Recovering 3D images of polymeric nanofibers in solution through theoretical analysis and Monte-Carlo simulations of their 2D TEM images†

Han Miao, Jianfeng Li* and Daoyong Chen*

Nanofibers are well-known nanomaterials that are promising for many important applications. Since sample preparation for the applications usually starts from a nanofiber solution, characterization of the original conformation of nanofibers in the solution is significant because the conformation affects remarkably the behavior of nanofibers in the samples. However, the characterization is very difficult by existing methods: light scattering can only roughly evaluate the conformation in solution; cryo-TEM is laborious, time-consuming, and challenging technically, and thus difficult to study a system statistically. Herein we report a novel and reliable method to recover the 3D original image of nanofibers in solution through theoretical analysis and Monte-Carlo simulations of TEM images of the nanofibers. Firstly, six kinds of monodisperse nanofibers with the same composition and inner structure but different contour lengths were prepared by the method developed in our laboratory. Then, each kind of nanofiber deposited on the substrate of the TEM sample was measured by TEM and meanwhile simulated by the Monte Carlo method. By matching the simulation results with the TEM results, we determined information about the nanofibers including their rigidity and the interaction between the nanofibers and the substrate. Furthermore, for each kind of nanofiber, based on the information, 3D images of the nanofibers in solution can be re-constructed, and then the average gyration radius and hydrodynamic radius can be calculated, which were compared with the corresponding values measured experimentally to demonstrate the reliability of this method.

1 Introduction

Nanofibers, especially core–shell polymeric nanofibers having good dispersity in solution, are very promising for constructing nano-devices and nano-reactors, reinforcing polymer matrices, preparation of functional materials with large surface area, biological applications (e.g. as drug nano-carriers with long blood circulation time), etc. In most cases of applications, the sample preparation starts from a nanofiber solution, thus the original 3D (three-dimensional) conformation of the nanofibers in the solution remarkably affects their behavior/states in the samples. Therefore, it is significant to know the 3D conformation of the nanofibers in the solution, which is difficult to realize by the existing methods. It is known that the \( \langle R_G \rangle / \langle R_H \rangle \) ratio (\( \langle R_G \rangle \), average gyration radius, and \( \langle R_H \rangle \), average hydrodynamic radius, obtained by static and dynamic light scattering respectively) of nanofibers can only roughly evaluate the 3D conformation (structure and morphology) in solution. Although cryo-EM (cryogenic electron microscopy) with tomography can reconstruct 3D images, it is laborious, time-consuming, and technically challenging, thus difficult to study a system statistically by analyzing a large number of objects in most cases, TEM (transmission electron microscopy) acquires only 2D (two-dimensional) images of the nanofibers, which are obviously different from the 3D images in the solution.

Herein, through theoretical analysis and Monte-Carlo simulations of TEM images, we developed a novel and reliable method to acquire information about nanofibers including their rigidity (at a certain temperature, the rigidity \( L_p k_B T \) of a nanofiber can be fully characterized by its persistence length in solution, \( L_p(3D) \) and the interaction between the nanofibers and the substrate of the TEM samples. Based on the information, the original 3D images of the nanofibers in solution were recovered, and the average gyration radius (\( \langle R_G \rangle \)) and the average hydrodynamic radius (\( \langle R_H \rangle \)) of the nanofibers were calculated, which were then compared with the values measured by laser light scattering to confirm the reliability of the method.
2 Experimental and theoretical methods

