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Calculation of the solution properties of flexible macromolecules: methods and applications

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Abstract While the prediction of hydrodynamic properties of rigid particles is nowadays feasible using simple and efficient computer programs, the calculation of such properties and, in general, the dynamic behavior of flexible macromolecules has not reached a similar situation. Although the theories are available, usually the computational work is done using solutions specific for each problem. We intend to develop computer programs that would greatly facilitate the task of predicting solution behavior of flexible macromolecules. In this paper, we first present an overview of the two approaches that are most practical: the Monte Carlo rigid-body treatment, and the Brownian dynamics simulation technique. The Monte Carlo procedure is based on the calculation of properties for instantaneous conformations of the macromolecule that are regarded as if they were instantaneously rigid. We describe how a Monte Carlo program can be interfaced to the programs in the HYDRO suite for rigid particles, and provide an example of such calculation, for a hypothetical particle: a protein with two domains connected by a flexible linker. We also describe briefly the essentials of Brownian dynamics, and propose a general mechanical model that includes several kinds of intramolecular interactions, such as bending, internal rotation, excluded volume effects, etc. We provide an example of the application of this methodology to the dynamics of a semiflexible, wormlike DNA.

Keywords Brownian dynamics · DNA dynamics · Flexible macromolecules · Monte Carlo method · Solution behavior

Introduction

Solution properties (hydrodynamic coefficients, scattering-related quantities, etc.) are among the main sources of information on the conformation of biological macromolecules. For a quasi-rigid macromolecule, the essential structural features are the dimensions and shape of the macromolecular particle. However, many macromolecules of biological relevance in solution are flexible entities and do not have a defined shape. For them, the essential structural feature is the flexibility itself, which may have an essential role in their function, and also determines their solution properties. During the last two decades, the extraordinary development of X-ray crystallography, structural NMR and cryo-electron microscopy has prompted the elucidation of the shape, even with atomic detail, of quasi-rigid molecules. Following these advances, the theory and methods for the calculation of hydrodynamic and other solution properties of rigid structures have evolved to cope with the level of complexity and detail revealed by the structural techniques (García de la Torre et al. 1994, 2000, 2001; Byron 1997, 2000; Spotorno et al. 1997; García de la Torre 2001; García de la Torre and Carrasco 2002).

However, many biological macromolecules present flexible conformations which do not have a defined shape. For them, the essential structural aspect is the type and extent of their flexibility that determines important aspects of their function, and is evidently reflected in their properties and behavior in solution. Synthetic polymers, which do not possess the richness of intramolecular, specific interactions that originate the peculiar shape of biological macromolecules, are very flexible macromolecular chains. The understanding of the solution behavior of flexible polymer chains was the

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aim of the pioneering works (Kirkwood and Riseman 1948; Riseman and Kirkwood 1949, 1956; Rouse 1953; Zimm 1956) in which concepts like bead models and hydrodynamic interaction were introduced and developed. Indeed, those concepts were later applied to and constitute the foundations of the hydrodynamics of rigid particles (Bloomfield et al. 1967; García de la Torre and Bloomfield 1977).

Some flexible biopolymers can be adequately represented by simple, classical models. One is the random coil, valid for fully flexible macromolecular chains. The other is the wormlike chain, which has a uniform, variable degree of flexibility and, depending on the value of the flexibility parameter, includes conformations intermediate between the limits of the random coil and the rigid rod. However, there are other biological macromolecules with more complex, specific kinds of flexibility (types and examples will be mentioned throughout this paper), and therefore it seems pertinent to develop theoretical formalisms and computational tools for these cases. Our intention is to describe methodologies that enable the calculation of solution properties of flexible macromolecules with arbitrary complexity. In this paper, we review briefly how flexibility can be modeled, and then we describe how to apply simulation methodologies, particularly the Monte Carlo and Brownian dynamics techniques, describing their computational schemes. Finally, we present some applications which are mainly intended to illustrate the practical aspects and possibilities of these techniques.

Theory and methods

Types of semiflexible macromolecules

The wormlike chain, in which flexibility is uniformly distributed through the whole contour length of the macromolecule, is one extreme (and extremely important) model for semiflexible macromolecules. Another important type is that of segmentally flexible macromolecules (Yguerabide et al. 1970; García de la Torre 1994), in which most regions within the macromolecule are essentially rigid, and flexibility is concentrated in a few, small portions that act as more or less flexible joints connecting rigid subunits. Between these two extreme kinds of semiflexible macromolecules, we will find in practice a variety of intermediate situations: for instance, multisubunit structures in which the connectors joining the rigid subunits are not localized hinges but appreciably long linkers that behave as flexible coils or wormlike chains. The methodologies described in this paper apply in general to any of these types of semiflexible entities.

