

Dynamics of Semi-Flexible Polymers

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(Dated: August 19, 2005)

Understanding the behavior of semi-flexible polymers when immersed in a nematic phase of a liquid crystal is vital to modeling certain biological systems. The polymers fluctuate as a result of the thermal motion and the coupling with the nematic field. By analyzing these fluctuations, we are able to test different theoretical models designed to explain their dynamics.

PACS numbers: 61.30.Eb, 82.35.Lr

INTRODUCTION

Research on semi-flexible polymers has been constantly expanding, and a lot of scientific interest has been directed to better explaining their dynamics. The reason for which we study semi-flexible polymers in a nematic background is that this configuration is encountered in many biological systems. For instance, the cytoskeleton of the eukaryotic cell contains networks of polymers such as actin, intermediate filaments and microtubules. The cytoskeleton controls the motility, shape and division of the cell ([8], [2]). Moreover, the sarcomeres of the muscles contain networks of actin, and the axons contain networks of neurofilaments, all immersed in nematic-like backgrounds. The properties of these systems can lead to new methods of achieving high alignment of biopolymers such as DNA. Because of the above-mentioned reasons, we are interested in how the nematic field influences the dynamics of the polymer. There have been two models proposed so far. The first one theoretically derives the free energy considering the coupling with the nematic field and has been tested for high order parameters of the liquid crystal ([2]). The other one generalizes the Edwards tube model and the reptation model for flexible polymers in entangled melts ([5]). The latter model has been verified for semi-flexible polymers constrained to a fixed geometry, such as microchannels. It has not been proved for semi-flexible polymers constrained in nematic fields. In this paper, we will investigate which of the models is applicable to nematic fields with low order parameter.

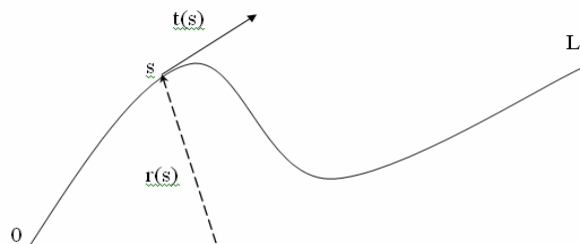


FIG. 1: The worm-like chain model (also known as the Kratky-Porod model) for linear polymers.

Semi-flexible polymers

Semi-flexible polymers are often theoretically modeled as worm-like chains. This model assumes the polymer to be an inextensible curve of length L parameterized by a variable s that follows the contour from 0 to L (see Figure 1). Central to understanding polymers is the concept of persistence length. This parameter measures the stiffness of a polymer, and it is defined as the arc length above which the tangents to the contour of the polymer become uncorrelated. Mathematically, the persistence length is derived from the tangent-tangent correlation function. To compute the tangent-tangent correlation function (TTCF), first take any two points that are a contour length s apart. Then take the tangent at these two points and compute their dot product. Lastly, take the average for all pairs of points that are a distance s apart. The resulting function is referred to as the tangent tangent correla-

tion function (TTCF). For a polymer moving in three dimensions in an isotropic solution the TTCF takes the form of an exponential decay ([5]). The inverse of the exponential decay constant defines the persistence length L_p .

$$\langle t(x)t(x+s) \rangle = e^{-s/L_p}$$

Depending on their persistence length, polymers are classified into : stiff, semi-flexible and flexible polymers. The stiff ones, for which the contour length is less than the persistence length, essentially behave like rigid rods. Semi-flexible polymers are those for which the persistence length and the contour length have the same order of magnitude. These polymers tend to bend and entangle. The last type, the flexible polymers are those for which the contour length is much less than the persistence length. These polymers are elastic, entangle and coil into themselves. Since we will be focusing on semi-flexible polymers, it is worth mentioning some examples, such as: microtubules, neurofilaments, actin, pf1 virus, fd virus, Kevlar. We will use actin and pf1 virus as case studies of the general properties of semi-flexible polymers.

