Anton Peterlin and DNA

Rudolf Podgornik[1]
[1]*Department of Physics, Faculty of Mathematics and Physics,*
University of Ljubljana, Jadranska 19, SI-1000 Ljubljana, Slovenia
Department of Theoretical Physics (F-1),
J. Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia
and
Laboratory of Physical and Structural Biology, NICHD, Bld. 9 Rm. 1E-116,
National Institutes of Health, Bethesda, MD 20892-0924, USA
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I will present an overview of Peterlin's work on the determination of DNA persistence length by analysing the Bunce - Doty light scattering experiments in terms of the Kratky - Porod wormlike chain model. I will describe the theory he used, the general ramifications of the experimental method and the unavoidable limitations of his result, as well as their impact on the development of DNA science.

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A. Historic context

1953 was a veritable *annus mirabilis* for DNA science in more respects then one. First of all, of course, in the April 24 issue of the premier science publication *Nature*, the structure of DNA in dense fibers was elucidated in three fundamental and epoch-making papers: the first one by Watson and Crick [1], the second one by Franklin and Gosling [2], and the third one by Wilkins, Stokes and Wilson [3]. Their work on molecular structure of DNA launched the era of the double-helix. This scientific breakthrough was based on one side on the detailed X-ray scattering studies of DNA fibers, prepared from calf thymus DNA by the Swiss scientist Rudolf Signer, started by William Astbury in 1937, picked up by Maurice Wilkins in 1951 and culminating in 1952 with the iconic *Photo 51* obtained by Rosalind Franklin and Raymond Gosling. The interpretation and the reading of these diffractograms was based heavily on the theory of the scattering intensity of helical molecules devised in 1951 by W. G. Cochran, F. Crick and V. Vand [4] (the CCV theory). Though the original aim of the CCV theory was to provide a theoretical foundation for the elucidation of the structure of helical proteins, such as the alpha helix discovered in 1952 by L. Pauling, it turned out that it could be profitably applied also to the X-ray scattering of DNA. In particular it helped to associate the broad features of the DNA diffractograms with the physical parameters of the helix, as well as to elucidate the fingerprint of the double helical, assymetrical nature of this helix, in the diffraction intensity.

The rest is history one would be inclined to say, were it not for the other discovery published in the same journal in the very same year, about two - actually in the February 7 issue - months before the papers on the double-helical nature of DNA. The paper I am referring to was submitted on June 5 the previous year and is entitled *Light Scattering by very Stiff Chain Molecules* [5], authored by Anton Peterlin, then the director of the "Josef Stefan" Institute of Physics in Ljubljana [6]. Peterlin was analysing the experimental data on light scattering of dilute DNA aqueous solutions and was applying the theory of light scattering to the DNA solution case, in the same way that Watson and Crick were applying the theory of X-ray scattering of helical molecules to the case of DNA fibers. The light scattering experiments on solution of DNA, or thymonucleic acid as it was refferred to at that time, were performed by Barbara H. Bunce [7], who in 1950-1951 worked as a National Institutes of Health Predoctoral Fellow in the lab of Paul Doty [8], the founder of the Department of Biochemistry and Molecular Biology at Harvard. P. Doty, together wit Bruno Zimm and Herman Mark, co-authored the seminal paper on determining absolute molecular weights of polymers by light scattering [9] and was interested in the physico-chemical characterisation of macromolecules in general [10]. Thus his Ph.D. student B. H. Bunce [11] used in particular the light scattering methods to determine molecular weight of DNA in aqueous solutions. They published their work in 1952 in two papers, one in Journal of the American Chemical Society and the other one in the Journal of Polymer Science [12].

In order to learn anything from the light scattering experiment on DNA aqueous solutions, of the type performed by Bunce and Doty, one has to evaluate the corresponding scattering function and compare it to what is seen in experiments. In particular Peter J. W. Debye, when at Cornell but also after his retirement in 1952, was using his early work on X-ray scattering and applying it to the determination of molecular weight of polymer molecules in solution. In his evaluation of the scattering function or the form factor of a polymer chain in a solution, he used the random walk or the free-filght chain model to describe the thermal statistics of a completely flexible polymer molecule [13]. A few years later, in 1949, O. Kratky and G. Porod presented their worm-like chain model of a polymer [14] which was supposed to describe semi-flexible polymers that show persistence in the direction of the chemical bonds. Otto Kratky is among the towering figures in the theory and design of X-ray scattering experiments. In 1940 he became the director of the X-ray department of the Kaiser-Wilhelm institute in Berlin and in 1943 he was appointed as professor at the Institute of physical chemistry in Prague, moving three years later to Graz as a professor of theoretical and physical chemistry. His most acknowledged research efforts were in the development of X-ray small-angle scattering methods, especially measurements of thermodynamic properties of polymers and biopolymers. From 1972 he was the head of the *Institut für Röntgenfeinstrukturforschung der Akad. d. Wiss. und des Forschungszentrums Graz.* Günther Porod studied physics and chemistry under Kratky. He was Kratky's most important coworker in X-ray small angle scattering. In 1965 he was appointed as professor of experimental physics at the University of Graz.

Around 1952 Peterlin had the idea that DNA should be much closer to the Kratky-Porod semi-flexible chain model then to the Gaussian chain model, appropriate for other, less locally stiff polymers. He thus used the Kratky-Porod model to evaluate the form factor of a long polymer chain. As we know today, this problem has a complicated analytical solution, but at that time Peterlin had to use an approximate approach in order to calculate it.

I will not say more about the particular circumstances of Peterlin's life at that time, since they will be covered by other contributors to this volume.

In what follows I will first present a modern view of the Kratky-Porod model, and evaluate the two limiting forms of the polymer form factor that follow from this model. Then I will discuss the way Peterlin approached this problem and how he analysed DNA light scattering data of Bunce and Doty in 1953. I will conclude with an outlook on the DNA work of Peterlin and its historical impact [15].

