulk, biopolymers gels and solutions can have a complex structure and inhomogeneities at length scales $\sim 1\mu\text{m}$, the typical size of the probes used in microrheology. Additionally, we could learn from our experience and the literature that there are several factors that can influence the Mean Squared Displacement (MSD) of the

probes. In biopolymer solutions these factors are the depletion of polymers close to the probe surface, the interactions between the filaments and probe, even the adsorption of the probes, the relaxation of the polymers, the hopping diffusion, the treadmilling, among less important others. All these factors can cause misinterpretation of data. Then, what are the main factors influencing the viscoelasticity observed in the microrheology measurements?

Figure 1.1.2: Hopping diffusion.

Scaling relations Interesting questions about the relation between the main parameters of the network, as the mesh size and the persistence length, comes from the comparison between the data available from different references. For example, in Fig 1.1.3 the MSD in entangled F-actin can be observed to increase with the mesh size and decrease with the probe diameter. Thus, we can ask: can the MSD be predicted by the relation between probe and mesh size for these high concentrated solutions?

Figure 1.1.3: MSD vs. time of probes embedded in entangled F-actin increases with the mesh size and decreases with the probe diameter. Left: Reference [7]. Right: Reference [8].

Improve analysis We can observe in Fig. 1.1.4 that the usual scaling of the MSD with the probe size does not provide coincident curves. Is there a more appropriate scaling for the MSD? Since the estimation of the complex shear modulus is based on the MSD, such scaling would make the modulus independent of the probe size. A model that correctly describes diffusion allows us to investigate these questions, also to interpret data and test hypothesis about other factors that may influence the results.

Figure 1.1.4: Plot of the MSD scaled by the probe radius vs. time from reference [7]. Curves does not superpose with this scaling.

Our project is in this context and its purpose had both experimental and theoretical scopes. The experimental part consisted in the evaluation of viscoelastic properties of F-actin, understanding the relation of the morphology of the actin networks with the mechanical properties of cell. The theoretical part had the purpose of reconstruct a system consisting of semi-flexible polymers, whose typical model is the F-actin, and study the diffusion of probes between their filaments. The approach is to start from the simplest situation with rigid filaments and then increase the complexity, adding fluctuations to the filaments and interactions between the particles.

With this, we try to find out the basic physics behind the microrheology measurements, then the final goal of this study is to figure out the main factors that influence the motion of the probes. As mentioned in reference [9], the mechanical response arises from different processes that are difficult to be isolated. For this reason viscoelasticity usually has a phenomenological treatment (see Appendix 1.A). This work makes a contribution to a

better understanding of the mechanical properties of F-actin in terms of a microscopic description.

1.2 Aim and outline of this Master thesis

The main objective of this work is to understand the main factors, both morphology and microstructure, that determine the viscoelasticity of F-actin solutions and have the tools to relate them with the mechanical properties of cells. This objective was pursued by experimental and computational studies, as previously mentioned. From now on, this thesis is divided into background sections, experimental part, numerical part and final considerations. The description of their content is given below:

Background The next sections provide the background to this thesis. In Sec. 1.3 there is a description of actin from the molecular to the cellular level. While introducing the main concepts, there will be given an overview of the research in the field. Sec. 1.4 presents some basic concepts of polymer physics and the main physical characteristics of actin filaments and networks. In Sec. 1.5 there are the details about microrheology and the general comparison with other available techniques. In Sec. 1.6 there is an emphasis on the phenomenological relations used to derive the viscoelastic moduli from the measurements of MSD. Some of the concepts of rheology and diffusion useful to the understanding of these methods are also presented in the Appendices 1.A and 1.B.

Experimental Part In Chap. 2 there are the description of the experimental methods used – involving details about the technique, the experimental apparatus used for the acquisition of data, and its analysis – and the results relative to the performed experiment. Sec. 2.1 describes the experimental apparatus used, how the samples were prepared, and the procedure for the data analysis. In Sec. 2.2 there are the results of the microrheology experiments with F-actin solutions. Sec. 2.3 describes an experiment with collagen gels, therefore a solid-like material, to make a comparison with the viscoelastic F-actin solutions and the viscous buffers previously studied.

Numerical Part Chap. 3 is relative to the numerical part of this Master thesis. Sec. has the models and the numerical techniques used in the simulation. A 2d lattice model was developed for first studies, as described in Sec. 3.2. The results related with the 3d models are in Sec. 3.3. The appendix at the end of the chapter explains the procedure to determine the `damp` parameter used in LAMMPS, since it does not seem to correspond to the value expected.

Final considerations The Chap. 4 has the conclusions and the outlook of this work, closing this Master thesis.

1.3 Actin: from the molecular to the cellular level

Formation of the filament The globular actin (G-actin) is a monomer formed by hundreds of aminoacids. G-actin has four subdomains arranged in the shape of letter U. When bounded by Adenosine Triphosphate (ATP), it can polymerize into F-actin, a double helix structure with ~ 10 nm of diameter and contour length that can be between 10 to 30 μm [8]. The ATP can then hydrolyse forming Adenosine Diphosphate (ADP). In Fig. 1.3.1 there is the representation of the G-actin and F-actin. Monomers associate faster at one end of the

Figure 1.3.1: Representation of the G- and F-actin. Left: G-actin monomer (filled with black), with the ATP bounded (filled with white). Center: Highlight of how the G-actin is inserted in the structure of the F-actin. Right: Double helix structure of the F-actin.

filament, called *barbed end*, when compared with the second end, called *pointed end*. The steady state of this process is called *treadmilling*. *In vitro*, the nucleotide hydrolysis is the main process for the transition between monomers and filament, but for the cytoskeleton

there are Actin Binding Proteins (ABP) that regulate the polymerization of F-actin. For this reason the treadmilling is not relevant to the dynamics of the cytoskeleton [1].

Actin binding proteins There are about sixty types of ABP divided in two groups. They both contribute to the formation of the filaments and networks of actin. The first group is of the *capping* protein, which can bind to the end of the chain and can either stabilize or disassemble the filament. Gelsolin is one example of capping protein. The second group is the group of the *cross-link* proteins. They can form more complex structures, depending on the specific type of the protein within the group, such as bundles and branches. The different morphologies of the F-actin networks seen inside the cell arise from the different angles between the filaments of actin the cross-link protein creates. α -actinin is one example of cross-link protein.

Formation of gels and entangled solutions Gels and solutions can be polymerized *in vitro* with the contribution of these two classes of proteins. Gels have their elastic properties very dependent on the cross-link concentration [8]. If the monomers are polymerized without them, depending on the concentration and if the polymer chain is long enough, entangled solutions are formed (see Sec. 1.4). The entanglements form a transient network structure, to which is attributed a weak viscoelastic behavior [10]. The modifications of the network formed by the entanglements are related with the thermal fluctuations of the polymer, that is $k_B T$, where k_B is the Boltzmann constant and T is the temperature.

Actin and the cytoskeleton In the cellular level, actin is found between the three components of the complex biopolymer network responsible for complex behaviors of eukariotic cells, namely the *cytoskeleton*. Fig. 1.3.2, represents the three components of the cytoskeleton: actin microfilaments, intermediate filaments and microtubules. In Fig. 1.3.3 the actin cytoskeleton and the actin polymerized *in vitro* can be visualized. Inside striated muscle cells, the actin network is extremely organized and forms a stable structure [12]. Inside other cell types, there are three morphologies of the actin network. One is the isotropic network beneath the cell membrane, namely *actin cortex*, that is present in both

Figure 1.3.2: The cytoskeleton of a cell consists of a complex network of biopolymers, whose average contour length are in the order of nanometers. Left: microfilaments of actin. Centers: microtubules formed by tubulin. Right: intermediate filaments formed by proteins such as vimentin. Image adapted from Scientific American [11].

adherent and suspended cells. The other two are the stress fibers (e.g. the fibers connected to the focal adhesions) and fiber bundles (e.g. the *filopodia* protrusion). The fibers are oriented in these cases and are present only in adherent cells. These networks present in the cell are very dynamic. They are continuously remodeling, specially when shear stress is applied on the cell.

Figure 1.3.3: A: fluorescence microscopy (LabM²/IFUSP) B: confocal microscopy (Incor/FMUSP). C: confocal microscopy, image of F-actina *in vitro* adapted from reference [8].

1.4 Physical characteristics of actin filaments and networks

Before introducing the main physical characteristics of the actin filaments and networks, we present some concepts of polymer physics that are relevant to them and will be mentioned again in this thesis.

Concentration regimes Polymer solutions have three concentration regimes depending on its relation with the critical mass concentration c^* , given by

$$c^* = \frac{\rho N v}{V},$$

where ρ is the polymer density, N is the degree of polymerization, v is the volume of the monomer, and V is the volume of the solution. The critical mass concentration is defined as the mass of the polymer chain over the volume of solution spanned by it, called the pervaded volume. If the mass concentration c is smaller than c^* , the polymer solution is in the dilute concentration.

If $c = c^*$ the solution is in the overlap concentration. If $c > c^*$, it is in the semi-dilute concentration regime [13]. Fig. 1.4.1 represents these regimes. These are the concentrations regimes that interest us in this study. The concentration of polymer can be increased even more, the solutions going to the concentrated regime, up to the point there is no solvent present, this regime is called polymer melt.

Figure 1.4.1: Concentration regimes of polymer networks. Left: Dilute regime, $c < c^*$. Center: Overlap regime, $c = c^*$. Right: Semi-dilute, $c > c^*$.

Types of polymer networks Ferry, in reference [10], classified the types of networks depending on the type of interaction between the filaments of the polymer. These types are represented in Fig. 1.4.2. Primary bonds are associated to covalent bonds between the different filaments. On the other hand, secondary bonds are due to non-covalent bonds, as hydrogen interactions, between the different parts of the same strand or between different filaments. This type is associated with self-healing gels, i.e. gels able to return to their previous conformation after being broken. Entanglements are topological constraints to the movement of one filament caused by a second one. Polymer gels are usually formed by the primary and secondary bonds, while entanglements are usually related with viscoelastic polymer solutions.

Figure 1.4.2: Types of networks for semi-dilute or more concentrated polymer solutions, depending on the type of bonds between the chains. Left: Primary bonds. Center: Secondary bonds. Right: Entanglements.

1.4.1 Persistence length

Persistence length is the distance in the filament from which the directions are not correlated. Considering $\vec{r}(s)$ as the position over the polymer chain and s as the length coordinate over the contour length of the filament, the tangent vector is defined by $\vec{u}(s) = \frac{\partial \vec{r}}{\partial s}$. The tangent vectors $\vec{u}(s)$ along the filament lose their correlation if their distance is larger than the persistence length:

$$\langle \vec{u}(s) \cdot \vec{u}(s') \rangle = e^{\frac{-(s'-s)}{l_p}} \quad (1.4.1)$$

where $\langle \dots \rangle$ represents the temporal average due the thermal fluctuations. The Young modulus ν is about 10^9 Pa, and it is possible to relate it with the persistence length by

the following equation:

$$l_p = \frac{\kappa_0}{k_B T}$$

where κ_0 is the bending stiffness of the filament. The F-actin has high bending stiffness when compared with syntetic polymers, specially when considering that the monomers have also comparatively weak bonds.

Figure 1.4.3: Filament parameters.

Measurement Experimentally, there are several ways of measuring the persistence length of biopolymers described in the literature. Here we summarize one of them, reported in the reference [14] for F-actin. The filament labeled with phalloidin was confined between plates coated with Bovine Serum Albumin (BSA) to restrict the fluctuations to two dimensions. For 20 minutes, the conformations of the filament was recorded. Each frame had the filament skeletonized and then reparametrized to be described as a succession of vectors. Using Eq. 1.4.1 for several vector lengths, the authors calculated the persistence length of $(16.7 \pm 0.2) \mu\text{m}$.