Actin is formed by polymerization of globular actin into two strands that twist in a helical fashion. We decided to study actin because it has a very high persistence length, around $16 \mu\text{m}$, and a contour length of $2\text{-}20 \mu\text{m}$, which allows us to directly observe it under the optical microscope ([2]). The other semi-flexible polymer used, pf1 virus is a polyelectrolyte with contour length of $2 \mu\text{m}$, and persistence length assumed to be on the same order. However, this has not been experimentally verified ([7]).

Most other semi-flexible polymers have very small persistence lengths, and we are not able to image them with an optical microscope. For example, DNA and neurofilaments have a persistence length of around $0.05 \mu\text{m}$ and $0.2 \mu\text{m}$ respectively ([2]).

Liquid Crystals

The other component of our samples, liquid crystals, have attracted increased attention from both academia and industry. Liquid crystals are a phase of matter with order parameters greater than liquids,

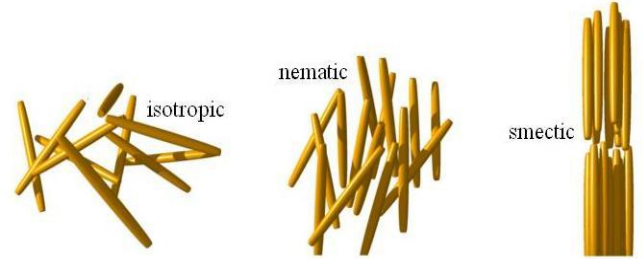


FIG. 2: With increasing concentration of the rods in the solution, we can increase the order in the sample and achieve different liquid crystalline phases. *Picture courtesy of Complex Fluids Group, Brandeis University.*

but less than solids. In liquids the molecules point in all directions with equal probability and they can move freely. In solids on the other hand, the molecules are in fixed positions of the lattice. However, in liquid crystals, the molecules can have orientational or positional order. For example, in a nematic phase of a liquid crystal, the molecules tend to point along one preferred direction. In a smectic phase, they are also constrained to pack into layers. Liquid crystals can only be formed by anisotropic molecules, for instance molecules that have one axis much longer than the other axes. ([6]) Our particular liquid crystal was obtained from fd virus, which can be modeled as a $1 \mu\text{m}$ rod. By increasing the concentration of the virus in our solution, we increased the order of the molecular arrangements. The order parameter is a measure of the alignment of the molecules inside the sample, and has a value of 1 for solids and 0 for liquids.

The increased concentration engenders entropically driven phase transitions, so we can obtain either one of the isotropic, nematic or smectic phases (see Figure 2). The phase that we are interested in is the nematic.

MATERIALS AND METHODS

Fluorescence microscopy

The method we used involved fluorescently labeling actin and then observing its fluctuations under an optical microscope. Fluorescence is the process

by which a molecule absorbs a high energy photon and emits in response a low energy photon. One of the problems with fluorescence is that the molecule in the excited state, before emitting the low energy photon, can interact with the molecular oxygen. Hence, with time the fluorescence signal fades. The fluorescence microscope operates as follows : a mercury lamp emits light, which reaches a filter that selects one particular wavelength, usually green. The green light is reflected into the sample, that absorbs it and emits orange light, that reaches the filter again and passed to the CCD and eyepiece. In our experiments, two microscopes have been used alternatively : an upright Nikon Eclipse E600 POL microscope and an inverted Nikon Eclipse TE2000-U microscope. These microscopes were equipped with cooled CCD cameras : Photometrics' CoolSnap cf, with a pixel pitch of $4.65 \times 4.65 \mu\text{m}$ and CoolSnap HQ, with a $6.45 \times 6.45 \mu\text{m}$ pixel pitch. The software used to acquire images was Universal Imaging Corporation's MetaVue and Roper Scientific RSIImage. A 100x Nikon oil-immersion objective was used for fluorescence detection. The experiments were performed at room temperature, as the TTCF is temperature independent.