B. The Kratky-Porod model

Here I will derive the Kratky-Porod model in its modern form and calculate the ensuing form factor of a polymer chain in a solution. Let us consider a polymer as a torsionally relaxed elastic filament, whose elastic energy does not depend on the torsional deformation. For a filament with a circular cross section one can write the elastic deformation energy in the form of an Euler-Kirchhoff elastic filament deformation energy as [16]

$$F = \tfrac{1}{2}K_c \int_0^L \dot{\mathbf{t}}^2 d\ell = \tfrac{1}{2}K_c \int_0^L \left(\frac{d\mathbf{t}}{d\ell}\right)^2 d\ell, \qquad \text{where} \qquad \mathbf{t} = \dot{\mathbf{r}}(\ell) \tag{1}$$

is the unit tangent vector of the polymer described with a parametric curve $\mathbf{r} = \mathbf{r}(\ell)$, with ℓ the natural parameter, *i.e.* the arc-length. The expression for the deformation energy looks similar to kinetic energy of a particle with "position" $\mathbf{t}$ and "time" ℓ, which is the essence of the Kirchhoff kinematic analogy [17]. In addition this partcle would have to move on a unit sphere since $\mathbf{t}^2 = 1$. For an elastic filament that is at a constant non-zero temperature one should study its free energy as opposed to its energy. It can be obtained from the partition function that is defined as

$$\mathcal{Z} = \int \mathcal{D}[\mathbf{t}(\ell)]\ e^{-\frac{1}{2}\beta K_c \int_0^L \left(\frac{d\mathbf{t}}{d\ell}\right)^2 d\ell} \qquad \text{with} \qquad \mathbf{t}^2(\ell) = 1. \tag{2}$$

where β is the inverse thermal energy, $1/\beta = k_B T$ and the integral has to be performed over all unit vector configurations. The partition function is thus analogous to the probability amplitude of a quantum mechanical particle living on a unit sphere, if one identifies the length of the filament with "imaginary time" [18]. The formal relationship can be written as

$$\mathcal{Z} = \int d\mathbf{t}_1 \int d\mathbf{t}_2 \mathcal{Z}(\mathbf{t}_2(L), \mathbf{t}_1(0)). \tag{3}$$

One can thus derive an equation for the "probability amplitude" $\mathcal{Z}(\mathbf{t}_2(L),\mathbf{t}_1(0))$ analogous to the Schrödinger equation in the form

$$\frac{\partial \mathcal{Z}(\mathbf{t}_2(L),\mathbf{t}_1(0))}{\partial L} = \frac{1}{2\mathcal{L}_p}\mathbf{L}^2 \mathcal{Z}(\mathbf{t}_2(L),\mathbf{t}_1(0)), \tag{4}$$

where $\mathcal{L}_p = \beta K_c$ is introduced as the persistence length and $\mathbf{L}^2$ is the angular part of the Laplace operator, given by

$$\mathbf{L}^2 = \frac{1}{\sin\theta}\frac{\partial}{\partial\theta}\left(\sin\theta\frac{\partial}{\partial\theta}\right) + \frac{1}{\sin\theta^2}\frac{\partial^2}{\partial\phi^2}. \tag{5}$$

Since the filament looks statistically the same along all of its contour one can write

$$<\mathbf{t}(\ell_2)\cdot\mathbf{t}(\ell_1)> = <\mathbf{t}(\ell_2-\ell_1)\cdot\mathbf{t}(0)> = <\cos\theta(\ell_2-\ell_1)>. \tag{6}$$

If we now take note of the statistical definition of $<\cos\theta(\ell_2-\ell_1)>$ [19] we can derive straightforwardly that

$$\frac{\partial <\cos\theta(\ell_2-\ell_1)>}{\partial L} = \frac{1}{2\mathcal{L}_p}<\mathbf{L}^2\cos\theta(\ell_2-\ell_1)> = -\frac{1}{\mathcal{L}_p}\cos\theta(\ell_2-\ell_1). \tag{7}$$

The last equality follows from the fact that $\mathbf{L}^2$ is the angular part of the Laplace operator and thus that $\mathbf{L}^2\cos\theta(\ell_2-\ell_1) = -2\cos\theta(\ell_2-\ell_1)$. Therefore one obtains that

$$<\cos\theta(\ell_2-\ell_1)> = e^{-(\ell_2-\ell_1)/\mathcal{L}_p}. \tag{8}$$

The directional correlations along an elastic filament decay exponentially, where the decay length is equal to the persistence length. The persistence length is thus the correlation length for elastic correlations along the filament. This can be seen straightforwardly from the length of the chain directed along the direction of the beginning of the chain *i.e.*

$$<\mathbf{t}(0)\cdot(\mathbf{R}(L)-\mathbf{R}(0))> = <\mathbf{t}(0)\cdot\left(\int_0^L \mathbf{t}(\ell')d\ell'\right)> = \int_0^L e^{-\ell/\mathcal{L}_p}\,d\ell' = \mathcal{L}_p\left(1-e^{-L/\mathcal{L}_p}\right). \tag{9}$$

Here we have taken into account that $\mathbf{R}(L)-\mathbf{R}(0) = \int_0^L \frac{d\mathbf{r}(\ell)}{d\ell}d\ell = \int_0^L \mathbf{t}(\ell)d\ell$. For long filaments the length of the chain directed along the direction of the beginning of the chain thus saturates at the value of the persistence length.

We are now in a position to calculate what is the statistical shape of an elastic filament in a brownian thermal bath. Let us just remind ourselves that the average square of the end to end separation of the filament is given by

$$\begin{aligned} <(\mathbf{R}(L)-\mathbf{R}(0))^2> &= \int_0^L\int_0^L d\ell d\ell' <\frac{d\mathbf{r}(\ell)}{d\ell}\frac{d\mathbf{r}(\ell')}{d\ell'}> = \\ &= \int_0^L\int_0^L d\ell d\ell' <\mathbf{t}(\ell)\mathbf{t}(\ell')> = \\ &= \int_0^L\int_0^L d\ell d\ell' <\cos\theta(\ell-\ell')>. \end{aligned} \tag{10}$$

Using now Eq. 8 for the angular average we remain with

$$<(\mathbf{R}(L)-\mathbf{R}(0))^2> = 2\mathcal{L}_p\left(L-\mathcal{L}_p+\mathcal{L}_p e^{-L/\mathcal{L}_p}\right). \tag{11}$$

This general result was first derived by Kratky and Porod in 1949, be it in a completely different fashion, and thus the model of an elastic filament based on their calculation is usually referred to as the *Kratky-Porod model* or the wormlike chain model or the semiflexible chain model, the nomenclature really varies with the author.