Our method was applied to a system containing monodisperse nanofibers prepared through self-assembly of poly(ethylene oxide)-block-poly(4-vinyl pyridine) (PEO113-b-P4VP) micelles and DNA chain, based on the method developed in our laboratory (S1 of ESI). Six kinds of monodisperse nanofibers with different contour lengths \( L_n \) were synthesized and dispersed in water, respectively. Then, the nanofibers were characterized by TEM and dynamic and static light scattering (Fig. 1 and Table 1). For each kind of nanofiber, by TEM image analysis, we could acquire the average apparent persistence length \( \langle L_{p(3D)} \rangle \) (the projected persistence length of nanofibers on the substrate), the average apparent gyration radius \( \langle R_{G(2D)} \rangle \) of the nanofiber, and the probability density function of \( R_{G(2D)} \) by light scattering, \( \langle R_{e} \rangle \) and \( \langle R_{h} \rangle \) were measured. We further used Monte-Carlo simulations to simulate the nanofibers in a layer with the layer height \( h \), the highest height the nanofibers might reach away from the substrate. For each specific nanofiber conformation on the substrate, its existing probability depends on the free conformational energy \( E \) and the nanofiber-substrate interaction potential \( U \), complying with the Boltzmann distribution; \( E \) is related to the rigidity derived from \( L_{p(3D)} \). We projected these conformations onto a 2D plane just like observing the sample through TEM. For each kind of nanofiber with a certain \( L_n \), about \( 10^8 \) ensemble nanofibers were produced by the simulation so that the projected \( \langle L_{p(3D)} \rangle \) and \( \langle R_{G(2D)} \rangle \) could be calculated statistically. There are three tunable model parameters in the simulation: \( L_{p(3D)} \) (the true persistence length), \( e \) (the depth of the potential well), from which \( U \) can be calculated, as explained below, and \( h \) (the highest height the nanofibers might reach from the substrate). By carefully tuning these parameters to minimize errors between the theoretical model and the TEM experiment, we could find out a set of optimized parameters when the simulated 2D results matched best with the results obtained from the real 2D TEM images. At this time, the three tunable desired model parameters \( L_{p(3D)} \), \( e \), and \( h \) were determined. Then, by simulating the nanofibers again in the solution (with the same \( L_{p(3D)} \) but without considering \( e \) and \( h \)), the gyration radius \( \langle R_{G(3D)} \rangle \) and the hydrodynamic radius \( \langle R_{h(3D)} \rangle \) of the nanofibers in solution could be calculated, which were compared to those measured by laser light scattering listed in Table 1, showing quite a good agreement, verifying the reliability of this method.

We chose the above PEO-b-P4VP core–shell monodisperse nanofiber system as an example mainly for the following reasons: (1) the 3D information recovery of nanofibers is significant since nanofibers dispersed in solution can be thought as enlarged linear polymer chains. (2) The monodispersity of the nanofibers makes theoretical simulation and statistical calculation possible and convenient. If the length distribution is broad, then the simulation will be difficult because the distribution function of the experiment cannot even be determined in the first place; also experimentally, the light scattering result would be inaccurate, more weighted on longer nanofibers, because bigger objects contribute much more to the scattering intensities. In contrast, if the nanofibers are monodisperse, then different nanofibers in solution can be effectively seen as a statistical ensemble of one nanofiber.

A program has been coded to measure the 2D apparent persistence length and the 2D apparent gyration radius of the nanofiber. By properly adjusting the image contrast, the program will firstly subtract the background from the original image that contains hundreds of nanofiber shapes, and then identify the contours of the nanofibers one by one. We measure (or calculate) the gyration radius of the shape as well as the angle between the tangent vectors of a nanofiber’s two ends, which can be further used to infer the persistence length \( L_{p(3D)} \) of a nanofiber according to the equation with \( L_n \) being the contour length.

\[
\cos \theta = \exp(-L_n/L_{p(3D)})
\]

The gyration radius of a specific nanofiber shape can be computed using the following formula,

\[
R_{G(2D)} = \sqrt{\frac{\int \rho(r)(r - r_c)^2 dA}{\int \rho(r) dA}}
\]
where the integration is integrated over the 2D vision of the snapshot of a particular nanofiber shape with \( \rho(r) \) being the grayness of the image and \( \mathbf{r} \) the mass center of the nanofiber shape. The position vector in the equation is measured in pixels of the image, so a ratio \( z = d_{sc}/\rho_{ac} \) has to be multiplied (\( d_{sc} \) is the real length that the scale bar represents and \( \rho_{ac} \) is the number of pixels of the scale bar). For each kind of monodisperse nanofiber with a certain \( L_n \), the conformational shapes of about three thousand nanofibers from several tens of images were unbiasedly analyzed, which are sufficient enough for the present experiment: the corresponding relative error is about \( 1/(3000)^{1/2} \approx 1.8\% \), which is smaller than the error caused by other factors. Statistics over all the \( L_{p(2D)} \) or \( R_{G(2D)} \) of the respective nanofibers lead to \( \langle L_{p(2D)} \rangle \) or \( \langle R_{G(2D)} \rangle \): the probability density function of \( \langle R_{G(2D)} \rangle \) was also calculated statistically.