Obtaining the nematic phase

Using a virus to obtain a liquid crystal has the advantage that, unlike most synthetic polymers, viruses are very monodisperse ([7]). Monodispersity means that the molecules in the polymer are uniform with respect to constitution and molecular mass.

The fd virus is a great choice to obtain a liquid crystal because it infects the bacteria *Escherichia coli*, and therefore is easy to cultivate and genetically modify. Bacteriophage fd is characterized by a length of $L = 880 \text{ nm}$, a diameter of $D = 6.6 \text{ nm}$, a persistence length $L_p = 2.2 \mu\text{m}$, and a charge per unit length of around $10e^-/\text{nm}$ ([7]). The fd phase behavior is easily explained using the theory proposed by Onsager for rods with hardcore repulsion. Since fd is a polyelectrolyte, the ionic strength of the solution is also an important factor in determining the phase transitions.

To prepare fd, we first need to grow a colony of the bacteria *E. Coli*, infect it with the virus and

then separate the virus culture by centrifugation. The yield is usually 10-100 mg fd virus per liter of infected bacteria solution.

Since we will be using different concentrations of fd, we need to be able to resuspend the virus. The procedure of resuspending the virus involves a first stage in which the solution is spun down in a Beckman Optima TLX Ultracentrifuge at 40,000 g for 15 min, to sediment the bacteria contained in the sample. In the next step, the supernatant is spun down at 85,000 g for 2 1/2 hrs, and the obtained pellet will contain only the fd virus. Resuspending the fd involves adding buffer to the pellet and leaving the sample in the refrigerator overnight.

To measure the concentration of fd, we used a Genesys 10 UV scanning spectrophotometer (Thermo Spectronic, Rochester, NY). The instrument measures the light absorption spectrum to determine concentration of the solution. It is based on the Beer-Lambert Law, that describes the relationship between the absorbance and concentration of a specimen. At a concentration of 10 mg/ml and at a wavelength of light of $\lambda = 269\text{nm}$, the fd virus has an optical density coefficient of $\epsilon_0 = 3.84 \frac{\text{cm}^2}{\text{mg}}$. With the spectrophotometer we can only measure optical densities for which $0 \frac{\text{cm}^2}{\text{mg}} < \epsilon < 3 \frac{\text{cm}^2}{\text{mg}}$. Therefore, we dilute the fd solution, record the new optical density and the initial concentration can be calculated using the formula:

$$C = \frac{d \times \epsilon \times 10 \frac{\text{mg}}{\text{ml}}}{\epsilon_0}$$

,where d represents the dilution factor. To probe that the concentration obtained corresponded to a nematic phase, we looked at the sample under crossed polarizers, using a 10x Nikon objective. Because of the orientational order of the molecules, the light is separated into two perpendicular components that travel at different speeds through the sample, phenomenon known as birefringence. If we rotate the sample 45° under the crossed polarizers, the dark regions become bright, and vice versa. As we were able to probe, all our samples exhibited birefringence.