1.4.2 Mesh size

For the description of polymeric solutions, the most important parameter is the mesh size ξ , that represents the typical spacing between the closest monomers of different filaments.

For the entangled solutions of F-actin the mesh size in micrometers is given by the relation

$$\xi = \frac{0.3}{\sqrt{c_A}}, \quad (1.4.2)$$

where c_A is the concentration of protein in mg/ml of the G-actin solution used to prepare the F-actin *in vitro* [8,15]. The average mesh size in solution varies between 0.1 to 2 μm .

Measurement Schmidt *et al* measured the mesh size of F-actin solutions using scaling laws [15]. The diffusion coefficient of spheres with diameter in the order of 1 to 100 nanometer was measured by Fluorescence Photobleaching (FRAP). The results showed that $\ln[\ln(D_0/D)]$ is proportional to $c_A^{(1.02 \pm 0.1)}$ and to $b^{(2.13 \pm 0.3)}$. Then the data was combined with the relation derived by de Gennes *et al* assuming that the diffusion is a process activated by the elastic free energy associated with the expansion of a mesh formed by Gaussian chains,

$$D = D_0 \exp \left[-\beta \left(\frac{b}{\xi} \right)^\nu \right], \quad (1.4.3)$$

where D_0 is the diffusion coefficient in the solvent, β is a dimensionless coefficient expected to be 1 and ν is expected to be 2.5 for cross-linked networks and 2 for entangled networks. The combination resulted in the Eq. 1.4.2 above.

Other physical characteristics If the contour length is much bigger than the mesh size, $l_c \gg \xi$, as is the case for the actin solution, the filaments will entangle. They have a elastic rigidity G between 0.05 and 3 Pa, for the frequency interval 0.005 to 30 rad/s. That is a large rigidity if compared with flexible polymers, indicating the semi-flexible nature is relevant to the mechanical properties of the network [8]. In the table below there is a summary of the main characteristics of the monomer and filament of actin.

1.5 Rheology and microrheology

Rheology is the study of how matter flows and deforms. Its objects of study are viscous fluids, elastic solids, and all the range of materials between them, called for this reason

	Symbol	Typical value	Description	References
G-actin	b_G	55 Å	Diameter	[1]
	m_G	42 Da	Mass	
F-actin	l_c	10–30 μm	Contour lenght	[8, 15]
	l_p	$\sim 17 \mu\text{m}$	Persistence length	[8, 14]
	b_F	$\sim 10 \text{ nm}$	Diameter	[8, 14]
	ν	10^9 Pa	Young modulus	[8]
F-actin solutions	ξ	0.1–2 μm	Mesh size	[8, 15]
	G	0.05–3 Pa	Elastic rigidity	[8]

Table 1.4.1: Main physical characteristics of the G- and F-actin

viscoelastic. Experimentally, the mechanical characterization of these materials is usually done with a rheometer. The required samples have their volume in the order of milliliters, what is considered a big amount of material if you are dealing with cells and purified biopolymers. Microrheology is very interesting for this special situation. As mentioned previously, in this technique colloidal spheres are embedded in the material to access its viscoelastic properties, as can be seen Fig. 1.5.1.

Figure 1.5.1: For the microrheology technique probes with size approximately equal or slightly bigger than the mesh size ξ of the polymer network.

In addition of requiring microliters of sample the microrheology techniques also provide smaller strains, that reduce the risk of damaging the material during the measurement. A

third advantage is that these techniques can evaluate the local or bulk viscoelastic properties of the medium in a frequency range not accessible by rheometers. It is convenient to probe local mechanics since biological materials can have inhomogeneities in the length scale in the order of the cells size. The frequency reveals structural changes in the material [16]. For a given frequency, if the material deforms quickly, it is solid-like. If it deforms slowly, it is a liquid-like material.

The colloidal spheres embedded in the material can have their movement driven by the thermal energy or an external force. This is the criterion to classify between passive and active microrheology. The spherical particles, that are the probes, are expected to get stuck between the filaments of the entangled polymer network since the microspheres have bigger diameter than the mesh size, that is different for each material and concentration. If the probe is smaller than the mesh size, the movement of the probe is related with the viscosity of the solvent and can provide some information about the microstructure of the network.

1.6 Passive microrheology

This work is focused in the passive microrheology. For this case, as the motion is driven by the thermal energy, the MSD of a set of probes is used to the estimation of the material viscoelasticity using the phenomenological generalized Stokes Einstein relation (GSE), that will be presented in Sec. 1.6.1. The MSD can be evaluated by three different methods. The Multiple Particle Tracking (MPT), the one used in this work, uses a microscope and a particle tracking software to observe and quantify the displacement of the probes. It also allows the observation of the inhomogeneities of the materials, so the local viscoelasticity can be measured. The other two are the Dynamic Light Scattering (DLS) and Diffusing Wave Spectroscopy (DWS). DLS has spatial resolution similar to video microscopy, better spatial and time resolution. On the other hand, it requires low viscosity medium. DWS has better spatial and time resolution, and allows measurements within high viscosity medium. But it requires larger samples. This method is based on the measurement of the

autocorrelation function of the light scattered by multiple microspheres embedded in the material [17].

Figure 1.6.1: Representation of the expected result for the passive microrheology technique, considering a viscous (red) and a viscoelastic (blue) fluid. Left: The MSD of probes embedded in the fluids. Right: The respective storage and loss moduli for each case. Image adapted from reference [18].

Fig. 1.6.1 represents the analysis to obtain the real and the imaginary components of the complex shear modulus $G^*(\omega)$, that are respectively the storage $G'(\omega)$ and the loss moduli $G''(\omega)$:

$$G^*(\omega) = G'(\omega) + iG''(\omega). \quad (1.6.1)$$

The concept behind $G^*(\omega)$ comes from mechanical measurements, usually using rheometers. In the next sections, we will focus on the procedure to get them in passive microrheology context. In reference [19], it is made the analogy of $G^*(\omega)$ as a elastic susceptibility, determining the stress induced upon the application of an oscillatory shear strain. The stress has an in phase and an out of phase component that can be related with the storage and the loss moduli. Therefore their meaning are related respectively with the elastic modulus of elastic solids and the viscosity of viscous fluids (see Appendix 1.A).

As represented in Fig. 1.6.1 by the curves in red, if the probe is in a pure viscous material, its MSD is linear with time and proportional to $6D$. The complex shear modulus will have only the loss modulus component. If the probe is in a viscoelastic material, the MSD can be subdiffusive or confined. The complex shear modulus will have both the

storage and the loss components.

1.6.1 Generalized Stokes-Einstein relation

The generalized Stokes-Einstein relation (GSE) was proposed to cover the range of materials between pure viscous fluids and the pure elastic solids. It is derived from the generalized Langevin equation:

$$m\dot{v}(t) = f(t) - \int_0^t \zeta(t-t')v(t')dt', \quad (1.6.2)$$

where m and $v(t)$ are the mass and the instantaneous velocity of the probe respectively, and $f(t)$ is the random force causing its brownian motion. The function $\zeta(t)$ is the time dependent memory function, then the integral corresponds to the viscous damping of the fluid. Once there is the storage of energy by the material, the temporal correlation of the random forces is given by

$$\langle f(0)f(t) \rangle = k_B T \zeta(t). \quad (1.6.3)$$

The velocity correlation and the time dependent memory function may be related to each other taking the unilateral Laplace transform of Eq. 1.6.2 and by the equipartition energy theorem, resulting in

$$\langle v(s)v(0) \rangle = \frac{k_B T}{ms - \zeta(s)}, \quad (1.6.4)$$

where s is the frequency of the Laplace domain. For the frequency range of the microrheology based on the multiple particle tracking, the inertial term can be neglected.

The Eq. 1.6.4 can be related with the MSD. In references [19,20], it is assumed that the memory function is related with the frequency dependent viscosity by

$$\tilde{\eta}(s) = \frac{\tilde{\zeta}(s)}{6\pi a},$$

that is exact for a pure viscous fluid, where a is the radius of the probe. Since the time

dependent memory function is given by

$$\tilde{\zeta}(s) = \frac{6k_B T}{s^2 \langle \Delta \tilde{r}^2(s) \rangle},$$

the usual Stokes Einstein relation is extended to its frequency dependent form

$$\tilde{G}(s) = s\tilde{\eta}(s) = \frac{k_B T}{\pi a s \langle \Delta \tilde{r}^2(s) \rangle}, \quad (1.6.5)$$

where $\langle \Delta \tilde{r}^2(s) \rangle$ represents the Laplace transform of the MSD. The details of the derivation GSE are presented in the references [19,20]. The assumptions that allows the use of this relation, given by Eq. 1.6.5, are that the complex fluid can be considered as a continuum and the structures causing the elasticity are small compared with the spherical probe [21].

1.6.2 Viscoelastic moduli from the GSE relation

This section is based on the paper of Mason [21] in which he derives the complex shear modulus $G^*(\omega)$ of viscoelastic fluids avoiding the Fast Fourier Transform of the MSD, which can lead to unphysical values at extreme frequencies. For this, Mason uses the following power law expansion of the MSD:

$$\langle r(\Delta t)^2 \rangle \approx \langle \Delta r(1/\omega)^2 \rangle (\omega t)^{\alpha(\omega)}, \quad (1.6.6)$$

and the assumption that the bulk viscoelastic and the local viscoelastic moduli close to the sphere are equivalents. Then he relates the complex shear modulus with the MSD by the equation

$$|G^*(\omega)| \approx \frac{k_B T}{\pi a \langle \Delta r(1/\omega)^2 \rangle \Gamma[1 + \alpha(\omega)]},$$

where $\Gamma[1 + \alpha(\omega)]$ is the gamma function approximated by $0.457[1 + \alpha(\omega)]^2 + 1.36[1 + \alpha(\omega)] + 1.90$ and $\alpha(\omega)$ is given by

$$\alpha(\omega) = \frac{d \ln \langle \Delta r(1/\omega)^2 \rangle}{d \ln t},$$

where a is the microsphere diameter. Using Eq. 1.6.1, we have that the storage and the loss moduli are respectively

$$\begin{cases} G'(\omega) = |G^*| \cos(\pi\alpha(\omega)/2), \\ G''(\omega) = |G^*| \sin(\pi\alpha(\omega)/2). \end{cases}$$

Using the relation given by Eq. 1.6.2 for the passive microrheology using the MPT, the spatial resolution is in the order of nanometers. For this reason the values that can be measured with this combination is in the order 100 Pa [20]. This can be verified by the expression 1.6.2 itself:

$$|G^*(\omega)| < \frac{10^{-23} \cdot 10^2}{1 \cdot 10^{-6} \cdot (10^{-9})^2} \approx 10^3 \text{ Pa}.$$

1.A Appendix: Concepts of rheology

Stress and strain Stress τ is defined as the ratio of a force and the area this force is acting across. Strain γ is the length variation related with the deformation caused by the stress. So stress and strain are given by

$$\tau = \frac{F}{A} \quad \text{and} \quad \gamma = \frac{\delta x}{x_0}.$$

The stress is usually expressed in Pa. Stress and strain are tensor quantities, despite being represented above as scalars for simplicity.

Elastic modulus and viscosity A Hookean solid has a linear relation between stress and strain. If small shear deformations are being considered, then

$$\tau = G\gamma,$$

where the proportionality constant G is called elastic modulus. The deformation holds until the stress is applied. For a fluid the analogous situation consists of a liquid between two surfaces separated by a small gap. One of the surfaces is fixed while the second one moves with constant velocity. This velocity can be related with the rate of strain $\dot{\gamma} = d\gamma/dt$. A Newtonian fluid has a linear relation between the stress and the rate of strain, being the viscosity η the constant of proportionality:

$$\tau = \eta \dot{\gamma}.$$

Linear viscoelasticity models The phenomenological treatment of the viscoelastic behavior is in terms of the combination of Hookean springs and Newtonian dashpots in complex combinations [9]. The linear viscoelasticity using these type of models implies that the springs and dashpots are in their linear regime as well. If the spring and dashpot are combined in parallel, they correspond to the Kelvin-Voigt model. On the other hand, if they are combined linearly, they correspond to the Maxwell model. These are the simplest configurations and are represented in Fig. 1.A.1.