The essence of the model is that there are persistent orientational correlations along the chain that die out exponentially along the contour length of the chain, see Eq. 8, with a characteristic length equal to the persistence length of the chain. Equivalently one could claim that within this model the orientation of the first link in the chain is preserved along the length of the chain again equal to its persistence length, see Eq. 9. The closed form expressions that we derived above have some very intuitive limiting forms that we discuss next.

C. The limiting forms of the Kratky-Porod model

The Kratky-Porod model has two important limits dependnig on the ratio $L/\mathcal{L}_p$ that we shall analyse in what follows. They are imbedded in the Kratky-Porod model but can be derived even without invoking it. First of all we have the Gaussian chain limit, that can be derived as

$$\lim_{L/\mathcal{L}_p \longrightarrow \infty} <(\mathbf{R}(L) - \mathbf{R}(0))^2> = 2\mathcal{L}_p L, \tag{12}$$

The length $2\mathcal{L}_p$ is usually referred to sa the *Kuhn length* and describes the independent unit of the polymer chain. Obviously in this limit the thermal bath completely destroys the correlations between far away segments along the filament.

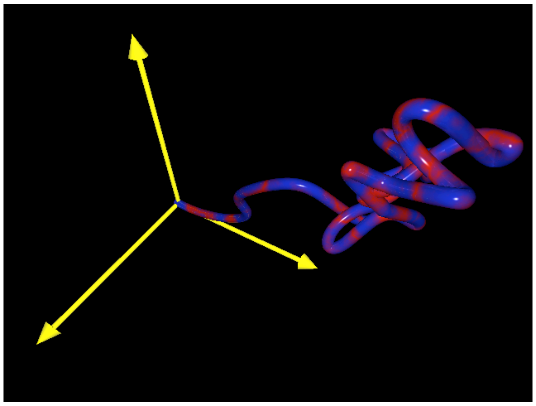

FIG. 1: Statistical shape (exaggerated) of an elastic filament in the Kratky-Porod model. Short filaments really look like stiff rods. The longer they are, the more convoluted they become, eventually becoming completely disordered Gaussian chains.

This result can be obtained also from a simplified consideration along the following lines. Assume the chain is composed of segments of length b directed along the local tangent $\mathbf{t}(\ell)$, so that the end-to-end vector is defined as

$$\mathbf{R}(L) - \mathbf{R}(0) = \int_0^L \mathbf{t}(\ell)\ d\ell.$$

The segments are assumed to be orientationally completely uncorrelated so that

$$<\mathbf{t}(\ell) \cdot \mathbf{t}(\ell')> = \ b\ \delta(\ell - \ell'). \tag{13}$$

This definition of the orientational correlation function gives for the length of the chain directed along the direction of the beginning of the chain *i.e.*

$$<\mathbf{t}(0) \cdot (\mathbf{R}(L) - \mathbf{R}(0))> = <\mathbf{t}(0) \cdot \left(\int_0^L \mathbf{t}(\ell') d\ell' \right)> = b$$

exactly the length of the segment b. It follows straightforwardly in this case that the average size of the chain squared is

$$<(\mathbf{R}(L) - \mathbf{R}(0))^2> = b \int_0^L \int_0^L \ \delta(\ell - \ell')\ d\ell d\ell' = b\ L. \tag{14}$$

Clearly by comparing Eqs. 12 and 14 we can identify the Kuhn length with the length of the statistically independent unit of the polymer chain, thus $b = 2\mathcal{L}_p$.

The other limit of the general Kratky-Porod result can be derived for a very stiff or a very short chain in the form

$$\lim_{L/\mathcal{L}_p \longrightarrow 0} <(\mathbf{R}(L) - \mathbf{R}(0))^2> = L^2, \tag{15}$$

Obviously the thermal bath does not play any role at all in this case. Let us derive this result again by a different route. For a very stiff chain the only configuration surviving the thermal average over all configurations Eq. 2 is the one that minimizes the elastic energy

$$F = \tfrac{1}{2} K_c \int_0^L \left(\frac{d\mathbf{t}}{d\ell} \right)^2 d\ell. \tag{16}$$

The minimization leads to the Euler - Lagrange equation of the form

$$\frac{d^2\mathbf{t}}{d\ell^2} = 0, \qquad \text{with solution} \qquad \mathbf{t}(\ell) = \mathbf{n}, \tag{17}$$

where $\mathbf{n}$ is a unit vector, $\mathbf{n}\cdot\mathbf{n}=1$, specifying the direction of the rod in space and thus $\mathbf{t}(\ell)=\mathbf{n}$ obviously describes a straight line in space. The corresponding square of the end-to-end separation si given by

$$<(\mathbf{R}(L)-\mathbf{R}(0))^2> = \int_0^L\int_0^L d\ell d\ell'\ \mathbf{t}(\ell)\mathbf{t}(\ell') = \int_0^L\int_0^L d\ell d\ell' = L^2. \tag{18}$$

Here we have ignored the thermal average since as we noted for a very stiff chain the only possible configuration is the one corresponding to the solution of the Euler-Lagrange equation Eq. 17. The statistical average thus reduces to a single value. In this limit the elastic properties of the chain obviously do not figure any more in its statistical description.