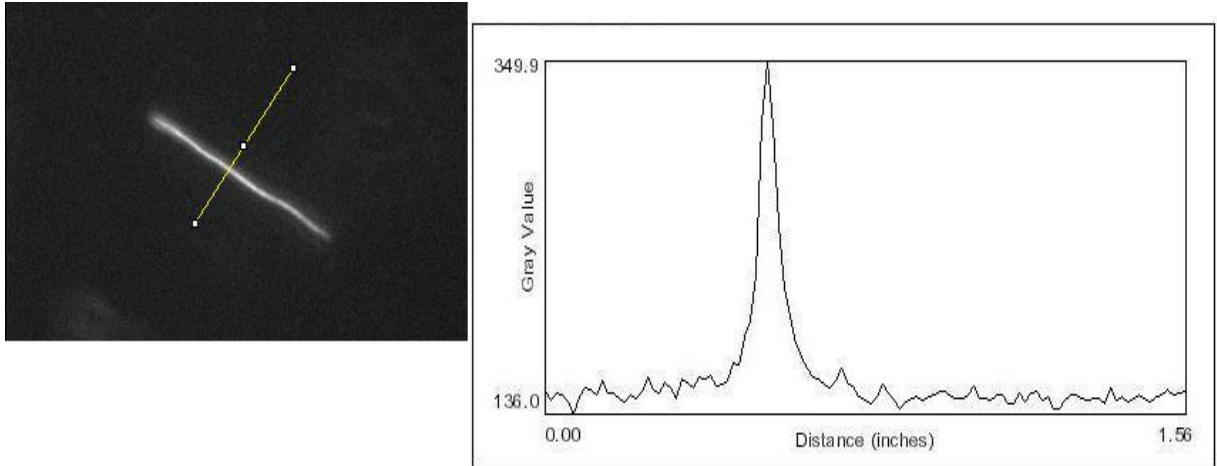


FIG. 3: Left : An image of an actin filament and a perpendicular to its contour.
Right : The intensity profile along the perpendicular to the filament. It is best approximated by a Gaussian function.

Sample Preparation

Our samples contain : fluorescently labeled actin, fd virus at nematic concentration, buffer, anti-oxidant to prevent photobleaching and bovine serum albumin to prevent sticking of the filaments to the glass surfaces. First, we label actin fluorescently with the dye Alexa 488 (Molecular Probes). The dye is non-covalently bound to the actin filament, so the sample must be kept on ice to prevent dissociation of the dye. We also used pf1 virus labeled with Alexa 488, but in this case the dye was covalently bound. We then add the fd solution at a nematic concentration, 20mM phosphate buffer at pH=7.5 because fd is pH sensitive and an anti-oxidant to prevent photobleaching. The anti-oxidant contains 2 mg/ml glucose, 360 U/ml catalase, 8 U/ml glucose oxidase and 0.25 vol % mercaptoethanol, all of which are stored at -70°C ([2]). Bovine serum albumin (BSA) was used to prevent sticking of the filaments to the glass surfaces. The concentration of BSA in the final solution was 0.5 mg/ml.

After the sample was prepared, it was placed between a cover slide and a cover slip. Because drift was an initial problem in our samples, we used a parafilm spacing between the cover slide and cover slip. The sample was filled by capillarity. To prevent the formation of air bubbles inside the sample, the slides and

slips were cleaned with Hellmanex and sonicated. The sample was sealed with Norland Optical Glue 81. To prevent undesirable wall effects, data was not taken from the areas surrounding the surface of the slide, instead we focused in the bulk of the sample.

Image Analysis

The recorded images were analyzed using programs written in IDL 6.0. A typical movie taken of a filament fluctuating contained 2000 frames. The movies were stream acquisitions, with exposure time of 100ms, binning of either 2x2 or 3x3 in which a region of interest was selected.

An image is basically a two dimensional vector of the light intensities recorded by the CCD camera. By taking a perpendicular to the filament and plotting the profile along that line, we obtain a bell-shaped curve. In order to determine to sub-pixel accuracy the position of the filament, we fitted Gaussians to this profile (see Figure 3). Usually, the perpendicular cuts were 20 pixels in length. Next, we filtered out the images where the filament gets out of focus, by imposing the maximum distance between two consecutive points along the filament be less than 3 pixels, and that the length of the filament should not deviate more than 6 pixels from the mean. Then we computed the corre-

lation and averaged as many filaments as possible to get better statistics. To obtain the real length of the filament from the pixel values, we used the formula:

$$L = \frac{\text{Pixel Pitch} * \text{Binning}}{\text{Objective Magnification}} \times \text{Length in Pixels}$$

Most filaments had lengths around $15 \mu\text{m}$ to $30 \mu\text{m}$.