The existing probability of each specific nanofiber conformation on the substrate depends on the free conformational energy \( E \) and the nanofiber–substrate interaction potential \( U \).

In order to calculate the free energy \( E \), we model the nanofiber as a discrete worm-like chain (DWLC) with \( N \) beads (\( N \) depends on the contour length), each with the radius \( r = 10.5 \text{ nm} \) (Fig. 1C, \( N \) and \( r \) of each kind of nanofiber were determined according to TEM images). According to the discrete worm-like chain model, the free conformational energy for a nanofiber can be expressed as

\[
E = \frac{L_p k_B T}{2} \sum_{n=2}^{N-1} \left| \mathbf{r}_{n-1} + \mathbf{r}_{n+1} - 2\mathbf{r}_n \right|^2 / \left| 2\mathbf{r} \right|^3
\]

(3)

where \( \mathbf{r}_n \) is the position of the \( n \)th bead.

As for the nanofiber–substrate interaction, it can be described by the 4–10 Lennard-Jones (L-J) potential

\[
U(d) = \varepsilon_0 \left( \frac{\sigma^6}{3d^6} - \frac{\sigma^4}{2d^4} \right)
\]

(4)

where \( \varepsilon \) is the depth of the potential well, \( d \) is the distance between the bead of the nanofiber and the substrate, and \( \sigma = 1.2 \, r \) with \( r \) being the radius of beads. Note that this 4–10 L-J potential describes the interaction between a sphere and a surface, which is obtained by integrating the 6–12 L-J potential over one spatial dimension. Second, we confine the nanofiber in a box with a height \( h \), which can be interpreted as the highest height the nanofibers might reach on the substrate. A reflective boundary condition is imposed for the upper boundary of the box and no boundaries for the other four sides.

There are three tunable model parameters: the true persistence length \( \langle L_p(3D) \rangle \), the L-J potential coefficient \( \varepsilon_0 \), and the highest height the nanofibers might reach away from the substrate \( (h) \).

For a given set of model parameters, for a certain kind of nanofiber with a certain contour length \( (N \) beads) and a radius \( r \) of 10.5 nm, Monte-Carlo simulations using the Metropolis algorithm and the off-lattice chain method are employed to obtain the corresponding nanofibers; for a specific nanofiber obtained by the simulation, its existing possibility was determined by the energy \( E \) and \( U \) (calculated using eqn (3) and (4), respectively), complying with the Boltzmann distribution. About \( 10^6 \) ensemble samples were produced by Monte-Carlo simulations to obtain reliable results. All the simulated nanofibers were projected onto the 2D substrate plane. Then, the simulated 2D values \( (\langle L_{p(2D)} \rangle, \langle R_{G(2D)} \rangle) \) and the probability density function of \( \langle R_{G(2D)} \rangle \) were computed by analysing the projected simulated images in exactly the same way as that for analysing the TEM images to obtain the experimental 2D values. By carefully tuning the model parameters and trying different sets of parameters, we could find out a set of three parameters that makes the simulated 2D values match the best with the experimental 2D values. At this time, the values of the desired model parameters \( (\langle L_{p(3D)} \rangle, \varepsilon_0 \), and \( h \) \) were determined.

The above theoretical simulations of the nanofibers on the substrate considered the following two factors: the intrinsic property of nanofibers in solution and the adhesive interaction between the nanofibers and the substrate. If we only consider the intrinsic property of nanofibers, such as rigidity, then the 3D images of nanofibers in solution can be obtained, and some other key properties can further be obtained from these 3D images, such as morphological properties \( \langle R_{G(3D)} \rangle \) and \( \langle R_{H(3D)} \rangle \). For each kind of nanofiber we studied, with the same discrete worm-like chain model settings and the \( L_p(3D) \) but without considering the nanofiber–substrate interaction, Monte-Carlo simulations of the nanofibers can be used to recover 3D original images of the nanofibers. \( \langle R_{G} \rangle \) and \( \langle R_{H} \rangle \) of the nanofibers in solution can also be computed. In simulations, \( \langle R_{G} \rangle \) is computed according to its definition, while \( \langle R_{H} \rangle \) is computed according to

\[
\frac{1}{\langle R_{H} \rangle} = \frac{1}{N(N-1)} \sum_{m \neq n} \frac{1}{|\mathbf{r}_m - \mathbf{r}_n|}
\]

(5)
3 Results and discussion

According to the method described in Section 2, we have tried different sets of model parameters to minimize errors between the theoretical model and the TEM experiment, and found that when \( L_{2D} = 490 \text{ nm}, \varepsilon = 0.2kT, \) and \( h = 294 \text{ nm} \), the simulated 2D values (curves in Fig. 2) matched best with the experimental 2D values (solid squares in Fig. 2).