Figure 1.A.1: Linear viscoelasticity models. Left: The Maxwell element. Right: The Kelvin-Voigt element.

In the first case, the Kelvin-Voigt model, the parallel connectors remain horizontal, resulting in equal strain being applied in the spring and the dashpot [22]:

$$\tau = G\gamma + \eta\dot{\gamma}.$$

This model has a very simple relation for the storage and loss moduli. The former being $G'(\omega) = G$ and the latter, $G''(\omega) = \eta\omega$.

For the the Maxwell model, the total rate of strain is the additive property, then:

$$\dot{\gamma} = \frac{\dot{\tau}}{G} + \frac{\tau}{\eta}.$$

The complex shear modulus for this case is given by

$$G^*(\omega) = \frac{iG\omega\iota}{1 + i\omega\iota},$$

where ι is the timescale of the material, $\iota = \eta/G$. Then, the storage and loss moduli are:

$$G'(\omega) = \frac{G\omega^2\iota^2}{1 + \omega^2\iota^2} \quad \text{and} \quad G''(\omega) = \frac{G\omega\iota}{1 + \omega^2\iota^2}.$$

Viscoelasticity of entangled solutions Ferry proposed the use of a modified Maxwell element to model fluid solutions of entangled polymers when the applied stresses are small [10]. It consists of one spring in parallel with one dashpot and this set in series with another dashpot, i.e. one spring and two dashpots in total. This configuration is called retarded Maxwell element. For this case, we have the complex shear modulus given by

$$|G^*(\omega)| = \frac{G\omega\iota_s\sqrt{1 + \omega^2\iota_p^2}}{\sqrt{1 + \omega^2(\iota_s + \iota_p)^2}},$$

where ι_s and ι_p is the timescale of the material, respectively $\iota_s = \eta_s/G$ and $\iota_p = \eta_p/G$. The η_s and η_p standing to the viscosity of the series and parallel dashpots [10]. Then, the storage and loss moduli are:

$$G'(\omega) = \frac{G\omega^2\iota_s^2}{1 + \omega^2(\iota_s + \iota_p)^2} \quad \text{and} \quad G''(\omega) = \frac{G\eta_s[1 + \omega^2\iota_p(\iota_s + \iota_p)]}{1 + \omega^2(\iota_s + \iota_p)^2}.$$

For the retarded Maxwell element, the spring is interpreted as the filament between to entanglements of the network. The parallel dashpot can be seen as the friction causing the resistance to the rearrangement of the chain. The series dashpot represents the relaxation of stresses caused by disentanglements or reconfiguration of the chains.

Figure 1.A.2: Retarded Maxwell element.

1.B Appendix: Brownian dynamics and diffusion

Brownian dynamics Let us consider a small sphere with radius between 0.1 and 1 μm , i.e. a colloidal particle, inside a simple viscous fluid. This sphere diffuses due to the constant and random hitting of the fluid molecules. The equation for the Langevin dynamics is

$$m \frac{d^2 \vec{r}}{dt^2} = -\nabla U(\vec{r}) - \alpha \frac{d\vec{r}}{dt} + F_r(t),$$

where $F(t)$ is a random force with $\langle F_r(t) \rangle = 0$ and $\langle F_r(t) F_r(t') \rangle = A \delta(t - t')$, and α is the friction coefficient. For this case, the Einstein's relation is

$$D = \frac{k_B T}{\alpha}. \quad (1.B.1)$$

Diffusion Diffusion is a collective transport phenomenon that can be treated with statistical mechanics using random walks. The MSD of the diffusing particles,

$$\langle r(\delta t)^2 \rangle = \langle (r(t + \delta t) - r(t))^2 \rangle,$$

where δt is the time interval between two measurements of the position of each particle. The MSD can be related with the diffusion coefficient D and the time t by:

$$\langle r(\delta t)^2 \rangle = 2dD\delta t,$$

where d is the number of dimensions considered in the computation of the MSD, since each coordinate contributes with $2D\delta t$. For the case of a two dimensional system, as $r^2 = x^2 + y^2$, $\langle r^2 \rangle = 4D\delta t$. Then, for this case, the diffusion coefficient can be calculated

dividing the angular coefficient of the MSD by $2d$.

If the process is not linear with time, it is called *anomalous diffusion*, that is related with disordered systems as cells and porous media. In this case,

$$\langle r(\delta t)^2 \rangle \propto \delta t^\alpha$$

for $\alpha > 1$ the anomalous process is called *super-diffusive*, $\alpha < 1$ is *sub-diffusive*, and $\alpha = 1$ is the aforementioned normal diffusion. Considering these cases of anomalous diffusion, reference [23] defines the diffusion coefficient as

$$D = \frac{1}{2d} \lim_{\delta t \rightarrow +\infty} \frac{\langle r(\delta t)^2 \rangle}{\delta t}. \quad (1.B.2)$$

Chapter 2

Experimental Part

In this chapter we describe the materials, the methods, and the results relative to the experimental part of this thesis. For our measurements with F-actin solutions we used the passive microrheology technique with Multiple Particle Tracking (MPT) to obtain the MSD of the colloidal microspheres embedded in the material, used as probes. In the laboratory there were two experimental setups available for the measurements of the probes displacements. Then using the procedure described in Sec. 1.6.1 and 1.6.2, the complex shear modulus could be calculated. Similar measurements were also performed with gels of collagen type I, that are presented at the end of this chapter.

2.1 Materials and methods

2.1.1 Experimental setup

The current experimental apparatus is used for the MPT methodology, i.e., to register the dynamics of embedded probes during a total time of about 100 seconds. We used two setups consisting of a charged-coupled device (CCD) camera connected to an inverted microscope. The Setup 1 used the Leica DMI 4000 B inverted microscope without fluorescence while Setup 2 used a second Leica DMI 4000 B equipped with fluorescence. Both microscopes were placed over anti-vibration workstations. For Setup 1 images were not recorded, they were directly transmitted to a computer using the National Instruments Firewire Port 800 board (Fig. 2.1.1). The tracking software was developed in C++ by the

Figure 2.1.1: Experimental Setup 1.

LabM² originally for the OMTC technique. The software is used to get the center of mass of each probe and then it creates a file where the xy position of each probe is recorded. More than 200 microspheres can be identified simultaneously. For the Setup 2 the images of 500 by 500 pixels are recorded and after the measurements they are analyzed in the particle tracking software. The image acquisition rate were 10 and 20 frames per second

(FPS), this determines the time resolution of the experiment. The microscope objective magnification were of 10, 20, and 40 $\times$. The setups used are detailed in the table below:

	Setup 1	Setup 2
Inverted microscope	Leica DMI 4000 B	Leica DMI 4000 B
Equipped with fluorescence	No	Yes
CCD camera	AVT Pike	CMOS Neo Andor
Refrigeration system in the camera	No	Yes
Workstation	TMC anti-vibration	Newport optical table
Image acquisition rate	20 FPS	10 and 20 FPS
Available objective magnification	10 and 20 $\times$	10, 20, 40 and 63 $\times$
Micro-incubator with temp. control	No	Yes
Magnetizer for OMTC	Yes	No
Software	Software OMTC	Micromanager

Table 2.1.1: Description of the different setups used for the measurements.

2.1.2 Preparation of F-actin and other solutions

F-actin solution The entangled solution of F-actin was prepared with the polymerization of lyophilized G-actin purified ($>95\%$) from rabbit skeletal muscle (#akl95, Cytoskeleton, Denver, USA). The protein have to be reconstituted with distilled water to the concentration of 10 mg/mL. Then aliquots of 1 $\mu\ell$ were put into Eppendorf tubes, snap frozen in liquid nitrogen, and stored in an ultrafreezer at -80°C . In Fig. 2.1.2 there is the fluxogram of this procedure. One aliquot is diluted with G-buffer (preparation described in the paragraph below) to the concentration of 0.4 mg/mL and incubated on ice for 1 hour. An optional step is to clarify the solution centrifuging it for 15 minutes at 16.000 g and 4°C . Then transfer the supernatant to a new Eppendorf tube. Add F-buffer in the volume correspondent to 10% of the total volume of the G-actin solution and incubate the final solution at room temperature for 1 hour.

G-Buffer 1x The lyophilized general actin buffer (#bsa01, Cytoskeleton, Denver, USA) was resuspended with 10 ml of MiliQ water, resulting in the 1× strength buffer with the composition: 0.2 mM CaCl_2 , 5 mM Tris-HCl pH 8.0. This final solution was stored at 4°C. Before being used, the buffer was supplemented with 0.2 mM ATP (#bsa04-001, Cytoskeleton, Denver, USA) and 0.2 mM DTT (43816, Sigma-Aldrich). The divalent cation, ATP, and DTT present in the buffer are used to stabilize the G-actin. The Tris HCl keeps the buffer in the pH of 7.5. G-actin is stable for pH between 6.5 and 8.5.

F-Buffer 10x The lyophilized actin polymerization buffer (#bsa02, Cytoskeleton, Denver, USA) was resuspended with 2 ml of 100 mM Tris-HCl pH 7.5 (93362, Sigma-Aldrich), resulting in the 10× strength buffer with the composition: 100 mM Tris-HCl, 20 mM MgCl_2 , 500 mM KCl, 10 mM ATP, 50 mM guanidine carbonate pH 7.5. Guanidine carbonate is a stabilizer for the ATP. Then aliquots of 20 μl were put into PCR tubes, snap frozen in liquid nitrogen, and stored in an ultrafreezer at -80°C.

ATP solution The lyophilized ATP (#bsa04-001, Cytoskeleton, Denver, USA) was resuspended with 1 ml of cold 100 mM Tris-HCl pH 7.5 (93362, Sigma-Aldrich). Then aliquots of 10 μl were put into PCR tubes, snap frozen in liquid nitrogen, and stored in a freezer at -20°C. ATP is required for the polymerization process.

Figure 2.1.2: Simplified fluxogram with the procedure to resuspend lyophilized actin.

BSA 10% solution This solution corresponds to a concentration of 100 mg/mL. Then 1 g of powdered BSA is dissolved by layering in 10 mL of distilled water inside a capped tube. This procedure avoids the clumping of the Bovine Serum Albumin (BSA). Gently rock the tube until dissolve completely. Make aliquots and store in a freezer at -20°C .

2.1.3 Sample preparation

For the sample preparation we used latex probes with diameter 2.4 and 0.6 μm . The latex microspheres of 2.4 μm were produced with nonionic polystyrene. Its surface is not charged, for this reason the microspheres do not aggregate in the presence of polyvalent cations as the calcium ions present in the buffer solutions. For the latex microspheres of 0.6 μm the polystyrene has chloromethyl groups bonded to the styrene monomers. These groups have an active surface. In this case, the formation of clusters was observed. The solution was to coat the probes with BSA, that is a stabilizer. Also the sample container was coated with BSA. For this, a BSA 10% solution was prepared (see Sec. 2.1.2). The walls of the container were covered with this solution for 6h, then the solution was discarded and the container dried. The probes were kept in the BSA solution for the same time before being used. Both sizes of the probes used are larger than the typical mesh size of F-actin solutions, usually between 0.1 and 0.2 μm . The specification of the probes used are displayed below:

Diameter [μm]	Material	Concentration	Fornecedor	Catalogue
2.4	Nonionic latex	4% w/v	Molecular Probes	N37453
0.6	Chloromethyl latex	4% w/v	Molecular Probes	C37347

Table 2.1.2: Specification of the probes used for the measurements.