Alignment with a magnetic field

When observing a sample under the fluorescence microscope, we noticed that the sample was divided into nematic domains with different director orientation. There was no uniform alignment throughout the entire sample. If the nematic is not uniform, it might be the case that the filaments fluctuate around a non-zero field with distortions. To make sure that a global alignment was not required to obtain a reliable TTCF, we decided to compare our data to that from a sample with a uniform nematic domain. We took data

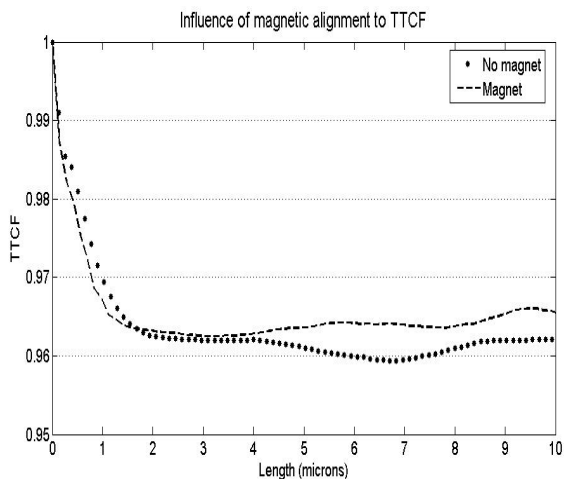


FIG. 4: TTCF is not influenced by aligning the nematic under a magnetic field of 1 Tesla.

on samples that were aligned using a Walker LDJ Scientific, Inc HV-7V electromagnet. The samples were placed parallel to the field, which had a magnetic field strength of $B = 1$ Tesla. The sample was left for 30 min between the magnetic poles. The applied magnetic field produced a magnetic moment per unit volume, which better aligned the fd molecules ([5]). Because of the non-covalent bonds between actin and the dye

Alexa 488, that allow dissociation of the dye with time (around half an hour if sample is not kept on ice), we used pfl virus samples. By inspection under crossed polarizers, the nematic domains in the sample were larger than before the exposure to the magnetic field. We collected data for 10 filaments and compared their average correlation to the data taken at the same fd concentration without the increased alignment.

Even though the domains were more uniformly aligned in the latter case, the data seemed to be reproducible and consistent (see Figure 4). For small length scales the TTCF curves taken for samples kept under a magnetic field and those with more nematic domains agree well. Therefore, we concluded that if we choose our filaments to be situated in a locally aligned nematic field, the data is reproducible.

Measuring persistence lengths

To probe that our routine yields results similar to those from literature ([4], [1]), we decided to measure the actin persistence length. In order to achieve that, we must prepare samples containing the labeled actin, the polymer dextran that will confine the motion of the actin filaments to a two-dimensional plane, BSA to prevent the filaments from sticking to the glass and anti-oxidant. Dextran is a flexible polymer that at the right concentration will exert a pressure on the actin filaments and push them to the glass interface. However, because of the BSA, the filaments will not stick to the glass, and they will be confined to move in two dimensions. The TTCF for this particular motion is an exponential decay with a decay constant equal to the inverse of twice the persistence length. From our measurements, we derived the persistence length of actin to be $16.5 \mu\text{m}$, which agrees well with the established literature. We also attempted to measure the persistence length of pfl, but because of its smaller contour and persistence lengths, the dynamics are much faster, so the filaments could not be confined. In response, we used slides and slips coated with MPEG-Silane-500 polymer. The process of coating involves leaving the slides and slips in NaOH for an extended amount of time, so that they would become hydrophilic. Subsequently, they are immersed in

ethyl alcohol, to eliminate inorganic materials. The polymer is dissolved in distilled water at pH=2.0, and later added to the slides and slips. Because of the hydrophilic nature of the slides and slips, the polymer solution will adhere to the glass. Then the glass was left to dry in an oven at 70°C. Unfortunately, we weren't able to acquire conclusive data for the persistence length of pfl.