D. Light scattering from a Kratki-Porod filament in solution

We now move to the most important consequence of the Kratky-Porod model, that was investigated and used by Peterlin in his analysis of X-ray scattering data on DNA solutions. Let us start from a definition of the total scattering intensity as the average of the square of the structure factor of a polymer filament in solution which is given by

$$\mathcal{I}(\mathbf{Q}) = <|\mathcal{F}(\mathbf{Q})|^2> \tag{19}$$

since we have to avaluate the statistical average $<\ldots>$ over all the conformations of the filament in solution. Assuming that the filament is infinitely thin, and thus does not posses any transverze dimension, this leads to

$$\begin{aligned}\mathcal{I}(\mathbf{Q}) &= <|\mathcal{F}(\mathbf{Q})|^2> = <\int_{(V)}\rho(\mathbf{r})\ e^{i\mathbf{Q}\cdot\mathbf{r}}\ d^3\mathbf{r}\ \int_{(V)}\rho(\mathbf{r}')\ e^{-i\mathbf{Q}\cdot\mathbf{r}'}\ d^3\mathbf{r}'> \\ &= \frac{1}{L^2}\int_0^L\int_0^L <e^{i\mathbf{Q}\cdot(\mathbf{r}(\ell)-\mathbf{r}(\ell'))}>\ d\ell d\ell'.\end{aligned} \tag{20}$$

Here we have conventionally normalized the result by dividing it with L^2. It is very difficult to calculate this quantity exactly for the Kratky-Porod chain, though there is no shortage of approximate results that interpolate between two obvious limiting cases given by Eqs. 14 and 18. Let us evaluate these limits explicitly.

For a Gaussian chain the probability distribution of segments with length $\mathbf{r}(\ell)-\mathbf{r}(\ell')$ is Gaussian due to the central limit theorem that follows from statistical independence of the segments. For a Gaussian distribution one can furthermore derive [20]

$$<e^{i\mathbf{Q}\cdot(\mathbf{r}(\ell)-\mathbf{r}(\ell'))}> = e^{-\frac{1}{2}<\left(\mathbf{Q}\cdot(\mathbf{r}(\ell)-\mathbf{r}(\ell'))\right)^2>}. \tag{21}$$

We already know that the statistics of a Gaussian chain is isotropic, which means that for each Cartesian component x_i one can write

$$<(x_i(\ell)-x_i(\ell'))^2> = \tfrac{1}{3}b|\ell-\ell'|.$$

where of course we took account of the fact that $<(\mathbf{r}(\ell)-\mathbf{r}(\ell'))^2> = b|\ell-\ell'|$. Therefore we finally remain with

$$\mathcal{I}(\mathbf{Q}) = \frac{1}{L^2}\int_0^L\!\!\int_0^L e^{-\frac{b}{6}Q^2|\ell-\ell'|}\ d\ell d\ell' = \frac{2(e^{-(QR_g)^2}-1+(QR_g)^2)}{(QR_g)^4} = f((QR_g)^2),$$

where $f(x)$ is the Debye scattering function and the radius of gyration R_g is given by $R_g^2=\frac{1}{6}\ bL$. The Debye scattering function can be often conveniently approximated by

$$\mathcal{I}(\mathbf{Q}) = \frac{1}{1+\frac{1}{2}Q^2R_g^2}$$

with about 15 % accuracy for the whole range of Q values. Debye derived this scattering function within the random walk model of a polymer chain, that accurately describes the thermal statistics

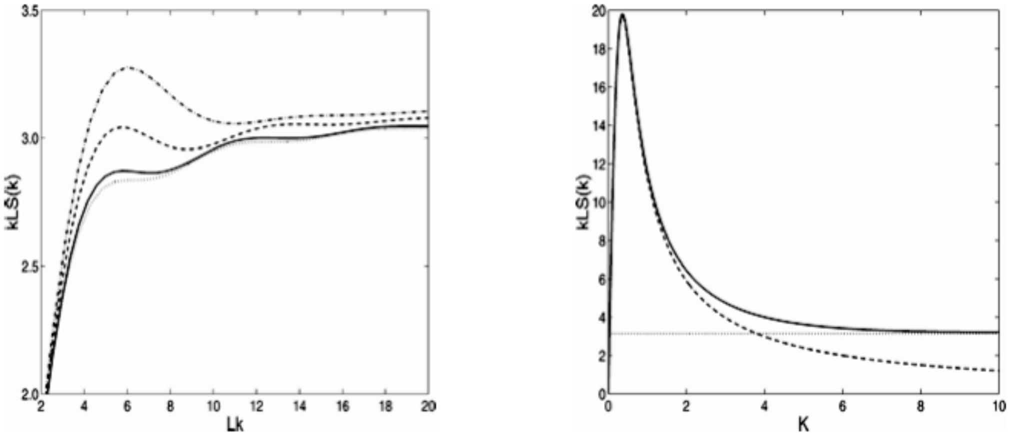

FIG. 2: Exact scattering intensity for a Kratki-Porod chain calculated by Spakowicz and Wang (Adapted from A.J. Spakowicz and Z-G. Wang, *J. Chem.Phys.* **37** (2004) 5814-5823). The l.h.s. graph shows $(kL)\mathcal{I}(k)$ as a function of the dimensionless wave vector $k = 2\mathcal{L}_p Q$ for different values of the dimensionless length of the chain $N = L/(2\mathcal{L}_p)$. (in this work the symbol $S(Q)$ is used instead of $\mathcal{I}(Q)$). $N = 0.1$ (solid line), $N = 0.5$ (dashed line) and $N = 1$ (dashed-dotted line). For comparison the figure also includes the rigid rod result Eq. 23 (dotted line). The r.h.s. graph shows $(kL)\mathcal{I}(k)$ for $N = 100$ for the exact form of the Kratky-Porod scattering function (solid line) and the Gaussian chain (dashed curve). The Kratky - Porod model closely agrees with the Gaussian chain model for small k; however, as k increases, these two models diverge. Since a wormlike chain is rigid at sufficiently small length scales, the structure factor for the wormlike chain model approaches the rigid rod limit for large k regardless of the stiffness of the chain. Such behavior is not captured by the Gaussian chain model since it has no (bending) stiffness at any length scales.