The entangled solutions of F-actin were prepared following the simplified procedure presented in Fig. 2.1.3, with the complete description in the Sec. 2.1.2. The probes were diluted in the F-buffer before they were added into the sample, in the ratio of 1:1000 of the final sample volume. Adding the probes before polymerization provide that the probes will be embedded in the sample, between the filaments of actin. To analyze the residual

Figure 2.1.3: Simplified fluxogram with the procedure to polymerize actin.

solution with the spectrophotometer and determine the protein concentration is an optional step. Usually the concentration was adopted as the initial dilution of material, since we worked with lyophilized and standardized materials.

Approximately 50 to 100 $\mu\ell$ of the solutions were polymerized in 20 mm glass bottom dish, covered with a glass coverslip, and sealed with clear nail polish for measurements in Setup 2 and in wells of 96 well plates for Setup 1. To avoid walls effects the measurements considered the center of the samples. Experiments did not take a long time because the exposure to the air dry the samples, causing the denaturation of the proteins. This changes the mechanical properties of the networks [8]. Measurements with F-actin were performed at the same day of the polymerization of the samples.

2.1.4 Analysis of data

For the data analysis a routine in the programming language C was developed to compute the MSD and the viscoelastic properties of the material being studied, as described previously in Sec. 1.6.1 and 1.6.2. The input file has the xy position of each probe tracked. Observing the displacement of each probe during one measurement we could observe eventually a preferential direction, a trend in the data. This is due a drift of the stage of the microscope. Then the average movement of the probes is subtracted from the displacement of each probe. The displacement is expected to look like a random walk. After this procedure, the MSD was calculated using Eq. 1.B. By this calculation, the MSD for small δt is obtained by the average of more data. For this reason the MSD was more accurate for smaller δt and final points were not considered in the calculations. The typical displacement in the plan xy of one probe before and after subtracting the average over the movement of all probes in one measurement, that is the way drift is calculated, are represented in Fig. 2.1.4.

Figure 2.1.4: Displacement of one probe. Left: example of the displacement observed. Inserted graph shows the drift calculated by the average movement of the total set of probes. Right: the same displacement with the subtraction of the drift.

In Fig. 2.1.5 an usual situation is represented, this situation is when we observe two types of probe movements. These two types of movements were probably due the formation of to regions where the probes were stuck between the filaments and regions without filaments where the probes could freely diffuse. For this reason the routine can separate these different types of movement before performing the calculations. This procedure is also useful to disregard the movement of probes whose z position goes out of the focal plane of the microscope.

Figure 2.1.5: Comparison between two situations found during measurements due to regions where the probes were stuck between the filaments and regions without filaments where the probes could freely diffuse. Up left and right: xy trajectories and MSD respectively of the situation where the probes movement are similar for the entire sample. Bottom left and right: xy trajectories and MSD respectively of the situation where two types of movement of the probe can be identified.

2.2 Viscoelasticity of F-actin solutions

2.2.1 Control

As a control to the measurements with entangled solutions of F-actin we performed the procedure to acquire the data of diffusion in the general actin and polymerization buffers. With this we could identify between our measurements which were relative to solvent only. This may be due, for example, to situations when the polymerization did not happen or the measurement is taken in a region of low concentration of filaments, resulting in a network unable to retain the probes. The measurements of the MSD and the respective moduli can be seen in Fig. 2.2.1. The measurements were performed at room temperature, objective of $10\times$, and frame rate of 10FPS. This measurement had to consider smaller time intervals because the freely diffusing probes does not remain for a long time in the field of view. The measurements were performed using the Setup 1 and the $2.4\mu\text{m}$ nonionic latex probes.

Figure 2.2.1: Data analysis from the diffusion of $2.4\mu\text{m}$ latex probes in G-buffer at 21.5°C , 13 probes, objective of $10\times$, and frame rate of 10FPS. Left: MSD vs. δt and fitting considering the interval $[0, 10]$. Right: Storage and loss moduli vs. ω . For $G'(\omega)$ the errorbars were small for the scale and were not represented.

As can be seen in the figure above, there is a deviation from the expected linear behavior for δt close to 10s. It was considered a systematic error since it is present in the other measurements. The linear fitting $Ax + B$ of the data, also presented in Fig. 2.2.1, got the following values for the coefficients: $A = (0.611 \pm 0.007)\mu\text{m/s}$ and

$B = (-0.009 \pm 0.002) \mu\text{m}$. With this and using the Stokes Einstein relation we got the viscosity of $(1.175 \pm 0.014) \text{pg}(\mu\text{m}\mu\text{s})^{-1}$ for the mixture of the G- and F-buffers. The loss modulus dominates over the frequency range measured as expected.

2.2.2 Results and discussion

The F-actin solution was prepared following the protocol presented in Sec. 2.1.2, then with final concentration of 0.36 mg/ml and mesh size of $0.5 \mu\text{m}$, calculated by Eq. 1.4.2. The measurement with the $2.4 \mu\text{m}$ nonionic latex probes considered the average over 60 probes at room temperature, about 21°C . The drift was calculated and represented in Fig. 2.2.2 (up right). The drift was subtracted from the displacement of each probe before the computation of the MSD and the $G'(\omega)$ and $G''(\omega)$ moduli, as described previously in Sec. 2.1.4. The value for the storage modulus forms a plateau between the frequencies of 0.7 a 10 rad/s which is consistent with the expected value, about 1.5 Pa , as mentioned before, in Sec. 1.3.

Studying the limits of the technique we could observe that for the objective of $10\times$ we were working close to the highest measurable values, that is about 2 Pa , that is very close to the elastic plateau measured. For this reason, a second measurement at room temperature used objective of $40\times$ and the same frame rate of 10 FPS . The probes were changed to the $0.6 \mu\text{m}$ chloromethyl latex. For this material, BSA was used to coat the surface of probes and sample holder. With this procedure both the aggregation of the microspheres and the adsorption of the probe by the actin filaments were prevented. The value for $G'(\omega)$ was lower for this case and we did not observe the formation of the plateau. The value of $G'(\omega)$ for this case was smaller than the previous one. Also a smaller number of probes could be measured for this case. As we used higher magnifications and the Setup 2 for the data acquisition, the pictures had, in average, 2 to 3 probes, demanding much more measurements to have comparable statistics to the previous one.

In Fig. 2.2.3 (left) we observe an exponent for the time dependence close to 0.5 for the MSD, configuring then a subdiffusive process (see Appendix 1.B). The storage modulus dominates over the loss modulus for frequencies over 0.3 rad/s and there is no indication of

Figure 2.2.2: Data analysis from the diffusion of $2.4\mu\text{m}$ latex probes in the F-actin solution at room temperature, objective of $10\times$, and frame rate of 10 FPS. Up left: Picture of the probes embedded in the material. Up right: Drift subtracted from each particle's movement. Bottom left: MSD vs. δt . Bottom right: Storage and loss moduli vs. ω .

the formation of a plateau of the storage modulus that increases slowly with the frequency. For the previous case the crossover of the storage modulus occurs about 0.2 rad/s with the immediate formation of a plateau.

2.2.3 Conclusions

The measurement with the G-buffer and F-buffer reported a higher viscosity than the value expected, that would be close to the viscosity of water. A possible explanation is that the probe adsorbed monomer of actin, changing the value of its radius. Another possibility is the effect of a residual drift, that would also explain the systematic error observed in

Figure 2.2.3: Data analysis of the diffusion of the $0.6 \mu\text{m}$ chloromethyl latex probes coated with BSA in F-actin entangled solution and using objective of $40\times$ at 27.9°C , and 10 probes. Left: plot of the MSD vs. δt at room temperature. Right: plot of the storage and loss moduli vs. ω .

data. From the experience with the apparatus, we have that the best setup for this type of experiment is Setup 2, since the magnification of the objective is fundamental for good data acquisition. For the measurement with the Setup 1 we considered a case in which $b/\xi > 1$, then we could measure the elastic modulus plateau of the material. This plateau is in agreement with the literature. The measurement performed with the Setup 2 had $b/\xi \approx 1$, for this reason we could observe a subdiffusive behavior for the MSD. For this case, the motion of the probe reports the network dynamics and the mechanics at the length scale of probe diameter [24]. The plot of the storage and loss moduli shows the transition of a liquid-like to a solid-like behavior.

2.3 Elasticity of gels of collagen type I

Collagen is the most abundant protein in the extracellular matrix (ECM). For its porosity, transport, and mechanical properties, collagen hydrogels has been used to mimic the ECM and to study the relation between the cell mechanics and its environment. Microrheological measurements are suitable to assess the inhomogeneities of the material, see Fig. 2.3.1, that are normally of the same length scale of cells and not captured by bulk rheology. For this measurement we prepared two different concentrations of collagen type I gel and

performed the measurement of their storage and loss moduli using passive microrheology with multiple particle tracking.

Figure 2.3.1: samples prepared in similar conditions presented variations in the homogeneity. Phase contrast images of the collagen samples with concentration of 1.5 mg/mL.

2.3.1 Sample preparation

Collagen type I of initial concentration 5 mg/mL was reconstituted on ice with Dulbecco's Modified Eagle's Medium (DMEM) and the Reconstitution Buffer (RB). The volume of DMEM and RB corresponded to 10% of the final solution each. The amount of collagen is determined by the final concentration and the final volume desired, which is reached completing with cooled Phosphate Buffered Saline (PBS) mixed with the probes in the ratio 1:1000, using probes with concentration ranging between 0.1 and 0.4% w/v. In an eppendorf tube the solution is homogenized for 1 min with the vortex. And for 10 min at 4°C. pH 7 is set with HCl or NaOH solutions. Over a glass plate the sample rest 1h at 37°C to polymerize. Temperature was kept $\sim 37^{\circ}\text{C}$ during the measurements.

2.3.2 Results and discussion

For the measurements we used Setup 2 (that is described in Sec. 2.1.1) with objective magnification of $40\times$ and image acquisition rate of 20 FPS. Measurements were performed using the Setup 2, since temperature control was very important for this case to keep the stability of the gel, otherwise there is the depolymerization of the network and the observation of hopping diffusion of the probes. The probe used was the 2.4 μm non-ionic

latex. Fig. 2.3.2 has the moduli for the collagen gels. The values of the elastic plateau for the concentrations of 2 and 3 mg/ml were approximately (134 ± 5) and (149 ± 5) Pa. The values are in the approximate order as can be seen in the literature [25–27], obtained by different methods.

Figure 2.3.2: loss and storage modulus of collagen type I gels of 2 and 3 mg/ml. Measurement at 37°C, frame rate of 20 FPS, average over 100 and 50 probes respectively.

2.3.3 Conclusions

In this study, we measured the elastic modulus of collagen type I gels for two concentrations and at the temperature of $\sim 37^\circ\text{C}$. In this situation we did not observe the cage-hopping of the probe despite the inhomogeneity of the material. With these results we concluded that these gels are, as expected, solid-like for the frequency range measured. We could get a good estimate of the viscoelasticity of the material despite working in the limit of the technique used. The method provides information of the bulk elasticity of these gels since we took the average of different probes and samples. The method can be adapted to quantify its heterogeneity separating the region of the sample observed in the microscope into subregions depending on the velocity of the probe, as described in reference [28].

Chapter 3

Numerical Part

This chapter has the numerical part of this thesis. Here we describe the techniques and the three models used to study the diffusion of spheres inside actin networks. The lattice model was developed as first studies. This type of model is specially useful for qualitative understanding of problems and concepts. The results of these simulations indicated that the relation of the radius and the mesh size can provide information about the structure of the network and, consequently, they were incorporated in this text. For the three-dimensional models we considered two approaches: statical and fluctuating filaments with the persistence length of F-actin. Some of the questions addressed in this study were: (1) What structural information can, in principle, be obtained from the microrheological measurements? (2) What is the appropriate definition of mesh size? (3) What is the appropriate scaling of the MSD? (4) What changes arise from the addition of fluctuations to the filaments? (5) Can we recover the viscoelasticity of the F-actin solutions and networks experimentally observed?