Monte Carlo simulation

The Monte Carlo (MC) method is a simulation technique that generates conformations of the flexible particle using Boltzmann statistics associated with the internal energy, V . The probability of a conformation is given by the Boltzmann exponential, $e^{-V/k_B T}$, where k_B is Boltzmann's constant and T the absolute temperature. The properties of interest are evaluated for a sample containing a sufficiently large number of conformations, and the results are the averages over the sample. A general implementation of the MC simulation is the importance sampling procedure (Metropolis et al.

1953), in which, starting from a previous conformation, another is generated adding small, random displacements to the coordinates of the former. The potential energy, V' , is evaluated for the resulting conformation, and if $V' < V$ the conformation is accepted as the following one. Otherwise, a random number, u , with uniform distribution in the interval (0,1) is generated. If $u \leq e^{-(V'-V)/k_B T}$, the resulting conformation is also accepted, but otherwise the conformation is rejected and the following conformation is a copy of the previous one.

Special implementations of the MC procedure can be devised for specific cases. For instance, a freely rotating chain (Flory 1969), with fixed bond lengths and bond angles, can be easily generated, bond-by-bond, simply by choosing randomly the internal rotation angle. Frequently, the essential source of the intramolecular potential is the excluded-volume effect, which avoids interpenetration of the elements or subunits comprising the macromolecular model. Such is the case when there are no other interactions, or when other short-ranged interactions, such as bond lengths and angles, are constrained in the procedure. Then the Monte Carlo procedure may be very simple: conformations are generated in a fully random manner, and are checked for overlapping. Conformations in which elements overlap are ignored, and for the remaining ones the properties are calculated, and finally the results are the sample averages. An example of this case will be illustrated below.

The Monte Carlo procedure is strictly applicable to equilibrium properties, like the radius of gyration and the scattering form factor. For dynamic properties, it is employed in the so-called rigid body approximation (Zimm 1980; García de la Torre et al. 1982; Iniesta et al. 1988), in which the generated conformations are treated as if they were instantaneously rigid particles. For each conformation, the hydrodynamic properties are evaluated using the procedures proper of rigid particles and, like for the equilibrium properties, the results are just the sample averages. The Monte Carlo procedure is applicable to the translational coefficients (diffusion and sedimentation) and the intrinsic viscosity. For fully flexible, random-coil macromolecules, the Monte Carlo rigid-body treatment has been shown to reproduce accurately the theoretical and experimental results (see, for instance, García de la Torre et al. 1982; García Bernal et al. 1990, 1991). In the case of a flexible particle, there is no rotational diffusion; each part of the molecule reorients as a consequence of the internal motion proper of a nonrigid entity. The description of the reorientational dynamics is complex and depends on the time- or frequency-dependent property that is being experimentally observed. The dynamics of the molecule are governed by a series of relaxation times, τ_k , $k = 1, 2, \dots$. Still, there is a utility of MC simulation: the longest relaxation time, τ_1 , can be obtained by this procedure. Actually, this has been proven by employing the alternative technique of Brownian dynamics simulation (García de la Torre et al. 1994). This characteristic time can be observed in the long-time or small-frequency region of the observable properties. However, for a full characterization of the properties that depend on reorientational dynamics, the choice of simulation methodology will be Brownian dynamics.

A generalized bead-and-spring model

The basic building elements of the hydrodynamic models are spheres (beads), because the description of the friction at one element or the hydrodynamic interaction between them is less complicated when such elements are spherical. A convenient way to connect the beads is by means of springs with an associated potential energy and tension force that will depend on the elongation through a particular relationship. In the classical works on hydrodynamics of flexible-chain polymers, the bead-and-spring model was introduced by Rouse (1953) and Zimm (1956) (Yamakawa 1971; Doi and Edwards 1986). Thus a linear polymer chain can be modeled as a string of beads connected to their neighbors by more or less flexible springs.