The six kinds of monodisperse nanofibers have six different contour lengths. However, they have a same \( L_{p(3D)} \), which is reasonable since the cross-sections of the nanofibers have the same area, the same structure and the same composition\(^{15} \) and thus have the same rigidity, and, as aforementioned, at a certain temperature the \( L_{p(3D)} \) depends on the rigidity of the nanofibers only. The dependence of \( L_{p(2D)} \) on the contour length (Fig. 2a) should result from the adhesive interaction between the nanofibers and the substrate: for a longer nanofiber, the interaction with the substrate becomes stronger, resulting in a higher existing probability of more stretched conformation of the nanofiber.

As aforementioned in Section 2, we recovered 3D images of the nanofibers in solution by simulating the nanofibers again in their free state considering the intrinsic properties only, excluding the nanofiber–substrate interaction (i.e. with the same \( L_{p(3D)} \) but without \( \varepsilon \) and \( h \)). The computed results of \( \langle R_{G(3D)} \rangle \) and \( \langle H_{3D} \rangle \) are given in Fig. 3a (black and red solid curves).

In order to demonstrate the reliability of our method, \( \langle R_{G(3D)} \rangle \) and \( \langle H_{3D} \rangle \) of each kind of monodisperse nanofiber simulated were compared with the corresponding values measured by laser light scattering. As indicated in Fig. 3a, the data obtained by the light scattering experiments show a good agreement with those obtained by the simulation. It should be mentioned here that, different from existing studies, in the present study, monodisperse nanofibers were used. As mentioned above, the use of monodisperse nanofibers makes the theoretical simulation convenient. Besides, \( \langle R_{G(3D)} \rangle \) and \( \langle H_{3D} \rangle \) values obtained by measuring the monodisperse nanofibers are more reliable because, when polydisperse nanofibers were used,\(^{23–26} \) the values should be biased to larger values (larger nanofibers contribute much more to the scattering intensity than the smaller ones). We noted that in the literature monodisperse rigid inorganic Au nanorods were used for dynamic light scattering measurements.\(^{27,28} \) Nevertheless, monodisperse Au nanorods cannot be used as the model for worm-like chains because conformation of the nanorods cannot be changed, which makes Au nanorods and worm-like chains intrinsically different. The ratio \( \langle R_0 \rangle / \langle R_4 \rangle \) is a characteristic parameter allowing us to roughly estimate the morphology of a nano-object in solution.\(^{8,9} \) For a random polymer coil (ideal chain), it is a constant, 1.505, and if the nanofibers were stiff rods, the theoretical \( \langle R_0 \rangle / \langle R_4 \rangle \) ratios of such rods of 100 nm and 600 nm would be 0.89 and 1.96.\(^9 \) Both the simulation and experimental results are in the range between random coil and stiff rod, demonstrating that the morphology of the nanofibers is
worm-like, similar to that of worm-like chains (Fig. 3b). The deviation between the simulated $\langle R_g \rangle/\langle R_H \rangle$ values and the experimental values at large nanofiber contour lengths may be due to the excluded volume effect.

4 Conclusions

In summary, through theoretical analysis and Monte-Carlo simulations, we successfully extracted key properties from 2D TEM images to build 3D original images of the nanofiber in solution, including the nanofiber rigidity and morphological properties, and even the nanofiber-substrate interaction. Based on the nanofiber rigidity, we recovered 3D images of the nanofibers in the solution and further obtained the nanofiber’s statistical shape information characterized by the average gyration radius ($\langle R_g \rangle$) and the average hydrodynamic radius ($\langle R_H \rangle$). The as-simulated average gyration radius and hydrodynamic radius were compared to those measured by laser light scattering, showing a good agreement, demonstrating the reliability of the method.

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