of a completely flexible polymer molecule, with only short range orientational correlations along the chain. The opposite limit within the Kratky-Porod model is again obtained by treating the filament to the lowest order as a rigid rod. In this case $\mathbf{r}(\ell) - \mathbf{r}(\ell') = \mathbf{n}(\ell - \ell')$. where $\mathbf{n}$ is the constant unit direction tengential vector of the rod. For the rod the statistical average is translated directly into the integral over all the orientations of the rod with respect to $\mathbf{Q}$. Thus we remain with

$$\begin{aligned}\mathcal{I}(\mathbf{Q}) &= \frac{1}{L^2}\int_0^L\int_0^L <e^{i\mathbf{Q}\cdot(\mathbf{r}(\ell)-\mathbf{r}(\ell'))}> d\ell d\ell' \\ &= \frac{1}{L^2}\int_0^L\int_0^L e^{iQ|\ell-\ell'|\cos\theta} d(\cos\theta)\, d\ell d\ell' = \frac{2}{L^2}\int_0^L\int_0^L \frac{\sin Q|\ell-\ell'|}{Q|\ell-\ell'|}\, d\ell d\ell'.\end{aligned} \tag{22}$$

One can now introduce an auxiliary variable $u = \ell - \ell'$. The domain of integration decomposes into a stripe from u to L for the variable ℓ and from 0 to L for u. We can thus finally derive for the total scattering factor of an orientationally averaged thin rod

$$\mathcal{I}(\mathbf{Q}) = \frac{2}{L^2}\int_0^L (L-u)\frac{\sin Qu}{Qu}\, du = \frac{2}{(QL)}\left(\int_0^{QL}\frac{\sin z}{z}\, dz - \frac{1-\cos QL}{(QL)}\right). \tag{23}$$

The two forms of the scattering function Eqs. 22 and 23 also present two asymptotic limits for the scattering from a semi-flexible Kratki-Porod filament. For small values of $L/\mathcal{L}_p$ we have the rigid rod result and for large values of $L/\mathcal{L}_p$ we have the Debye Gaussian chain result. The limiting values of the two scattering intensities for large Q are $\pi/2$ for the rigid rod and 2 for the Gaussian chain.

Exact calculations of the scattering function for the Kratki-Porod model, *i.e.* its form between the limits of Eqs. 22 and 23, are extremely difficult to calculate and there have been various attempts at its explicit evaluation. In the Kratki-Porod model the scattering function can be obtained exactly from

$$\begin{aligned}\mathcal{I}(\mathbf{Q}) &= \frac{1}{L^2}\int_0^L\int_0^L <e^{i\mathbf{Q}\cdot(\mathbf{r}(\ell)-\mathbf{r}(\ell'))}> d\ell d\ell' = \\ &= \frac{1}{L^2}\int_0^L\int_0^L \mathcal{G}\left(\mathbf{Q};\ell-\ell'\right)\, d\ell d\ell'.\end{aligned} \tag{24}$$

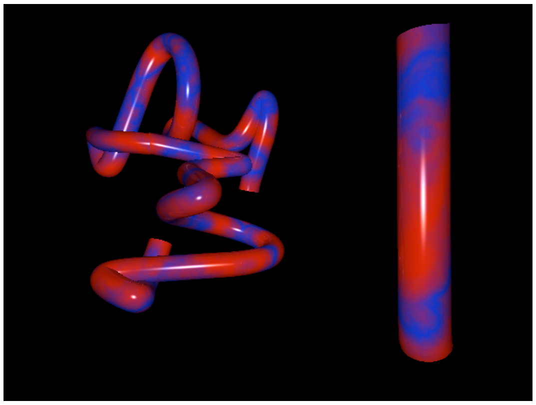

FIG. 3: Two limiting shapes - schematically - used to calculate the light scattering off a flexible polymer chain in solution. A Gaussian, completely disordered, coil and a stiff rod. They represent two extremes of the Kratky-Porod statistics of a filament in solution. The exact scattering form factor of a Kratky-Porod chain can interpolate between these two limits.

Here $\mathcal{G}\left(\mathbf{Q};\ell-\ell'\right)$ is the Fourier transform of the Green function of the Kratki-Porod chain defined as

$$\begin{aligned}&\mathcal{G}\left(\mathbf{r}-\mathbf{r}';\ell-\ell'\right) = \\ &= \int \mathcal{D}(\mathbf{t})\ \delta^3(\mathbf{r}-\mathbf{r}'-\int_{\ell'}^{\ell}\mathbf{t}(s)ds)\ e^{-\frac{1}{2}\beta K_c\int_0^L\left(\frac{d\mathbf{t}}{d\ell}\right)^2 d\ell}.\end{aligned} \tag{25}$$

where the summation is over all the configurations of the chain with $\mathbf{t}^2(\ell) = 1$ as in Eq. 2. This average is difficult to evaluate explicitly.

There are two ways to proceed from here. Harris and Hearst [21] consider an exact series expansion of the scattering function in terms of the weighting function method. An alternative approach was prosecuted vigorously by Spakowicz and Wang [22]. In a veritable mathematical *tour de force* they again obtained an exact result for the scattering function in the form of infinite continued fractions, which are obtained by exploiting the hierarchical structure of a moment-based expansion of the partition function. In both cases the final dependence of the scattering function $\mathcal{I}(\mathbf{Q})$ on the magnitude of the wavevector $\mathbf{Q}$ can only be evaluated numerically. On Fig. I D we compare the results of the exact calculation by Spakowicz and Wang and the limiting forms of the scattering form factor for a rigid rod and for a Gaussian chain calculated above.