This concept can be extended in a number of ways to treat a variety of effects such as intramolecular (excluded volume) interactions, branching, chain stiffness, electrical charges, etc. In an instantaneous conformation, the system has a potential energy

which is the sum of intramolecular contributions that are usually pairwise, and other external contributions that could be, for instance, due to an applied field. Thus V is the sum of intramolecular V_{ij}^{int} and external contributions V_i^{ext} :

$$V = \frac{1}{2} \sum_{i=1}^N \sum_{j=1}^N V_{ij}^{\text{int}} + \sum_{i=1}^N V_i^{\text{ext}} \quad (1)$$

and, associated with this potential energy, there is a force acting on each bead, \mathbf{F}_i , which similarly has internal $\mathbf{F}_{ij}^{\text{int}}$ and external contributions $\mathbf{F}_i^{\text{ext}}$:

$$\mathbf{F}_i = \sum_{j=1}^N \mathbf{F}_{ij}^{\text{int}} + \mathbf{F}_i^{\text{ext}} \quad (2)$$

There are a number of possible contributions; here we merely mention those that are most frequently employed:

1. Springs intended to describe the main connectivity. In a linear chain, each bead except those at the ends is connected to its two neighbors. However, there is no restriction in this sense, and other nonlinear structures (rings, branched topologies) can be described using more connectors between the beads. Depending on the kind of flexibility that is being represented, these springs may be of various types: Gaussian (as in the original Rouse-Zimm models), hard Hookean (Fraenkel 1952), finitely extensible (FENE) (Warner 1972), etc.
2. Interactions that depend on the angle subtended by two successive connectors joining three consecutive beads. These “angular springs” are intended to describe semiflexible structures: a wormlike chain (particularly, a moderately short DNA) can be modeled introducing a partial bending flexibility in this way (Hagerman and Zimm 1981b; Allison 1986), as we will show later on.
3. The concept can be indeed applied to molecules modeled with atomic detail, and in these cases, in addition to the two previous interactions that would correspond to fluctuating bond length and bond angles, we need to include also the internal rotation potential, and the corresponding forces, associated with three consecutive bonds that join four consecutive beads.
4. Interactions between elements that, being far apart along the chain contour, may be instantaneously close in space. This class of interactions describe the excluded volume effect, which in addition to the simple description (impenetrable elements) mentioned in the previous subsection, can also be described by continuous functions (for instance, the Lennard-Jones equation).
5. Interactions between molecular charges, which could model polyelectrolyte effects.
6. There may be a variety of external agents, such as electric fields, which can also be included.

The full description of this paradigm, which we name the “generalized bead-and-spring model”, with such a variety of interactions that, in turn, can be represented in different ways, is obviously out of the scope of the present paper. A more detailed description can be found elsewhere (Cifre 2000; manuscript in preparation). A schematic overview of the model is displayed in Fig. 1. This model can be treated by the Monte Carlo methods described above, particularly with the importance sampling procedure, to calculate equilibrium and (in an approximate manner) hydrodynamic properties. However, the full description of forces acting at each beads makes this model most suitable for an in-depth study of dynamic properties, which can be accomplished with the Brownian dynamics simulation technique and which is described next.

Brownian dynamics simulation

The Brownian dynamics (BD) simulation method is intended for the description of the Brownian motion of a collective of frictional elements, the beads, each of which interacts with all the others in the various ways described by the generalized bead-and-spring

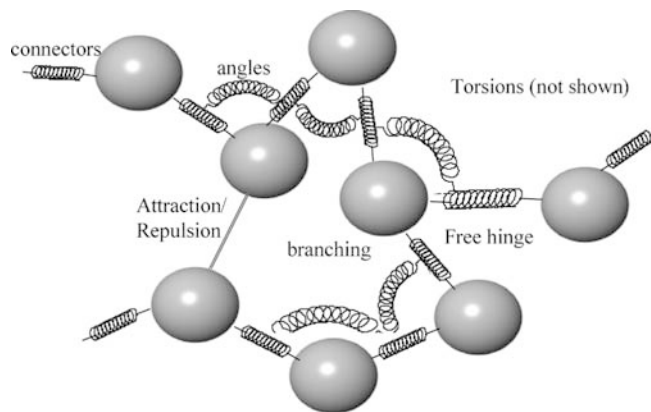


Fig. 1 Pictorial representation of the generalized bead-and-spring model, showing the various kinds of interactions

model. In addition to the deterministic effect of the internal and external interactions, each bead also experiences the peculiar, random influence of the solvent, which is typical of Brownian motion. Furthermore, the method can consider cases when the solution is not macroscopically at rest, but experiencing some type of flow, shearing, extensional, or of any other class. For the sake of brevity, we do not present a full description of Brownian dynamics theory and methodology, but we mention the essential aspects. In practice, the simulation procedure is based on an algorithm that generates a trajectory of the model, i.e. a series of positions (Cartesian coordinates) of the beads, along time. The procedure is discretized in time steps, Δt . From the position vector of each bead at time t_0 , \mathbf{r}_i^0 , the position vector at time t , \mathbf{r}_i , is evaluated adding some displacements, according to an expression of the form:

$$\mathbf{r}_i = \mathbf{r}_i^0 + [\Delta \mathbf{r}_i]^{(\text{forces})} + [\Delta \mathbf{r}_i]^{(\text{Brow})} + (\Delta t) \mathbf{v}_i \quad (3)$$