RESULTS

Comparison between nematic and isotropic phases

In an isotropic solution, where the molecules have no preferred direction or position, the actin filaments diffuse, visibly fluctuate and entangle. However, in the nematic phase the actin filaments tend to align parallel to the nematic director (see Figure 5). The white filaments represent the actin filaments, and they are surrounded by fd rods that aren't visible under fluorescence. The surrounding fd rods constrain the motion of actin. As the filaments fluctuate in the



FIG. 5: The actin filaments align with the nematic director.

nematic background, they maintain the same preferred direction with time. Also, the fluctuations are small on longer length scales compared to the isotropic fluctuations because the polymer is constrained by the background nematic. We are interested in measuring the tangent tangent correlation function because it will give us insights into the motion of the filament.

Since in an isotropic solution the filaments point in random directions and move and wiggle much more

as opposed to the nematic, the dynamics in the two cases are obviously very different.

The tube model

As mentioned before, the actin filaments are constrained by the fd filaments. We wanted to check if the motion of actin can be effectively thought of as that of a filament restricted to move in a tube-like environment, created by the surrounding fd rods (see Figure 6). The tube model has been a good way to understand motion of flexible polymers in melts and in explaining the motion of linear entangled polymers, also known as the reptation model ([5]). As described by Dogic et. al in [2], for high concentrations of fd, the dynamics of the filaments are well understood. At high order parameters of the nematic, the TTCF can be used to determine the Odijk deflection length, the elastic constant of the background, and the coupling constant with the nematic field. However, for lower order parameters of the nematic, there was no indication to what the dynamics of the filaments are.

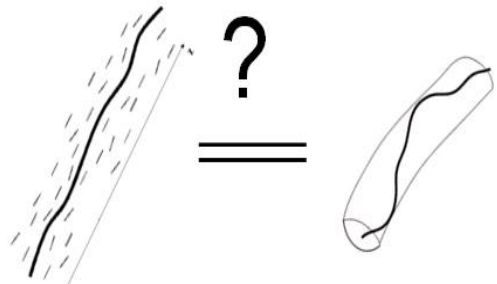


FIG. 6: The hypothesis predicts that the fluctuations of the actin filaments can be associated to those of a semiflexible polymer constrained to a tube. *Picture adapted from Frey [9]*

In the case of semi-flexible polymers, the tube model predicts, from experiments on actin confined to microchannels, that the TTCF will be a decay with superimposed oscillations. To verify if the model is correct, we will determine the tangent tangent correlation function for the filaments and inspect its behavior.

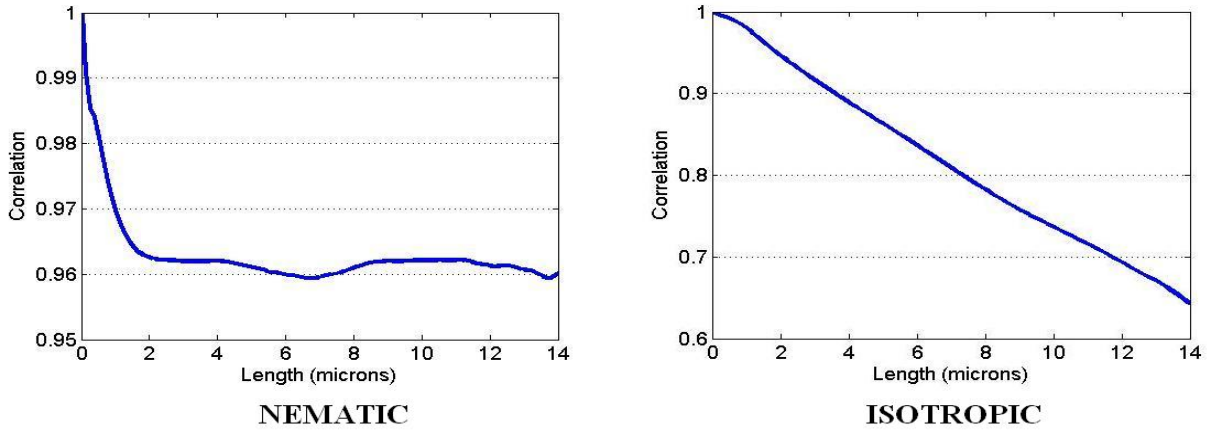


FIG. 7: Comparison between the nematic and isotropic TTCF decay. The nematic decay reaches a plateau, while the isotropic exponentially decays to zero.