E. Peterlin's analysis of DNA light scattering data

Peterlin published his investigations on the light scattering of semi-flexible polymers in five other publications, apart from his *Nature* paper [23]. They are all based on his approximate treatment of the scattering integral Eq. 20 that he was not able to evaluate exactly. The explicit and exact evaluation of the scattering function within the Kratky - Porod model was evaluated later by Harris and Hearst as well as Spakowitz and Wang (see above). Peterlin writes Eq. 20 in the form

$$\begin{aligned}\mathcal{I}(\mathbf{Q}) &= \frac{1}{L^2}\int_0^L\int_0^L <e^{i\mathbf{Q}\cdot(\mathbf{r}(\ell)-\mathbf{r}(\ell'))}> d\ell d\ell' = \frac{1}{L^2}\int_0^L\int_0^L e^{-\frac{1}{2}<(\mathbf{Q}\cdot(\mathbf{r}(\ell)-\mathbf{r}(\ell')))^2>} d\ell d\ell' = \\ &= \frac{1}{L^2}\int_0^L\int_0^L e^{-\frac{Q^2}{6}<(\mathbf{r}(\ell)-\mathbf{r}(\ell'))^2>} d\ell d\ell'.\end{aligned} \tag{26}$$

by implementing the Gaussian *ansatz* Eq. 21 and the fact that the statistical distribution of the polymer chain is isotropic. The magnitude of the scattering wave vector Q in the above formula is given by

$$Q^2 = \left(\frac{2\pi}{\lambda}\ 2\sin\frac{\theta}{2}\right)^2,$$

with λ the wavelength of light and θ the scattering angle. The difficult part in the above integration is to get the appropriate form of $<(\mathbf{r}(\ell) - \mathbf{r}(\ell'))^2>$ in the Kratky-Porod model. One can either evaluate it explicitly and remain with a complicated integration, or one can come up with some suitable approximation and hopefully evaluate the integral analytically. It was the latter path that was pursued by Peterlin.

The approximation embraced by Peterlin on purely intuitive grounds was to take

$$<(\mathbf{r}(\ell) - \mathbf{r}(\ell'))^2> = 2{\mathcal{L}_p}^2 \left(u - 1 + e^{-u}\right), \tag{27}$$

with x the ratio of the separation between the segments $\mathbf{r}(\ell)$, $\mathbf{r}(\ell')$ along the chain and its persistence length, *i.e.* $u = |\ell - \ell'|/\mathcal{L}_p$. The essence of the Peterlin approximation was to use the Kratky-Porod form of the end-to-end separation of the chain also for the local segment-to-segment separation among any two segments along the chain. Though this is not valid exactly, it is certainly plausible. Peterlin never explored systematically the range of validity of this approximation, he does however state [24] that the statistical deviation from this result, expected for a Kratky - Porod chain, should have little consequences on his final conclusions. In his opinion the local approximate relation Eq. 27 should be the more accurate the larger the ratio $L/\mathcal{L}_p$.

Taking this closed form expression for $<(\mathbf{r}(\ell) - \mathbf{r}(\ell'))^2>$ the two integrals in Eq. 26 can now be evaluated analytically, yielding the following closed form expansion for the scattering intensity

$$\mathcal{I}(\mathbf{Q}) = P(p,x) = e^{-u}\left[F(p,x) - \frac{p}{1!}F(p+1,x) + \frac{p^2}{2!}F(p+2,x) + \dots\right] \tag{28}$$

Here we used the following abbreviations

$$p = \tfrac{1}{3}\left(\frac{4\pi}{\lambda}\,\mathcal{L}_p\right)^2 \sin^2\frac{\theta}{2} \qquad \text{and} \qquad F(p,x) = \frac{2}{(px)^2}\left(px - 1 + e^{-px}\right),$$

where $x = L/\mathcal{L}_p$. As shown by Peterlin the above scattering intensity reduces directly to the Debye result valid for a Gaussian chain and corresponding to the limit $x \longrightarrow \infty$ as

$$\lim_{x\longrightarrow\infty} P(p,x) = \frac{2}{w^2}\left(w - 1 + e^{-w}\right) \qquad \text{with} \qquad w = \tfrac{1}{6}\left(\frac{4\pi}{\lambda}\,R\right)^2 \sin^2\frac{\theta}{2},$$

which is the Debye result with R^2 the average square end-to-end distance for a Gaussian chain.

Since experimentalists usually plot the scattering data in terms of the *s.c.* Zimm plots, where one plots not the scattering intensity but rather its inverse as a function of $\sin^2\frac{\theta}{2}$. Peterlin thus rewrote his results in an alternative form that would be in accord with this convention. He thus evaluated the expansion of the inverse scattering intensity, noticing that the Zimm plot should show a convex curvature close to the origin for a Gaussian chain and should show a concave curvature for a stiff rod. His results should fall right somewhere in between these two limits. He derived the following form for the inverse scattering intensity

$$\frac{1}{P(p,x)} = 1 + \frac{p}{3\;1!}\left[x + 3(F(1,x) - 1)\right] + \dots. \tag{29}$$

This form is obviously linear in $p = \frac{1}{3}\left(\frac{4\pi}{\lambda}\,\mathcal{L}_p\right)^2 \sin^2\frac{\theta}{2}$ and its L dependent coefficient, *i.e.* $[x + 3(F(1,x) - 1)]$, should be easily extractable from the Zimm plot. Peterlin also checked the limiting forms of this expression. First of all he writes down again the Debye limit of a Gaussian chain which now has the form of

$$\lim_{x\longrightarrow\infty}\frac{1}{P(p,x)} = 1 + \frac{w}{3} + \dots \qquad \text{again with} \qquad w = \tfrac{1}{6}\left(\frac{4\pi}{\lambda}\,R\right)^2 \sin^2\frac{\theta}{2}. \tag{30}$$