The term due to the forces requires the calculation of a force field and a $3N \times 3N$ mobility tensor. The term representing the Brownian displacement contains a diffusion tensor which is merely equal to the mobility tensor multiplied by a factor of $k_B T$. If there is a flow, we will have the final term, in which \mathbf{v}_i is the flow velocity at the point occupied by the i -th bead. All these quantities are evaluated at the initial position of the step, which must be sufficiently small so that they do not change appreciably over the step. Of course, the three contributions depend on the step duration Δt .

A well-known and simple algorithm is due to Ermak and McCammon (1978), which is a first-order solution of the stochastic differential equations that describe the fundamentals of this problem. Iniesta and García de la Torre (1990) have proposed an improved method which is of pseudo-second-order (Ottinger 1996) and has a greater computational efficiency.

The direct outcome of the BD simulation is the time series of bead positions, i.e. the Brownian trajectory. From this the properties of interest can be evaluated, usually in terms of the so-called correlation functions. A simple example is the determination of the translational diffusion coefficient, based on the famous Einstein expression for the square displacements of the center of mass, in a time t , from the position occupied at some initial time, $\mathbf{r}_{\text{cm}}(t_0)$, to that occupied when time t has elapsed, $\mathbf{r}_{\text{cm}}(t_0 + t)$:

$$\langle \mathbf{r}_{\text{cm}}(t_0) \mathbf{r}_{\text{cm}}(t_0 + t) \rangle_{t_0} = 6Dt \quad (4)$$

where $\langle \dots \rangle_{t_0}$ means the average over all the possible choices of the initial instant. Many other solution properties, from single-valued coefficients to more complex experiments like dynamic light scattering or transient birefringence, can be evaluated from the Brownian trajectory. A most interesting feature of the techniques is that it allows the simulation of the behavior of an individual molecule. Nowadays, this is of great importance due to the emergence of “single-molecule” techniques, including single-molecule hydrodynamics (Perkins et al. 1997).

Brownian dynamics is rather costly in terms of computing time. The operations that have to be carried out with the $3N \times 3N$ mobility tensor require a CPU time proportional to N^3 . The reason for the mobility tensor to have $3N \times 3N$ components is the hydrodynamic interaction (HI) effect: the displacements of all the N beads in the three directions are coupled. If HI could be neglected, such coupling disappears and the algorithm adopts a much simpler and faster form: without HI, the CPU time is just proportional to N . Unfortunately, HI is a must when one is calculating hydrodynamic properties, or in general something related to the time course of the molecular properties. However, if one is just interested in equilibrium (not dynamic) properties in steady-state conditions (for instance, radius of gyration and other molecular dimensions in a quiescent solution, static scattering, steady-state birefringence, etc.), Brownian dynamics without HI (BD-noHI) is useful and correct; indeed, the Brownian dynamics samples correctly the configurational space. Thus, BD-noHI is an alternative to (and in some situations may be more efficient than) standard MC simulations; some authors see BD-noHI as a *smart* Monte Carlo method (Rosky et al. 1978). On the other hand, Brownian dynamics with hydrodynamic interaction (BD-HI) has been proved to predict adequately the dynamic properties of fully flexible, random-coil macromolecules (see, for instance, Rey et al. 1989, 1991).

We are presently developing a new suite of computer programs, whose preliminary name is BROWFLEX, that will contain tools for both Brownian simulation and trajectory analysis. The project is ambitious, due to the diversity of interactions that can be included in the generalized bead-and-spring model, and the variety of properties and experimental situations that can be simulated. Some progress has already been done (Cifre 2000), and we will show an example here.

Applications

Model for a protein with two domains and a flexible linker: a Monte Carlo calculation

In order to illustrate how a MC procedure can be easily programmed and coupled to the programs in the existing HYDRO suite, we describe now an example which considers a particle having two rigid ellipsoidal subunits joined by a flexible linker chain. This model is representative of a number of proteins which possess two globular domains connected by a chain containing a certain number of residues that behave as a random coil. A schematic view of the model is presented in Fig. 2.