3.1 Numerical methods

3.1.1 Lattice simulations

There were performed simulations of a probe hopping between the pores of a polymer network by a simplified model in which the probe performs a random walk in a two-dimensional squared lattice. It can move to the next position only if the probe diameter b is smaller or equal to the position size ξ . This size is picked from a normal distribution with average $\bar{\xi}$ corresponding to the designated mesh size of the network and dispersion $\sigma = 0.1$ and $0.3 \mu\text{m}$, as represented in Fig. 3.1.1. The next position is chosen picking an integer between 0 and 3. We consider that the probe starts always from the same position. The MSD as function of time is calculated by the average over N_r runs. The simulations considered $N_r = 1000$ for the total time of 100 s, with 1000 steps each second and lattice size with 2000×2000 sites.

Figure 3.1.1: Distribution of sizes for each position in the lattice, mean value $\bar{\xi} = 1 \mu\text{m}$, dispersion $\sigma = 0.1$ and $0.3 \mu\text{m}$

Two cases were considered. In **case A**, the size was picked every new step. So if the probe returns to a previous position, its size would be different from the first time. For **case B**, the size for each position was established previously and did not change for all runs performed to calculate the average MSD. For this case, the averages are representative only of that specific network because each simulation is unique. In Fig. 3.1.2 there is a representation of how the lattice was seen by the probe at each probe size, from 0.2 to

$2.0\text{ }\mu\text{m}$ and $\sigma = 0.3\text{ }\mu\text{m}$. Purple color represents the prohibited positions and green the available ones.

Figure 3.1.2: Prohibited and available positions for each bead size in the two dimensional lattice. Purple color represents the prohibited positions and green the available ones

Case A can be thought of having an annealed disorder. On the other hand, case B can be thought of as having a quenched disorder. Disorder means that the elements of a system are not correlated. Here we mentioned the two types of such disorder: the *quenched* and *annealed*. Both of them have random variables as parameters which the difference consists that they are time independent for the quenched disorder. For this reason they are very difficult to analyze mathematically. It is not the case for the annealed disorder whose parameters evolve over time, because the average disorder and the thermal average can be analyzed together.

3.1.2 Random walk simulations

For these simulations we considered a three-dimensional box of side L with N_f line segments of length l_c representing the filaments of actin and a sphere of diameter $b = 2a$ representing a colloidal probe. The diameter of the filament is neglected because its size is on the order of few nanometers while the probe size is in the range of micrometers. A first approximation to the filaments is by assuming that they are rigid segments. It is reasonable for the contour length adopted, $l_c = 15\text{ }\mu\text{m}$, which is smaller than the persistence

length of F-actin. The probe performs a random walk with fixed step size s . If the probe moves through one of the filaments after one step, such movement is not accepted and the probe returns to its previous position. For this colloidal particle, its MSD is given by

$$\langle \vec{r}^2(t) \rangle = \langle \vec{r}(t) \cdot \vec{r}(t) \rangle = \sum_{i=0}^N \sum_{j=0}^N \langle \vec{r}_i \cdot \vec{r}_j \rangle = s^2 \sum_{i=0}^N \sum_{j=0}^N \langle \cos \theta_{ij} \rangle,$$

where $\langle \cos \theta_{ij} \rangle = 0$ for $i \neq j$ and $\langle \cos \theta_{ij} \rangle = 1$ for $i = j$. The relation between the diffusion coefficient D and the step size s is then:

$$\langle \vec{r}^2(t) \rangle = 6Dt = s^2 N \Rightarrow D = \frac{s^2}{6\delta t}, \quad (3.1.1)$$

where $\delta t = t/N$ is the time step used in the simulations. This equation is used in Fig. 3.1.3 which is compared with the simulation results.

Figure 3.1.3: Verification of the relation between probe radius and the step size, providing the correct values for the diffusion coefficient as predicted by the Stokes-Einstein equation. Left: diffusion coefficient vs. probe radius, given by the Stokes-Einstein relation. Right: diffusion coefficient calculated for the simulations for different step sizes and plot of Eq. ??.

By using Eq. ??, the step size may be written in function of the time step and the probe radius through the Stokes-Einstein relation to incorporate the colloid size dependence in the diffusion process:

$$s = \sqrt{\frac{k_B T \delta t}{\pi \eta r}}.$$

An interesting distribution of filaments for additional investigations was performed with the filaments parallel to the faces of the box and regular spacing between them, as represented in Fig. 3.1.4. This distribution of filaments is called *regular cubic network*. The number of filaments is $N_f = 3n^2$, where $(n-1)$ is the number of partitions of each side

Figure 3.1.4: One of the geometries proposed for the random walk simulations: Regular cubic network with $n = 6, 8, 10, 12, 14, 16, 18$ and 20 ; and the correspondent number of filaments given by $N_f = 3n^2$

of the box. For this network the mesh size is well defined and given by

$$\xi = \frac{L}{n-1}.$$

As explained before, the mesh size ξ is defined as the average distance between neighbor filaments. Considering a box of size ξ , which contains one filament for each dimension, the relation of the concentration ρ with the mesh size is given by

$$\rho = \frac{3\xi}{\xi^3} = \frac{3}{\xi^2} \Rightarrow \xi \approx \rho^{-1/2}. \quad (3.1.2)$$

This means that the mesh size depends only on the number of filaments N_f and the contour

length l_c , since the concentration of filaments, is calculated by the relation:

$$\rho = \frac{N_f l_c}{L^3}. \quad (3.1.3)$$

Then, the relations above are equivalent for large amounts of filaments:

$$\rho = \frac{3n^2 L}{L^3} = \frac{3n^2}{L^2} \Rightarrow \xi = \frac{L}{n}.$$

A variation of the network was obtained by the incorporation of random spacing between group of filaments resulting in the random cubic network represented in Fig. 3.1.5.

Figure 3.1.5: Second geometry proposed for the random walk simulations: Cubic network with random spacing between filaments, and total amount of filaments $N_f = 3n^2$

3.1.3 Generating Mikado networks

The Mikado network geometry is created by taking a random position inside a box of size L , then a random direction is taken to determine the position of the second extremity of the filament of contour length l_c . The Sphere Point Picking [29] was used to obtain the angles uniformly distributed over a sphere which consists in taking two random numbers

u and v within the interval $[0, 1]$ and calculating the θ and ϕ angles:

$$\theta = 2\pi u$$

$$\phi = \cos^{-1}(2v - 1).$$

If we pick θ and ϕ as random numbers from the intervals $[0, 2\pi[$ and $[0, \pi]$ respectively the resulting distribution will be non-uniform, consequently there will be more points close to the poles of the sphere. Then the procedure is repeated N_f times, that is the amount of filaments distributed through the box. The visual output of the network created is represented in Fig. 3.1.6. In the left side is represented the network used for the random walk simulations. For the periodic boundary conditions the filaments that cross the box were replicated in the opposite side of the box. In the right one is represented the adapted network for the coarse-grained MD simulations. Particles were added along the filament to represent groups of monomers bonded by an harmonic potential. More details are illustrated in Sec. 3.1.4.

Figure 3.1.6: Three-dimensional Mikado network with 100 filaments. This geometry was the third studied by the random walk simulations and was also used in the coarse-grained MD simulations where the atoms along the filaments represent the segments of the F-actin molecule. Left: Configuration for the random walk. Right: Configuration for the coarse-grained MD.

3.1.4 Molecular dynamics simulations

The previous simulation considered rigid filaments. In order to have thermal fluctuations, molecular dynamics (MD) were performed using LAMMPS [30]. MD simulations are based on the time integration of the Newton's equations of motion of a set of particles. The simulations does not correspond to full atomistic MD but instead larger length and time scales are considered, additionally the double strand F-actin molecule has its structure simplified to a bead-spring chain keeping the persistence and typical contour length of the original filament. For this reason, we are working *coarse-grained* simulations.

Some thermodynamical parameters have to be kept constant to perform numerical calculations. Depending on the parameters chosen we have different ensembles. The simulations described in this thesis considered the NVE ensemble, in which the number of particles, volume, and total energy are kept constant. The Brownian dynamics is used to compute the implicit solvent. In this case the solvent is considered a continuous medium, a procedure that reduces computational cost since thousands of solvent molecules will not be considered in our system. The simulations were performed with the `micro` style as unit set in order that the output could be compared with experiments. Our system consists in a box of size L with periodic boundary conditions used to minimize surface effects.

There were two types of particles: one type corresponds to the spherical probe diffusing, the second one corresponds to a group of monomer of the actin filaments. There is no interaction between the probes. This assumption for experiments can also be considered if a low concentration of probes – about 0.004 %w/v – is used in the sample.

Figure 3.1.7: Representation of the actin filament in the coarse-grained MD simulations.

The group of monomers are linked by bonded interactions. We considered two of them: the bond stretching and the angle bending. The bond stretching is represented by an

harmonic spring potential:

$$U_{bond}(\vec{r}_i, \vec{r}_j) = \frac{1}{2} k_{ij} (r_{ij} - r_{ij}^0)^2,$$

where k_{ij} is the spring constant, $r_{ij} = ||\vec{r}_i - \vec{r}_j||$ is the distance between two consecutive groups of monomers, and r_{ij}^0 is the equilibrium distance. The F-actin is considered nonextensible, for this reason, k_{ij} was determined in a way that high value would lead to negligible variations of the bond length. The angle bending is given by

$$U_{angle}(\vec{r}_i, \vec{r}_j, \vec{r}_k) = \frac{1}{2} \kappa_{ijk} (\theta_{ijk} - \theta_{ijk}^0)^2,$$

where κ_{ijk} is the bending rigidity, $\theta_{ijk} = \sin^{-1} (\vec{r}_i - \vec{r}_j) \cdot (\vec{r}_k - \vec{r}_j)$ is the angle formed by three consecutive groups of monomers, and θ_{ijk}^0 is the equilibrium angle. The value of κ_{ijk} was set in a way that the persistence length of the filament would be $\sim 17\mu\text{m}$, the known value of the persistence length of F-actin [8]. The procedure to estimate the persistence length is explained in Sec. 3.1.5.

The interaction between the filament particles and the probes were given by the Lennard-Jones (LJ) potential shifted to zero from the minimum for $r_c \leq 2^{1/6}\sigma$ and zero for $r_c > 2^{1/6}\sigma$, as given by the equation below:

$$U_{LJ}(\vec{r}_i, \vec{r}_j) = \begin{cases} 4\epsilon \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right] + \epsilon & \text{if } r_{ij} \leq 2^{1/6}\sigma \\ 0 & \text{if } r_{ij} > 2^{1/6}\sigma. \end{cases}$$

This description corresponds to an approximation to the hard core potential. The graphical representation of the Lennard-Jones potential and its main parameter are represented in Fig. 3.1.8. From the figure, σ can be interpreted as the addition of the interacting particles radius. Since the probe is in the order of micrometers and the filament thickness is in the order of nanometers, the thickness can be neglected and σ can be considered only as the radius of the probe.

Figure 3.1.8: Representation of the Lennard Jones potential and its main parameters.

Initially taken as rigid rods constituted by a sequence of spheres, the actin filaments are randomly distributed inside a three-dimensional box resembling the Mikado model. At the first approach considered a static network and the beads moving according to Langevin dynamics to establish the relation of the diffusion coefficient with parameters as the contour length and the polymer concentration. In subsequent refinements, we incorporated fluctuations to the filaments, allowing them to undergo elastic and viscous deformations for the evaluation of the local mechanical properties of the network.