Here again R is simply the mean square end-to-end separation of a Gaussian chain. Then he derives also the form of the Zimm plot for a stiff rod. This limit corresponds to the general case of small length of the chain in comparison with its persistence length. Formally this limit is obtained by taking $x \longrightarrow 0$ in the general formula Eq. 28 yielding the following expression

$$\lim_{x\longrightarrow 0}\frac{1}{P(p,x)} = \frac{(QL)}{2}\left(\int_0^{QL}\frac{\sin z}{z}\,dz - \frac{1-\cos QL}{(QL)}\right)^{-1} = 1 + \frac{y}{9} + \dots \qquad \text{with} \qquad y = \left(\frac{4\pi}{\lambda}\,L\right)^2 \sin^2\frac{\theta}{2}, \tag{31}$$

which is completely in accord with Eq. 23. He notes that in the Zimm plot for all x his scattering curves remain concave. After obtaining the scattering intensity for a semi-flexible chain, which incidentally were calculated numerically by Mrs. Bibijana Čujec - Dobovišek of the J. Stefan Institute, Peterlin started comparing them to experiments by Bunce and Doty, which were the only ones at that time involving DNA. He took the data from Bunce's thesis and obtained a graph showing the comparison between theory and experiment, see Fig. I E. Different variants of this graph appear in several of Peterlin's papers dedicated to the scattering of light in dilute DNA solutions. They vary only in regard to which data sets Peterlin chose to include in the graph. The graph presented in the *Nature* paper [25] contains only data sets by Singer, Bunce-Geiduschek, Bunce-Geiduschek I and Gulland. The paper in *Die Makromoleküläre Chemie* [26] contains the data sets by Singer, Bunce-Geiduschek, Bunce-Geiduschek I and Gulland, Bunce-Geiduschek II, Bunce-Geiduschek III, Varin I, Varin II (pH = 2.6) and Varin III. The last three data sets were not obtained by Bunce [27]. The paper in the *Annals of the New York Academy of Science* [28] contains data sets by Singer, Bunce-Geiduschek, Bunce-Geiduschek I and Gulland, Bunce-Geiduschek II, and Bunce-Geiduschek III. The paper in the *Journal of Polymer Science* [29] contains only theoretical calculations. The paper in *Die Makromoleküläre Chemie* thus represents the most thorough set of experimental data and their comparison with Peterlin's theoretical calculations.

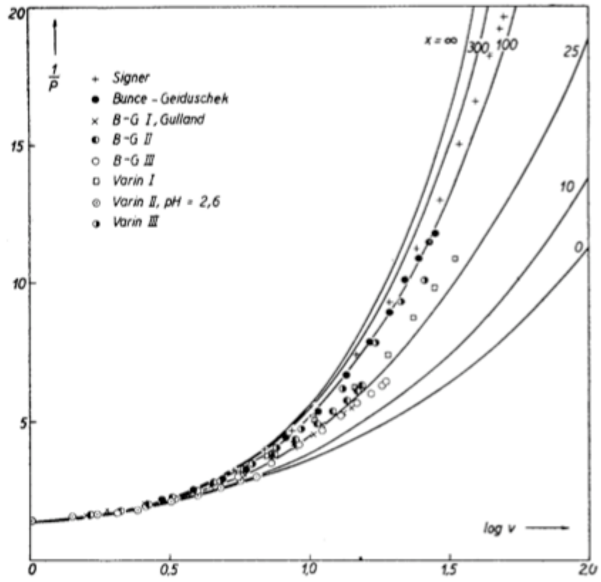

FIG. 4: Comparison between theory and experiment for a set of DNA data. Taken from A. Peterlin, Lichtzerstreuung an ziemlich gestreckten Fadenmolekulen, *Die Makromoleküläre Chemie* **9** 244-268 (1953). Instead of plotting $v \sim \sin^2 \frac{\theta}{2}$ on the abscissa, he chose to plot $\log v$. Other five papers by Peterlin on the same topic contain partial versions of this figure, showing only some, not all, sets of data presented above.

Obviously all the experimental data on Fig. I E fall between the Debye Gaussian result and the stiff rod result, indicated by the values of $x = \infty$ and $x = 0$.

Peterlin now used his expression Eq. 29 and fitted the length of the chain L as well as the persistence length $\mathcal{L}_p$ to the data. One should note here that the experimental data were not obtained for monodisperse DNA solutions and thus the length estimate should be considered as a polydispersity average. He assembled all his results in a table. There are different variants of this table in the various papers referred to above, the most thorough one again exhibited in the *Die Makromoleküläre Chemie* paper. In these tables he presents the molecular mass of the DNA used in the data set, the fitted persistence length, the fitted length, the average end-to-end separation obtained from the fitted values of the persistence length and the total length of DNA, and the linear mass of the DNA molecule obtained as the ratio between the molecular mass and fitted DNA length. The average persistence length of DNA obtained by Peterlin is thus given by

$$\mathcal{L}_p = 40.6\ (1 \pm 0.28)[\mathrm{nm}] \text{ or approximately 120 base pairs,} \tag{32}$$

where the contour length of the base pair is taken standardly as 0.34 nm. This makes DNA a moderately stiff molecule. The Peterlin value is indeed very close, but somewhat smaller, then the modern accepted value [30] of $46 - 50$ nm or $140 - 150$ base pairs, thus very close to the length of the nucleosomal DNA fragment. However, as it has been realized for a while [31], it depends crucially on the ionic solution conditions and can vary significantly with these conditions [32]. The most accurate values for DNA persistence length are obtained from atomic force spectroscopy (AFM) [33] which is the modern method of choice for measuring elastic properties of single macromolecules.

Data set	DNA preparation	$M \times 10^{-6}$	$\mathcal{L}_p$[nm]	L[nm]	R[nm]	M/L[M/0.1 nm]
1	Signer	6.7	28.5	4300	490	156
2	Bunce-Geiduschek	4	26	2600	370	154
3	Gülland	4	40	1000	280	400
4	Bunce-Geiduschek I	2.64	40	1000	280	264
5	Bunce-Geiduschek II	2.1	37	1100	280	190
6	Bunce-Geiduschek III	2.7	54	1080	340	250
7	Varin I	7.7	60.6	2100	500	370
8	Varin II (pH=2.6)	7.7	∞	2800	280	2750
9	Varin III	7.7	39	2820	470	270

TABLE I: Fitted persistence length, length, coil size (obtained from the Kratky-Porod formula Eq. 11) and linear mass of the various sets of DNA data in light scattering experiments. This table is taken from A. Peterlin, Lichtzerstreuung an ziemlich gestreckten Fadenmolekulen, *Die Makromoleküläre Chemie* **9** 244-268 (1953). Other papers by Peterlin analysing the DNA light scattering data contain partial versions of this table. The polydispersity of DNA samples is not indicated. Data set 8 has a very short range and can only be fitted as a stiff rod, indicated by the value ∞ for $\mathcal{L}_p$. This data set is thus not counted in the statistics for the values of $\mathcal{L}_p$. The anomalously high linear density of the data set 8 is probably due to the self-association of DNA molecules in very acid (pH=2.6) ionic solutions. The chemical value of the linear mass density is around 100 [M/0.1 nm].