The computer program HYDROSUB (García de la Torre and Carrasco 2002) is available for the calculation of solution properties of particles composed by ellipsoidal and cylindrical subunits, which are modeled as shells of “minibeads”. The program repeats the calculation for various decreasing values of the minibead radius σ , and the results are obtained by extrapolation to the shell model limit of $\sigma = 0$. In the latest version of the program we have added a new feature: the model may contain some additional spheres that are considered as beads in the strict sense (Carrasco and García de la Torre 1999), represented as mere spheres of some given, fixed radius, that are not shell-modeled. In some regard, the new version is a hybrid of the previous HYDROSUB and the HYDRO (García de la Torre et al. 1994) programs.

Figure 3 shows typical input data files for the HYDROSUB calculation. Many structures or conformations

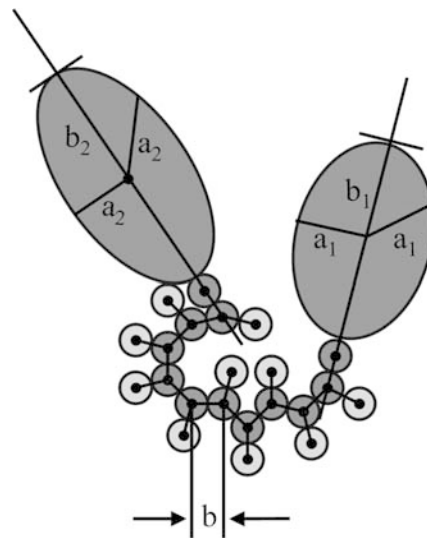


Fig. 2 Two-dimensional schematic representation of the model with two rigid subunits connected by a flexible, random-coil chain

can be processed in a single run. The file hydrosb.dat contains, for each case, the primary data of the solute/solvent system and the name of the structural file. The structural files, here named conform-xxx.txt, where xxx goes from 1 to the number, n_{conf} , of conformations, contain the data (size, position, and orientation) pertaining to the two ellipsoids, and the Cartesian coordinates and radii of the non-extrapolated spheres. In the HYDROSUB output, apart from a detailed results file for each conformation, we have a file, summary.txt, organized in columns, one for each property, containing the values of the solution properties calculated for the n_{conf} conformations. This file can be easily exported to a spreadsheet for posterior calculations, like the averages and other statistics proper of the Monte Carlo method.

Regarding now the physical details, the flexible linker in our model is a flexible array having a total of N_{tot} beads, and is intended to represent a polypeptide chain in a random coil conformation. The skeleton of the connector is a freely jointed chain (Flory 1969; Cantor and Schimmel 1980) constituted by N_{skel} tangent spheres of radius r , so that the length of the segment that connects the center of two contiguous beads is $b = 2r$. All the beads except the terminal ones have another neighbor, tangent bead (in addition to their neighbors in the skeleton), that is intended to represent an amino acid side chain, and for simplicity we assume that their radius is the same. There will $N_{\text{side}} = N_{\text{skel}} - 2$ side beads, and the total number of beads is $N_{\text{tot}} = N_{\text{skel}} + N_{\text{side}} = 2N_{\text{skel}} - 2$. The excluded volume condition is imposed on this chain: nonbonded spheres cannot overlap. The two subunits are represented by revolution ellipsoids with semiaxes (a_1, a_1, b_1) and (a_2, a_2, b_2) , placed at the ends of the chain, which are intended to represent two globular protein domains. For convenience, we suppose that the orientation of the symmetry axes of the two ellipsoids

Fig. 3 Files needed for a series of HYDROSUB calculations for multiple (100) conformations. Only the first and last blocks of hydrosb.dat, for the two first and last conformations, are displayed. We also show the first lines of one of the conformxxx.txt. The first three lines contain the data pertaining to the two ellipsoids (as in the previous version of HYDROSUB); in the next line, 38 is the number of extra beads in the connector, and the following 38 lines contain their coordinates and radii

File hydrosb.dat

```

conform001                                !Name of conformation
conform001                                !Main results file (OUTPUT)
conform001.txt                             !structure file (INPUT)
5,                                          !NSIG
1.3,                                       !Minimum radius of beads in the shell (SIGMIN)
3.0,                                       !Maximum radius of beads in the shell (SIGMAX)
293.,                                     !T (temperature, K)
0.01,                                     !ETA (Viscosity of the solvent in poises)
53107.,                                   !RM (Conformular weighth)
0.720,                                    !Partial specific volume, cm3/g
1.0,                                      !Solvent density, g/cm3
1                                          !IDIF
.....
etc.....
.....
conform100                                !Name of conformation
conform100                                !Main results file (OUTPUT)
conform100.txt                             !structure file (INPUT)
5,                                          !NSIG
1.3,                                       !Minimum radius of beads in the shell (SIGMIN)
3.0,                                       !Maximum radius of beads in the shell (SIGMAX)
293.,                                     !T (temperature, K)
0.01,                                     !ETA (Viscosity of the solvent in poises)
53107.,                                   !RM (Conformular weighth)
0.720,                                    !Partial specific volume, cm3/g
1.0,                                      !Solvent density, g/cm3
1                                          !IDIF
*                                          END-OF-FILE MARK