There were some parameters of the model that were empirically determined as the number of group of monomers per filament. For different amounts of groups per filament, they showed small differences in the calculated diffusion coefficient but there is no trend in them, Fig. 3.1.9. For reducing the computational costs, we chose the smallest number of particles per filament which avoid the probe moving through the filament. Concerning the choice of the parameter ϵ , the MSD was calculated for computational runs using $1 \leq \epsilon \leq 1000$ and no significant difference was observed as expected since ϵ only modifies how sharp is the hard core potential adapted from the LJ potential.

Figure 3.1.9: Diffusion coefficients vs. number of filaments for different values of particles per filament. Average over 1000 probes of radius $a = 1\mu\text{m}$ within a cubic box of size $L = 30\mu\text{m}$.

3.1.5 Numerical estimation of the persistence length

A first estimate of the persistence length can be obtained from a simplified way of Eq. 1.4.1:

$$\langle \cos \theta \rangle = e^{-s/l_p}.$$

By using the first approximation of Taylor expansion for the first neighbors in the polymer chain, where s is the bond length r_{ij} , we have that

$$\langle \cos \theta_{ijk}^* \rangle \approx 1 - \frac{r_{ij}}{l_p},$$

where θ_{ijk}^* is the supplementary angle of the bond angle θ_{ijk} , $\theta_{ijk}^* = \pi - \theta_{ijk}$. Therefore we reach this relation for the persistence length:

$$l_p = \frac{r_{ij}}{1 + \langle \cos \theta_{ijk} \rangle}.$$

3.2 Preliminary studies with the two-dimensional lattice

3.2.1 Results and discussion

For these simulations we considered a squared lattice in two situations, as described in Sec. 3.1.1, for case A, normal diffusion was observed for the simulations where the probe diameter was smaller than the mesh size $b/\bar{\xi} \leq 1$, as showed in Fig. 3.2.1 (left) below, because of the statistical spacial homogeneity of the network and the diffusion coefficient decays with the increase of the probe size as expected. For this case, confined diffusion was obtained only for the simulations where $b/\bar{\xi} > 1$. Similar results arose for case B, but situations of non-linear relation between the MSD and time, and situations of confinement of the probe were also observed. Note that $b/\bar{\xi} \approx 1$ in Fig. 3.2.1 (right).

Figure 3.2.1: MSD vs. time for the two cases considered. Left: case A, with changing configuration of the lattice. Right: case B, with fixed configuration of the lattice. While for the first case the MSD was linear for all situations, it was not for the fixed configuration ($b \approx \bar{\xi}$).

This is because of the formation of confined regions in the lattice, as can be seen in Fig. 3.2.2. In the image, purple color represents the prohibited positions and green the available ones. Therefore, for case B when $b \approx \bar{\xi}$, depending on the occupation of the sites and the path taken by the probe, the MSD can correspond to the non-linear relation or the confinement situation. If the movement is confined, the plateau in the MSD is related with the average free distance available for that specific probe size in the network. In other case, the MSD vs. time will correspond to a subdiffusive process when considering

large time scales (see Appendix 1.B). The behavior of the MSD with time can be seen in Fig. 3.2.3 for both linear and log-log scales.

Figure 3.2.2: Example of confined displacement of the bead in the lattice. Purple color represents the prohibited positions and green the available ones.

Figure 3.2.3: MSD vs. time for $b/\bar{\xi} \approx 1$ Left: linear scale. Right the same plot in the log-log scale.

The diffusion coefficient was calculated using Eq. 1.B.2 and related with the ratio probe size over mesh size for both cases as represented in Fig. 3.2.4. For case A, the fitting was proposed to be proportional to the amount of available sites in the lattice. From the expression of the pore size distribution,

$$P(\xi, \sigma) = \frac{1}{\sigma\sqrt{2\pi}} \exp \left(-\frac{(\xi - \bar{\xi})^2}{2\sigma^2} \right), \quad (3.2.1)$$

the probability that the probe would be stuck in the site is the integral up to the probe size of Eq. 3.2.1 as represented in Fig. 3.3.14, called the probability of occupation

$$p_{\xi,\sigma,b} = \int_0^b P(\xi) d\xi.$$

Figure 3.2.4: Diffusion coefficient vs. the probe size for the two cases considered. Left: case A, with changing configuration of the lattice. Right: case B, for a fixed configuration of the lattice. Each point corresponds to simulations in a 2000x2000 lattice taking the average over 1000 computational runs, for total time of 100 s with 1000 steps each second.

The distribution of the diffusion coefficient for case A is given by

$$D(b) = D_0(1 - p_{\xi,\sigma,b}).$$

For case B, a trial to describe the data is performed through the equation below

$$D(b) = \frac{D_0}{S(p_{\xi,\sigma,b})},$$

where $S(p_{\xi,\sigma,b})$ is the mean cluster size and expressed by

$$S(p_{\xi,\sigma,b}) = \sum_{s=1}^{\infty} s w_s = \frac{p_c + p_{\xi,\sigma,b}}{p_c - p_{\xi,\sigma,b}},$$

where w_s is the probability that a site belongs to a cluster containing s occupied sites, given by $w_s = \frac{s n_s}{p}$. p_c is the critical occupation probability, this is 0.592746 for a two-dimensional squared lattice.

Figure 3.2.5: The relation between the diffusion coefficient with the probe size is governed by the pore size distribution. Diffusion coefficient is proportional to the number of sites were the probe cannot access.

Most simulations took $\sigma = 0.3 \mu\text{m}$. High values of σ represent systems that do not show sensitive changes to the probe size, for this reason, the available path is usually the same for the probe size interval considered.

3.2.2 Conclusions

We have considered two situations for the simulations in the two-dimensional lattice: case A, in which the size attributed to each position changes, and case B, in which each position has a fixed size. For case A, the MSD of the probe is linear with time for all $b/\bar{\xi}$ in the interval $[0,1,2]$. For case B, the probe presented subdiffusive behavior for $b/\bar{\xi} \approx 1$ as b increases we observed situations with confinement of the probe to a region of the lattice. According to reference [31], it is expected to see anomalous diffusion in disordered systems. In a diffusion with obstacles, anomalous diffusion requires immobile obstacles, while for mobile ones, the diffusion is approximately normal. These are the analogous situations of case B and A, respectively.

In simulations for case B we could also observe the main behaviors in the diffusion inside polymer networks, depending on the relation between probe and mesh size. These are: free diffusion, diffusion, hopping diffusion, and confined diffusion. The first one occurs for very dilute concentrations or $b/\bar{\xi} \approx 0$. The diffusion is when the concentration of polymers is still not enough to retain the probe between the filaments, but sufficient to cause a measurable

modification of the viscosity of the solution. The normal diffusion corresponds to the values $0 < b/\bar{\xi} < 1$ in which we do not observe the anomalous diffusion. The hopping diffusion is the situation in which the probe size is comparable with the mesh size, $b/\bar{\xi} \approx 1$, then there will be situations of constricted movement in one cage formed by the filament and hopping to a adjacent pore of the network, this is characterized by the subdiffusion process. The analogous situation for the simulations in the 2d lattice is also for $b/\bar{\xi} \approx 1$, without being confined in the time interval considered. The confinement of the probes depends on the their size and the configuration of the lattice. For experiments with polymer solutions the situation corresponds to the probe retained by the filaments or entanglements. The plateau in the MSD is converted into the elastic plateau of the storage modulus after the calculation of the complex shear modulus. Because of the networks fluctuations and the constant depolymerization and repolymerization process, these behaviors could be better represented by an intermediate situation between the two cases described.

These results helped us to interpret data as described above. The model is still very simplified since it does not consider the different diffusion coefficients for probes of different sizes and other characteristics, as for instance, the dynamics of the network and the hydrodynamic interactions. The main insight that came from these results is that the relation between the diffusion coefficient and the ratio $b/\bar{\xi}$ can provide information about the pore size distribution, as we observed through the fittings proposed in plots of Fig. 3.2.4.

3.3 Simulations with three-dimensional models

3.3.1 Static approach

The static approach consists in the simulations with the static filaments. For these simulations we used the random walk and coarse-grained MD. The MSD was similar to those of the main behaviors observed experimentally in the diffusion inside F-actin solutions and networks as described previously in Sec. 3.2.2, Fig. 3.3.1. These MSD are represented in the range of 10^{-4} – 10 s. Next plots will be represented in the time range of experiments to make the comparison better.

Figure 3.3.1: MSD vs. time for different probe sizes, representing the behavior of the MSD for the static networks. Left: Diffusion in the regular cubic network. Right: Diffusion in the Mikado network.

In Fig. 3.3.2 we show the data from the random walk simulations with probes of radius $1\mu\text{m}$ in the three different types of organization of filaments proposed. The error bars were small compared with numerical values and were not represented. We expected less regular

Figure 3.3.2: Diffusion coefficient vs. filament concentration for different organizations of the filaments: regular cubic (+), random cubic (x), and mikado network (*). All the simulations correspond to the average over 1000 probes with trajectories starting from different points picked randomly. The total simulation time for each point is 10 s.

networks show less diffusivity. Although the expectation is true for the Mikado network, the same does not hold for the random spaced filaments network. Our hypothesis is that the increase of the diffusion coefficient is related with the increase of the inhomogeneity of the

network. On the other hand, the decrease in the organization of the network decreases the diffusion coefficient. This idea is reinforced by the plots of the diffusion coefficient vs. the frequency of collisions between spheres and line segments (called *bumps*) in Fig. 3.3.3. The random and regular cubic network, that have similar structures, showed similar relation to the number of bumps with the filaments, while the Mikado network exhibit a much larger number of bumps for similar MSD calculated.

Figure 3.3.3: Diffusion coefficient vs. frequency of collisions between spheres and line segments. These plots indicate that we can distinguish different structures. Left: Regular (+) and random cubic network (x). Right: Mikado network (*).

Consequently, these results give us insights about the relation of diffusion with structure, homogeneity, and organization of the networks. However, this relation deserves a more detailed look to enable a conclusion and verify the experimental viability of measuring these differences when, for example, considering different structures related with the cross-link proteins. The diffusion coefficient deviates from the Stokes-Einstein relation with the increase of the radius and the concentration of filaments, as shown in Fig. 3.3.4 (left). For a better visualization, the data relative to the Mikado network were added in a different plot as represented in Fig. 3.3.4 (right).

It was done a plot of the diffusion coefficient for the previous data as can be seen in Fig. 3.3.5 in function of the ratio radius over mesh size, similar to Fig. 3.2.4, in which an approximate overlapping of the data was obtained. By considering the scaling of the MSD by the probe radius, as argued in reference [7], it is expected to be the same for different probe sizes. In the same reference, measurements with BSA-coated PS beads, that is

Figure 3.3.4: Diffusion coefficient vs. radius for different amounts of filaments for the regular cubic network.

usually considered a nonsticky probe, of different sizes in 0.34 mg/ml F-actin solutions showed that the scaled MSD do not collapse and are ordered with the scaled MSD increasing slightly with the radius. In the static model the same non-collapsing behavior can be observed but with inverse ordering as represented in Fig. 3.3.6. To avoid the smaller probes crossing the filaments for these simulations the number of particles per filament was increased to 150.

Figure 3.3.5: Diffusion coefficient vs. the ratio radius over mesh size for different amounts of filaments for the regular cubic network. Random walk simulation within cubic box of size 30 μm , and average over 1000 probes.

Figure 3.3.6: MSD and scaled MSD vs. time for different radius of the probe. MD simulation within a cubic box of size $30\mu\text{m}$ with 500 static filaments, 150 particles per filament, and average over 1000 probes. Left: MSD vs. time. Right: scaled MSD vs. time.

3.3.2 Nonstatic approach

Figure 3.3.7: Representation of the system for the nonstatic approach. Frame generated using the software Ovito.