Data

The TTCF curve we obtained are very different in the case of nematic and isotropic phases (see Figure 7).

In the isotropic case, the correlation decays slowly exponentially, and compares well to the theoretical prediction and the accepted value for the persistence length of actin. Over a length of $14 \mu\text{m}$, the decay reaches the value of 0.65. In contrast, the TTCF for a nematic background starts as a decay, but quickly reaches a plateau. The plateau suggests that for values greater than $2 \mu\text{m}$, the filament behaves like a rigid rod (because rigid rods have a constant correlation function). This behavior is explained if we consider the constraining fd background.

Comparing the scales, the plateau is reached very fast, while the exponential decays gradually. The plateau is reached after $2 \mu\text{m}$ and maintains a value of approximately 0.96. Our data is unreliable for small length scales because of our finite microscopic resolution and for long length scales we don't have enough statistics. As an example of small scale aberrations, the TTCF for an isotropic should smoothly extrapolate to 1, but instead it flattens out.

To inspect the mechanisms of reaching the plateau, we inspected samples at different fd concentrations, in that way increasing the order parameter

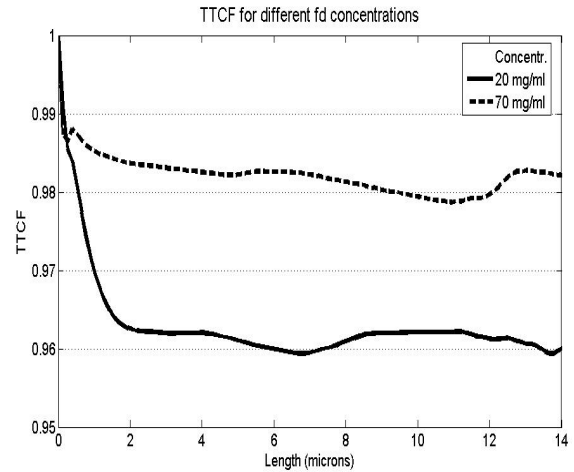


FIG. 8: The TTCF for two concentration of $20 \frac{\text{mg}}{\text{ml}}$ and of $70 \frac{\text{mg}}{\text{ml}}$. The plateau is reached faster, which means the actin acts as a rod for shorter wavelengths for the solution with increased concentration.

of the liquid crystal. Two concentrations have been inspected : one in which the fd is at $\frac{\text{mg}}{\text{ml}}$ and one at $70 \frac{\text{mg}}{\text{ml}}$. The plateau should be reached faster for the higher concentration, because the rods are more closely packed and restrict the motion of the filament (see Figure 8).

CONCLUSIONS

From what we have observed so far, the tube model fails to explain the dynamics, because we didn't observe any oscillations in the correlation function. To be more certain of that, we first need to account for the nematic background. We assumed our filaments fluctuate around a straight line, as in the average filament is straight, but there may be a local tangent we do not take into account. We also need to take more data for other concentration of fd (which implies other order parameters). And lastly, we can test the theoretical model which works for high fd concentrations ([2]), and check if it fits our data for lower concentrations too.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Dr Zvonimir Dogic for continuous support and guidance and Edward Barry for all his laboratory and software advice. I also thank Dr. Kathryn Hollar and Dr. Robert Graham for their dedication and I acknowledge support from the Rowland Institute, Harvard University and the NSF-REU program.

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