```

File conform001.txt

```

2
1      0.0      0.0 -29.3      0.0      0.0  27.0  18.0
1 -20.8  29.0  25.2  84.6  105.5  36.0  18.0
38,
  0.000,    0.000,    0.000,    1.900,
  0.000,    0.000,    3.800,    1.900,
 -3.482,   -0.550,    2.382,    1.900,
 -4.249,   -3.857,    4.091,    1.900,
 -1.696,   -6.552,    3.281,    1.900,
 -2.682,  -10.163,    3.937,    1.900,
  ....
  ....
  ....

```

coincide with those of the segment joining the first and the last pairs of beads, respectively, as indicated in Fig. 3.

The construction of the models or, in practical terms, writing a separate computer program that generates conformations of the model (producing the files to be later used by HYDROSUB), is the user's task. In our simple model, the coordinates of the flexible chain are generated following the statistics of the freely jointed chain. With the first sphere placed at an arbitrary point, the coordinates of the following skeletal spheres are obtaining by adding to those of the previous one the segment vector ($b\sin\theta_i\cos\phi_i$, $b\sin\theta_i\sin\phi_i$, $b\cos\theta_i$), where $\cos\theta$ is an uniformly distributed random uniform number in $(-1, +1)$ and ϕ is an uniformly distributed random number in $(0, 2\pi)$. The side beads are located on the external part of the bisector of the angle subtended by a skeletal bead and its two skeletal neighbors. The position of the centers and the orientations of the ellipsoids are located in such a way that they are tangent to the

first and last skeletal beads, and their symmetry axes are aligned with the first and last segment in the chain. The excluded-volume condition is fulfilled by checking, using geometric relationships, whether there is overlap between the two ellipsoids, between any of the ellipsoids and the spheres in the chain, or between the chain spheres themselves. In this simple version of the Monte Carlo procedure, conformations with overlapping are rejected, and the others are accepted. In this way, a sufficiently large number of conformations are generated. We have written a computer program, MONTE-SUB, which implements all the generation procedure and writes the files needed for HYDROSUB. We have attempted to make the MONTESUB program, which is available as a FORTRAN 77 source code, well structured so that it shows the main aspects of the Monte Carlo scheme. Of course, the program has modules (FORTRAN subroutines), specific for the present example, that can be replaced by others, still keeping the structure of the program and some parts of the code

useful for a different application. As mentioned, a single run of MONTESUB carries out all the generation of conformations and generates the files (Fig. 3) that are next used in a single run of HYDROSUB to compute the properties for each of them, that are written to file summary.txt. The final results for the observable properties are the averages over the individual properties for each conformation. This is simply accomplished by opening the file summary.txt with a spreadsheet, with which the averages and other statistical operations can be easily made.

The data that we employ may be typical of what one would expect for this type of two-segment, semiflexible protein, but do not correspond to any specific case. We take $a_1 = 18$ Å, $b_1 = 27$ Å, $a_2 = 18$ Å, and $b_2 = 36$ Å. This way, the volumes of the subunits are 3.64×10^{-20} cm³ and 4.88×10^{-20} cm³. These hydrodynamic volumes correspond to the hydrated volume of the protein domains. For typical values of the partial specific volume, $\bar{v} = 0.72$ cm³/g, and the degree of hydration, $\delta = 0.3$ g/g; we have molecular weights of 21.7 and 29.0 kDa, respectively, which correspond to about 183 and 244 amino acid residues in each domain. In this hypothetical example, the linker contains 18 amino acids. The chain in the model has 20 skeletal beads and 18 lateral ones. The connector length is identified with the distance between neighbor C $^\alpha$ atoms, so that we take $b = 3.8$ Å. Hanging from all the beads except the terminal ones we have 18 beads that represent the side chains. A summary of the files employed in the calculations is shown in Fig. 3, and a snapshot of the molecule is displayed in Fig. 4. The root mean square length of our linker chain model (obtained from separate Monte Carlo simulation) is $\langle r^2 \rangle = 892$ Å². For a real polypeptide chain, this value is given by $\langle r^2 \rangle = C_\infty (N_{\text{skel}} - 1) b^2$, where the characteristic ratio, C_∞ , is a number not too far from unity that depends on the sequence; typical values are 2.2