The nonstatic approach consists in the simulations with the fluctuating filaments with the persistence length of the F-actin, about $17\mu\text{m}$. For these simulations we used the coarse-grained MD using LAMMPS as described in Chap. 3. After the incorporation of fluctuations to the polymer the same initial configuration may be compared with the static filaments and fluctuating filaments. As shown in Fig. 3.3.8, after the incorporation of the fluctuations the MSD was increased because the filaments can now slide to accommodate the moving probe. The simulation considered 300 filaments.

Figure 3.3.8: MSD vs. time for the same mikado network in two situations: static filaments (+) and fluctuating filaments ($\times$). Left: Plot in the linear scale. Right: Log-log scales. The static and fluctuating filaments are represented close to their respective MSD.

For the nonstatic approach the number of probes was reduced in the simulation box. Despite the fact that, the probes do not interact with each other, their own movement can displace the filament being indirectly influenced by the nearest probes. The found solution reduce the number of probes and perform more simulations to take their average. By using 100 probes within a cubic box of size $15\mu\text{m}$ we note that it corresponds to a similar concentration compared to the one used experimentally. In Fig. 3.3.9 we show the average over 9 simulations.

Figure 3.3.9: MSD vs. time within a cubic box of size $15\mu\text{m}$ with 300 filaments, taking the average over 9 simulations with 100 probes each instead of using 900 probes in one simulation.

3.3.3 Comparison with experimental data

By considering that the diffusion coefficient of the sphere is proportional to the available space for its movement, a simple approximation is to take the volume of the box reduced by the volume of N_f cylinders of length l_c and diameter b

$$D \sim \frac{L^2}{\tau} \left(1 - \frac{\pi b^2}{4} \frac{N_f l_c}{L^3} \right)^{2/3},$$

where τ is the mean time to move through the box. By using Eq. 3.1.3 and 3.1.2 it follows that

$$D \sim D_0 \left(1 - \frac{3\pi}{4} \left(\frac{b}{\xi} \right)^2 \right)^{2/3}. \quad (3.3.1)$$

The data relative to diffusion in the regular cubic network which has a well defined mesh size can be displayed in a linear form if plotted like $\sqrt{1 - (D/D_0)^{3/2}}$ as shown in Fig. 3.3.10 (left). By applying the logarithm and doing a first-order Taylor series approximation around 1, i.e. considering small values of b/ξ

$$\log \left(\frac{D}{D_0} \right) = \frac{2}{3} \log \left(1 - \frac{3\pi}{4} \left(\frac{b}{\xi} \right)^2 \right) \approx -\pi \left(\frac{b}{\xi} \right)^2,$$

then

$$D = D_0 \exp \left(-\frac{\pi}{2} \left(\frac{b}{\xi} \right)^2 \right). \quad (3.3.2)$$

This is a very similar relation obtained from the experimental data by Schmidt *et al* in reference [15] as described in Sec. 1.4.2, which also presents a prediction of de Gennes *et al* derived with arguments of friction and elastic deformation of Gaussian chains, Eq. 1.4.3. This and Eq. 3.3.2 differ only by the factor $\pi/2$ in the exponent.

He and Tang [7] showed that PEG-coated PS probes between a set of coating and probe materials provides the less probe size dependent measurements. These results were considered the standard values for the viscoelasticity of entangled F-actin. Their measurements considered the concentrations of 0.34 and 1.00 mg/mL. By using Eq. 1.4.2, the correspondent mesh sizes of the solutions are respectively 0.51 and 0.30 μm .

Figure 3.3.10: MSD and scaled MSD vs. time for different radius of the probe diffusing in the regular cubic network. MD simulation, box size of $30\mu\text{m}$ with 500 static filaments, 150 particles per filament, and average over 1000 probes. Left: Plot of MSD vs. time. Right: Plot of scaled MSD vs. time.

The present data does not consider the same concentrations and analogously does not use the same probe sizes. For lower concentrations, similar simulations to previous ones were also performed for the fluctuating filaments. See Fig. 3.3.11 and 3.3.11. Reference [9] describes that the elastic response dominates at higher frequencies because of the motion of the polymer is too slow to release from the entanglements. There will be a point that none of the entanglements can relax and a plateau of the elastic modulus is formed.

Figure 3.3.11: MSD vs. time for different probe radius. Left: Plots in the linear scale. Right: Plot in the loglog scale.

Comparing the results of Fig. 3.3.13 with the experimental result displayed in Fig. 2.2.3 we observe a qualitative similarities between them. If we consider the trend of the curves in Fig. 3.3.13 we expect to obtain similar results increasing the number of filaments

Figure 3.3.12: MSD vs. time for different number of filaments. Simulation with static filaments, $a = 1 \mu\text{m}$, $L = 15 \mu\text{m}$, $N_p = 15$. Left: Plot in the linear scale. Right: Plot in the log-log scale. For $N_f = 500$ and 700 the error bars were small and were not represented.

inside the simulation box. This means that our experimental results are due mainly to the concentration and the persistence length of F-actin and the entanglements are not the main factor in determining the viscoelastic properties for this case.

We can observe the tendency to form a plateau in the loss modulus curve to become independent of the frequency for high values of ω . It is known that fluid-like materials will have the $G'(\omega)$ approximately proportional to ω^2 , while $G''(\omega)$ proportional to ω . For a solid-like behavior, both of them are approximately independent of ω . Those are the curve behaviors observed for the loss modulus, representing the transition from a fluid-like to a solid-like material. As expected, for the elastic modulus this transition is not observed since the model does not allow the entanglement of the filaments.

In the frequency range of 10^{-7} – 10^3 rad/s and 10^{-1} – 10^4 Hz, measurements of the viscoelasticity of F-actin solutions by methods as power spectra fluctuations and DWS respectively [17, 32] reported $G \propto \omega^{3/4}$ for both storage and loss moduli in F-actin solutions for high frequencies. Since they are obtained by using Eq. 1.6.5 this corresponds to MSD with proportionality $t^{3/4}$ for small times [17]. These frequencies are not accessible by the multiple particle tracking technique. Gittes reports the same scaling for F-actin, proportional to $\omega^{3/4}$, and for the Polyacrylamide (PAAm) he found the scaling proportional to $\omega^{1/2}$, which is the counterpoint between a semi-flexible and a flexible polymer.

Figure 3.3.13: Storage and loss moduli vs. frequency for different numbers of filaments. The error bars are small for the plot scale, then they were not represented. Up left: $N_f = 200$. Up right: $N_f = 400$. Bottom left: $N_f = 500$. Bottom right: $N_f = 700$.

For the simulations performed using the coarse-grained MD for both the static and the fluctuating filaments we could observe a different slope at small times for the MSD as can be seen in Fig. 3.3.15. As we have been representing the data for the time and the frequency range compatible with the experimental limitation, the different slope for the MSD was not visible. For the fluctuating filaments, the slope of the MSD for large times is approximately $1/2$, very similar to the ones obtained in the experimental part. For small times, we have not calculated the slope since we obtained few points, but with tendency in the slope range $1/2 - 1$. From the same Fig. 3.3.15 we observe that this slope changes earlier in time with higher concentrations of filaments. This feature can be better explored by considering the result frequently found in the literature.

Figure 3.3.14: Storage and loss moduli vs. frequency for $N_f = 200$ (grey) and 700 (black). The error bars are small for the plot scale, then they were not represented.

Figure 3.3.15: MSD vs. time. Left: For different probe diameters and static filaments, $N_f = 500$, $L = 30 \mu m$, $N_p = 150$. Right: For static and different number of filaments, $a = 1 \mu m$, $L = 15 \mu m$, $N_p = 15$.

3.3.4 Conclusions

Entangled meshworks of F-actin can be formed at semi-dilute concentrations. By the definition of the concentration regimes given in Appendix 1.4, our system can be considered semi-dilute since the box size was usually the same size of the contour length, $L \sim l_c$. As reported in [17], for concentrations $< 48 \mu M$, that corresponds to approximately 2 mg/ml , the solutions of F-actin form isotropic liquids. The concentrations considered here are

lower than this, from the numerical estimates of the possible correspondence of the real concentration and the simulational one. This means that our system consists of semi-dilute concentrations of isotropic solutions of filaments with the persistence length of F-actin.

The model proposed in this chapter correspond to the system we want to understand and make predictions in the future. The approach of adding the physical characteristics of F-actin solutions gradually revealed appropriate to reduce the complexity of the system and capture the main factors determining the mechanical properties of the network. In this chapter we considered the first two approaches to understand the main properties of F-actin solutions that can provide insights about the relation of microstructure and viscoelastic properties of F-actin solutions. These were to study the diffusion of colloidal probes inside networks with static and fluctuating filaments.

We summarize the goals and conclude this chapter reminding the questions proposed previously. (1) What structural information can, in principle, be obtained from the microrheological measurements? The static approach indicates that we can get information about the organization and homogeneity of the networks. For small values of b/ξ we could establish similar equation to the one used to calculate the mesh size of networks proposed by de Gennes, but derived using a geometrical argument. Comparing both, Eq. 1.4.3 and Eq. 3.3.2, we observe that they differ by the factor $\pi/2$ in the exponential. (2) What is the appropriate definition of mesh size? The concentration of the solutions can be a misleading parameter for determining the mesh size, since the polymerization efficiency can vary depending on the situation. Establish the mesh size from the diffusion coefficient may be a complementary procedure. For example, using multiple non sticky probe sizes and observe the confined diffusion of the bigger ones. (3) What is the appropriate scaling of the MSD? The scaling of the MSD is important to avoid that the methods lead to probe size dependent results. We could observe that scaling by the ratio b/ξ may help in this sense, but just for low values of this ratio. For the concentrations that interest us, more investigations are necessary. (4) What changes arise from the addition of fluctuations to the filaments? The simulations using the coarse-grained MD did not show a qualitative difference. But the increase in the MSD is still easily noticeable; (5) Can we recover the

viscoelasticity of the F-actin solutions and networks experimentally observed? The model proposed can already reproduce some properties qualitatively of our experimental data. There are several improvements that can be incorporated to the model. But even this very simplified version could already be related to scaling relations and reproduce some of the expected behavior. It is then a tool to help us to interpret data.

3.A Appendix: The damp parameter

The damp parameter corresponds to the time the temperature needs to relax. For this reason can be thought as proportional to the inverse of the viscosity of the implicit solvent. Comparing the frictional force F_f , the way it is computed by LAMMPS, with the Eq. 1.6.2 we obtain the correspondence with α ,

$$F_f = - \left(\frac{m}{\text{damp}} \right) \frac{d\vec{r}}{dt} \implies \alpha = \frac{m}{\text{damp}}.$$

Then, using Eq. 1.B.1, the expected value to the damp is given by

$$\text{damp} = \frac{Dm}{k_B T}.$$

For 20°C and 1 atm, the density ρ and viscosity η of water are approximately $1\text{pg}\mu\text{m}^{-3}$ and $1\text{pg}(\mu\text{m}\mu\text{s})^{-1}$ respectively.