F. Historic impact

The number of citations of Peterlin's six papers on the persistence length determination of DNA amounts to only 185 [34] in the years following 1970. For the years previous to that, I was not able to obtain any citation data but I would assume it is safe to conclude that Peterlin's work on the persistence length of DNA was not widely appreciated. I do not find this particularly surprising since it was tailgating the veritable explosion of molecular biology that sprung from the epoch-making paper by Watson and Crick published in the very same year as the *Nature* paper by Peterlin.

It was only much later that measurements of persistence length of DNA became fashionable. This timeframe coincides almost exactly with the introduction of the new experimental method of optical tweezers into the physics of single molecules. Bustamante and his coworkers [35] in a remarkable series of physical manipulation experiments on DNA since 1992 made the DNA persistence length respectable again and launched it into the forefront of the single molecule physics. By measuring the force *vs.* extension curves for a single DNA molecule, chemically attached by one end to a glass surface and by the other end to a magnetic bead, they verified that the random thermal flopping of about 100-kb [36] double helix led to an "entropic elasticity", consistent over a thousand-fold range of force, with the elastic equation of state obtained from the Kratky-Porod model, also used by Peterlin in his extraction of the persistence length from light scattering experiments. They found out that it took about 0.1 pN to pull the ends of a DNA apart a distance of half its contour length. This 0.1 pN force scale comes from the energy associated with a thermally excited degree of freedom divided by the DNA persistence length, the contour length of DNA over which a single appreciable bend occurs. The main conclusion of this work is that for small stretching forces (double stranded) DNA behaves as a linear spring with a Hookes constant $k_{DNA} = 3k_BT/2\mathcal{L}_pL$, that is, inversely proportional to the length of the molecule (L) and its persistence length $\mathcal{L}_p$. A 10 μm DNA molecule, for example, has a spring constant of approximately 10^{-5} pN/nm.

This atomic force spectroscopy, as it came to be referred to, thus uses the same physics as Peterlin's analysis, except that it does not describe the scattering properties of light of a Kratky - Porod chain, but its elasticity. Bustamante and coworkers were able to fit the Kratky - Porod elastic equation of state derived by Marko and Siggia [37] with a persistence length of 50 nm, which is very close to the Peterlin's value. Later these experiments were repeated at various ionic conditions of the bathing solution leading to the measurement of a variation in the persistence length with *e.g.* ionic strength of the solution [38].

The Kratky - Porod wormlike chain model thus provided the means of accurate determination of the persistence length of DNA from both the light scattering as well as force spectroscopy experiments. By the advent of the force spectroscpy the light scattering technique of persistence length determination was out of date, since the latter gives a much better accuracy and does not have any drawbacks of the light scattering method. Recently however, Philip Nelson of the University of Pennsylvania and colleagues used high-resolution AFM to critically assess the applicability of the Kratky - Porod model to DNA in such contexts as how it recognizes and binds to other molecules, *e.g.* proteins, and also for the way it packs into cellular components or viral capsids [39]. In all these cases DNA has to bend substantially, contrary to soft thermally induced local bending that guides its behavior in light scattering or force spectroscopy experiments. In a series of experiments [40] they came to a conclusion that on a very short scale DNA is a lot more bendable then suggested previously by the Kratky - Porod model. Nelson and colleagues used high-resolution AFM to image the curvature in a large number of double-stranded DNA molecules over distances as short as 5 nm, about ten times less then the scale set by the persistence length. The molecules in their experiments were gently adsorbed onto a negatively charged mica surface with the help of small concentrations of $MgCl_2$. When they analysed the statistical frequency of various DNA conformations in the AFM images, the number of highly bent segments was much greater than predicted by the Kratky - Porod model. Their analysis on these length scales suggests that DNA elasticity in general does not follow Hookes law. Moreover, they were able to fit their data to a new general model that they have named the sub-elastic chain model which differs radically from the Kratky - Porod model.

The fact that the elastic energy at length scales much shorter than the persistence length does not obey Hooke's law and thus can not be described by the Kratky - Porod model does not disqualify it for describing light scattering or force spectroscopy experiments on single DNA molecules. On the length scales probed by these mathods, the details of the elastic properties of the segments are washed out by thermal fluctuations, and the molecule as a whole follows the predictions of the Kratky - Porod model. At much smaller scales, however, the effect of thermal fluctuations is small and it is possible to observe nonlinear elasticity that is not captured by the Kratky - Porod model. In solid and soft matter, there are numerous examples of effective energies depending on the length scale at which they are studied, so it is no surprise to find similar behaviour in semiflexible molecules such as DNA. The new model of DNA elasticity proposed by Nelson *et al.* implies that the elastic restoring force is constant when the molecule is bent on small length scales and is not proportonal to the local curvature as implied by the Kratky - Porod model. The constancy of restoring forces in this experiment is apparently a consequence of the thermodynamic equilibrium between two types of differently stretched links. In a similar fashion, the small-length-scale bending of DNA observed and quantified by Nelson *et al.* could be due to an equilibrium between DNA molecules with different values of the local bend angle.

One could thus conclude that Peterlin's breakthrough determination of DNA persistence length in 1953 was overshadowed by the birth of molecular biology, while the possible stronger impact of his work in later years was sidetracked by the introduction of new experimental techniques that gave not only a much more accurate value of the persistence length, but also elucidated the limits of the elastic model of DNA used so successfully by Peterlin. Nevertheless it should not be forgotten that he was the first one to come up with a reliable value for DNA persistence length that withstood the test of time.

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