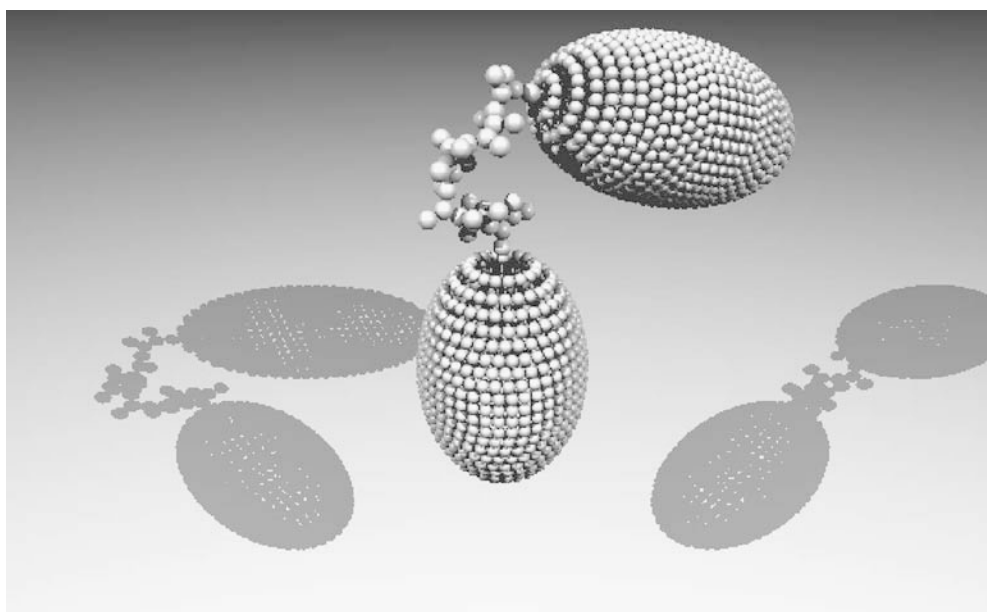
for polyglycine and 9.3 for poly(L-alanine). The result for $\langle r^2 \rangle$ of our model chain corresponds to that for a polypeptide chain with $C_\infty = 3.6$, which seems reasonable. The fully extended length of the chain would be 65 Å, which is rather close to that of a polypeptide chain having 18 residues. In the MONTESUB run we generated 100 valid conformations; one of them is displayed in Fig. 4. In order to obtain an estimate of the statistical error of the final averages, the set of individual results contained in summary.txt was divided into five subsets, each with 20 conformations, obtaining for each the mean values. The average of the five means is the final result, and from the standard deviation we obtain the errors. The calculated results are: radius of gyration, $R_g = 12.8 \pm 0.4$ Å; translational diffusion coefficient, $D_{20,w}^0 = (6.18 \pm 0.07) \times 10^{-7}$ cm²/s; sedimentation coefficient, $s_{20,w}^0 = 3.77 \pm 0.03$ S; intrinsic viscosity, $[\eta] = 5.50 \pm 1.4$ cm³/g; and longest relaxation time, $\tau_1 = 82 \pm 3$ ns.

Our model is intentionally simple in order to emphasize the methodology rather than the model itself, and the data are somehow fictitious. Of course, more complex models may require more elaborated programming and parameterization, but we hope that the present example shows clearly the scheme to be followed for a Monte Carlo calculation based on the HYDRO programs.

Brownian dynamics simulation of a moderately short (144 bp) DNA molecule

When the contour length, L , of a duplex B-DNA molecule is of the order of (not much larger than) the persistence length $P \approx 500$ Å, the DNA adopts the typical wormlike conformation of the Kratky-Porod model. In the original model the curvature is continuous, but an

Fig. 4 Snapshot of the model with two rigid subunits connected by a flexible, random-coil chain, showing the shells that represent the ellipsoidal subunits. Note that the chain is modeled as a string of beads of fixed radius, which is the same while the minibead radius in the shells is varied



acceptable discontinuous version of the wormlike chain (Schellman 1974; Hagerman and Zimm 1981b; Allison 1986; Schellman and Harvey 1995) can be made as a chain of N elements and $N-1$ bonds of length b . Each element will be regarded as a sphere of radius $b/2$, so that the contour length is $L = Nb$. BD algorithms that constrain distances between elements are available (Allison and McCammon 1984), but we and other authors (Allison 1986) prefer an alternative [and more efficient (Cifre 2000)] representation, in which bonds are represented by stiff springs, so that the bond length presents small fluctuations around the equilibrium value, b_{equil} . The force constant for the connector, \mathbf{b}_i , which joins beads i and $i+1$, is $\mathbf{F}_i = Q_b(b_i - b_{\text{equil}})(\mathbf{b}_i/b_i)$, where Q_b is the Hookean constant, for which a sufficiently large value is $Q_b = 100k_B T/b_{\text{equil}}$.