The other values used, converted to the micro units set of LAMMPS are:

Boltzmann constant: $k_B = 1.380\,648 \cdot 10^{-8}\text{pg}\mu\text{m}^2(\mu\text{s}^2\text{K})^{-1}$,

Temperature: $T = 293\text{K}$,

Probe radius: $r = 1\mu\text{m}$,

The typical value expected to the diffusion coefficient at 20°C:

$$D = \frac{k_B T}{6\pi\eta r} = \frac{1.380\,648 \cdot 10^{-8}\text{pg}\mu\text{m}^2(\mu\text{s}^2\text{K})^{-1} \times 293\text{K}}{6 \times 3.141\,592 \times 1\text{pg}(\mu\text{m}\mu\text{s})^{-1} \times 1\mu\text{m}} = 0.214 \cdot 10^{-6}\mu\text{m}^2/\mu\text{s}$$

The mass was set as

$$m = \rho \frac{4}{3} \pi r^3 = 1 \text{pg} \mu\text{m}^{-3} \times 1.333\,333 \times 3.141\,592 \times 1 \mu\text{m}^3 = 4.188 \text{pg}.$$

Then, the damp for this case value should be

$$\text{damp} = \frac{4.188 \text{pg} \times 0.214 \cdot 10^{-6} \mu\text{m}^2 \mu\text{s}^{-1}}{1.380\,648 \cdot 10^{-8} \text{pg} \mu\text{m}^2 (\mu\text{s}^2 \text{K})^{-1} \times 293 \text{K}} = 0.217 \mu\text{s}.$$

With this value for damp, however, it is not possible to recover the diffusion coefficient desired. On the other hand, with a value $100\times$ bigger the diffusion coefficient is the expected one, as can be seen in the Fig. 3.A.1 (left).

Figure 3.A.1: Left: Diffusion coefficient for different organizations of the filaments. Right: Relation between the diffusion coefficient and the damp parameter in LAMMPS

Plotting some diffusion coefficients *versus* the respective damp, Fig. 3.A.1 (right), it can be seen that the line given by

$$f(\text{damp}) = \frac{k_B T}{100m} \text{damp}$$

fits quite nicely to the data.

»

Chapter 4

Final considerations

In this chapter we present the conclusions and the outlook of the Master thesis. It is a summary of the questions addressed in this thesis. The next steps that can be followed to further developments are also presented.

4.1 Conclusion

This work resulted in the development of a framework for the study of specific biopolymers relevant to the mechanical properties of cells. In the experimental part of the project we could set the adjustments in the apparatus and the protocol for the preparation of samples and the performance of the future measurements, with F-actin and other biopolymers. We could make the mechanical characterization of the entangled solutions of F-actin for a frequency range between 10^{-1} to 10^1 rad/s, using the passive microrheology technique based in the multiple particle tracking. The values obtained for the $G'(\omega)$ and $G''(\omega)$ for the F-actin solutions agree with the literature. Our setups allowed us to determine the elasticity of polymeric networks up to 200 Pa, that depends on the configuration of lens and diameter of the probe. We performed the characterization of two concentrations of collagen type I gel. With this and the control measurements using the G-buffer and F-buffer, we could compare results relative to F-actin solutions with the elastic and purely viscous material.

In the numerical part we performed simulations with two and three-dimensional models. Despite the simplicity, they already showed interesting properties. We concluded with the simulations that the approach considering the static filaments can already reproduce the main behaviors observed for the MSD of entangled F-actin and other networks, as represented schematically in Fig. 4.1.1. If the probe is small compared with the mesh size,

Figure 4.1.1: Schematic representation of the main behaviors of the MSD of probes diffusion in polymer solutions.

it is not possible to assess the viscoelastic properties of the network but it can provide structural information. Our results indicate that we can get information about changes in the organization and homogeneity of the networks and the distribution of mesh sizes.

We could identify different diffusion coefficients for the different geometries proposed, but with the same density of filaments, which also means the same mesh size. The insight that we can have from this relation is that disorganization of the filaments decreases the diffusivity while increasing the inhomogeneity we observe an increase of diffusivity, that is one type of structural information about the network. The MSD for these cases were linear with time. As mentioned in [33], an inhomogeneous system can have normal diffusion if they are varied and large scales are being considered. If the probe is of comparable size with the mesh size or slightly bigger, for the static network the probe can be confined at certain point, the plateau will be proportional to the average available free path. For the fluctuating network the data indicate an increase of this available path, as expected. If the probe is bigger it still diffuses for the fluctuating network. The experimental data shows the modification of the inclination with time in the loglog scale, which was not observed in the fluctuating network simulations up to now.

The representation as rigid and semi-flexible filaments showed a difference in the MSD of up to $\sim 27\%$ for the concentration $\rho = 1.33 \mu\text{m}$. It is expected that this difference starts to be relevant for $b/\xi \approx 1$, since we could find a relation between diffusion coefficient, probe diameter and mesh size similar to empiric and theoretical relation, but deduced from a geometrical argument with rigid filaments. An appropriate scaling relation for the MSD and definition for the mesh size are interesting to eliminate or reduce the probe size dependence of the microrheology data, the ratio between the probe size and the mesh size is most likely to be this factor as indicated by the simulations and data for low concentration of filaments. Going back to the experimental procedure we considered interesting to use multiple probe sizes for the measurements. This allows a quick verification of the probes stickiness to the polymers, since the ones with diameter smaller than the mesh size are supposed to freely diffuse. Non sticky probes with diameter bigger than the mesh size are the suitable ones for the microrheology measurements.

4.2 Outlook

With the development of the framework to measure and analyse the viscoelastic properties of biopolymers we can continue the studies of relevant materials to understand the mechanical properties of cells, specially the F-actin solutions and more complex networks. Also test the action over the networks of components whose action is being studied with cells. The apparatus available allows the use of magnetic fields to determine the elastic and viscous moduli of more rigid materials. This could be an alternative method to study the viscoelasticity of F-actin networks and solutions. Keeping the attention if the material is damaged by the force applied, which would alter its mechanical properties. The next step concerning the simulations are to model the entanglements of the network. With this, we will be able to investigate the elastic plateau of F-actin solutions. It also complements our framework since we will have the tool to evaluate the feasibility of experiments.

Bibliography

- [1] Roberto Dominguez and Kenneth C. Holmes. Actin structure and function. *Annu Rev Biophys*, 2011.
- [2] M. L. Gardel, F. Nakamura, J. H. Hartwig, J. C. Crocker, T. P. Stossel, and D. A. Weitz. Prestressed f-actin networks cross-linked by hinged filamins replicate mechanical properties of cells. *Proc Natl Acad Sci U S A*, 103(6):1762–1767, Feb 2006.
- [3] Norman Y. Yao, Daniel J. Becker, Chase P. Broedersz, Martin Depken, Frederick C. Mackintosh, Martin R. Pollak, and David A. Weitz. Nonlinear viscoelasticity of actin transiently cross-linked with mutant α -actinin-4. *J Mol Biol*, 411(5):1062–1071, Sep 2011.
- [4] Juliane Zimmermann, Claudia Brunner, Mihaela Enculescu, Michael Goegler, Allen Ehrlicher, Josef Käs, and Martin Falcke. Actin filament elasticity and retrograde flow shape the force-velocity relation of motile cells. *Biophys J*, 102(2):287–295, Jan 2012.
- [5] Hidetaka Yamaoka, Shinji Matsushita, Yoshitaka Shimada, and Taiji Adachi. Multiscale modeling and mechanics of filamentous actin cytoskeleton. *Biomech Model Mechanobiol*, 11(3-4):291–302, Mar 2012.
- [6] Mohammad R. K. Mofrad and Roger D. Kamm, editors. *Cytoskeletal mechanics: models and measurements*. Cambridge University Press, 2006.
- [7] Jun He and Jay X. Tang. Surface adsorption and hopping cause probe-size-dependent microrheology of actin networks. *Phys Rev E*, 2011.

- [8] Margaret Lise Gardel. *Elasticity of F-actin Networks*. PhD thesis, Harvard University, 2004.
 - [9] Jim W. Goodwin and Roy W. Hughes. *Rheology for Chemists: An Introduction*. RSCPublishing, 2008.
 - [10] John D. Ferry. Viscoelastic properties of polymer solutions. *Journal of Research of the National Bureau of Standards*, 1948.
 - [11] D. E. Ingber. The architecture of life. *Sci Am*, 278(1):48–57, Jan 1998.
 - [12] Erwin Frey, Klaus Kroy, and Jan Wilhelm. Viscoelasticity of biopolymers networks and statistical mechanics of semiflexible polymers. *Advances in Structural Biology*, 5:135–168, 1998.
 - [13] Michael Rubinstein and Ralph H. Colby. *Polymer Physics*. Oxford University Press, 2003.
 - [14] A. Ott, M. Magnasco, A. Simon, and A. Libchaber. Measurement of the persistence length of polymerized actin using fluorescence microscopy. *Phys Rev E*, 1993.
 - [15] C.F. Schmidt, M. Bärmann, G. Isenberg, and Sackmann E. Chain dynamics, mesh size, and diffusive transport in networks of polymerized actin. a quasielastic light scattering and microfluorescence study. *Macromolecules*, 1989.
 - [16] Aleksander Y. Malkin and Avraam I. Isayev. *Rheology: concepts, methods, and applications*. ChemTec Publishing, 2006.
 - [17] Andre Palmer, Thomas G. Mason, Jingyuan Xu, Scot C. Kuo, and D. Wirtz. Diffusing wave spectroscopy microrheology of actin filament networks. *Biophys. J.*, 1999.
 - [18] <[http://www.lsinstruments.ch/technology/diffusing wave spectroscopy dws/microrheology/](http://www.lsinstruments.ch/technology/diffusing_wave_spectroscopy_dws/microrheology/)>, visited in June 2015.
 - [19] T. G. Mason and D. A. Weitz. Optical measurements of frequency-dependent linear viscoelastic moduli of complex fluids. *Phys Rev Lett*, 74:1250–1254, 1995.
-

-
- [20] T. G. Mason, K. Ganesan, J. H. van Zanten, D. Wirtz, and S. C. Kuo. Particle tracking microrheology of complex fluids. *Phys Rev Lett*, 79, 1997.
- [21] T. G. Mason. Estimating the viscoelastic moduli of complex fluids using the generalized stokes-einstein equation. *Rheol Acta*, 39:371–378, 2000.
- [22] H. A. Barnes, J. E. Hutton, and K. Walters. *An introduction to rheology*. Elsevier, 1989.
- [23] Anne Marie S. Niehaus, Dionisios G. Vlachos, Jeremy S. Edwards, Petr Plechac, and Roger Tribe. Microscopic simulation of membrane molecule diffusion on corralled membrane surfaces. *Biophys. J.*, 2008.
- [24] Long Hui Wong, M. L. Gardel, D. R. Reichman, Eric R. Weeks, M. T. Valentine, A. R. Bausch, and D. A. Weitz. Anomalous diffusion probes microstructure dynamics of entangled f-actin networks. *Phys Rev Lett*, 2004.
- [25] Long Hui Wong, Nicholas A. Kurniawan, Heng-Phon Too, and Raj Rajagopalan. Spatially resolved microrheology of heterogeneous biopolymer hydrogels using covalently bound microspheres. *Biomech Model Mechanobiol*, 2014.
- [26] Richard C. Arevalo, Jeffrey S. Urbach, and Daniel L. Blair. Size-dependent rheology of type-i collagen networks. *Biophys. J.*, 2010.
- [27] Ya-li Yang, Lindsay M. Leone, and Laura J. Kaufman. Elastic moduli of collagen gels can be predicted from two-dimensional confocal microscopy. *Biophys. J.*, 2009.
- [28] <[http://web.mit.edu/savin/public/tutorial v1.2/](http://web.mit.edu/savin/public/tutorial.v1.2/)>, visited in June 2015.
- [29] Wolfram Math World. <<http://mathworld.wolfram.com/spherepointpicking.html>>, visited in April 2015.
- [30] S. Plimpton. Fast parallel algorithms for short-range molecular dynamics. *J Comp Phys*, 1995.
-

- [31] M. J. Saxton. Anomalous diffusion due to obstacles: A monte carlo study. *Biophys. J.*, 1994.
 - [32] F. Gittes, B. Schnurr, P.D. Olmsted, F.C. MacKintosh, and C.F. Schmidt. Microscopic viscoelasticity: Shear moduli of soft materials determined from thermal fluctuations. *Phys Rev Lett*, 1997.
 - [33] Igor K. Sokolov. Anomalous diffusion on fractal networks. *Mathematics of Complexity and Dynamical Systems*, 2011.
-