The partial bending flexibility is the second part of the mechanical model that is simulated by BD. In the discrete version of the wormlike chain, the angle subtended by two successive bond vectors, \mathbf{b}_{i-1} and \mathbf{b}_i , is given by $\cos \alpha_i = (\mathbf{b}_{i-1} \cdot \mathbf{b}_i)/b_{\text{equil}}^2$. This restricted bending flexibility can be represented in the generalized chain by a potential associated with each of three consecutive beads, $i-1$, i , and $i+1$, which for a rather stiff chain can be a quadratic form, $V_i^{\text{bend}} = \frac{1}{2} Q_\alpha \alpha_i^2$, depending the deviation angle α_i^2 from the equilibrium value $\alpha_i^{\text{equil}} = 0$ (with $i=2, 3, \dots, N-1$). The bending forces $\mathbf{F}_i^{\text{bend}}$ have a complex aspect and we refer the reader to the original literature (Díaz and García de la Torre 1988; Iniesta et al. 1991). The relationship between the constant of the angular springs and the persistence length of the wormlike chain is (Schellman 1974):

$$Q_\alpha = Pk_B T/b_{\text{equil}} \quad (5)$$

For practical work, one will decide the number of beads, N , in the model, which cannot be large because, as commented above, the CPU time for BD with HI grows as N^3 . The equilibrium bond length is obtained from the contour length as $b_{\text{equil}} = L/N$. Then, knowing the persistence length P , the bending parameter Q_α is obtained from Eq. 5. Thus, DNAs with any number of base pairs can be modeled and simulated with chains with a reasonably small number of beads (Lewis et al. 1988; Allison et al. 1990).

In the present example, we are interested in a quite short B-DNA and we proceed in a different manner. The DNA double helix is acceptably represented by a cylinder (straight or curved, depending on the length) with a diameter, d_{cyl} , of about 20 Å. In Brownian dynamics we cannot represent the continuous surface of a cylinder, but instead we employ spherical beads. For the representation of short DNAs, following Hagerman and Zimm (1981b), the cylinder is replaced by a string of nearly touching beads, whose diameter d is the same as the (equilibrium) distance between neighbors, b_{equil} . As the string of beads must have the same contour length and the same volume as the cylinder, the two conditions give $b_{\text{equil}}^2 = 6d_{\text{cyl}}^2/4$, or approximately, $b_{\text{equil}} = 1.22d_{\text{cyl}}$,

with $b_{\text{equil}} \approx 24.5$ Å for B-DNA. We will simulate a model chain with $N=20$ beads, and therefore the contour length is 490 Å, which corresponds to about 144 base pairs. The ratio of the contour length to the persistence length is $L/P \approx 1$, so that the conformation is a typical, weakly bending rod.

A sufficiently large constant for the spring connectors is $Q_b = 100k_B T/b_{\text{equil}}^2$, which gives $Q_b = 67.3 \times 10^{-3}$ J/cm² at a temperature of 20 °C. With this value the connectors are rather stiff: the root-mean-square fluctuation in their length is about 5%. The bending constant is determined by Eq. 5, which for $P=500$ Å and $b_{\text{equil}} = 24.5$ Å gives $Q_\alpha = 8.25 \times 10^{-20}$ J/rad². (Parenthetically, we note that our Brownian dynamics program works internally with reduced quantities: energy is expressed in units of $k_B T$, length in units of b_{equil} , and time in units of $\zeta b_{\text{equil}}^2/k_B T$, where $\zeta = 6\pi\eta_0 b_{\text{equil}}/2$ is the frictional coefficient of the beads, and η_0 is the solvent viscosity (we assume water at 20 °C). In reduced form, the values for the parameters are $b_{\text{equil}}^* = 1$, $Q_b^* = 100$ and $Q_\alpha^* = 20.4$.

The total time of the BD trajectory was 342 μs, composed of 50 million steps of duration $\Delta t = 6.84$ ps (in reduced form $\Delta t^* = 0.0002$), which is suitable for stiff springs. Some snapshots of the model taken along the

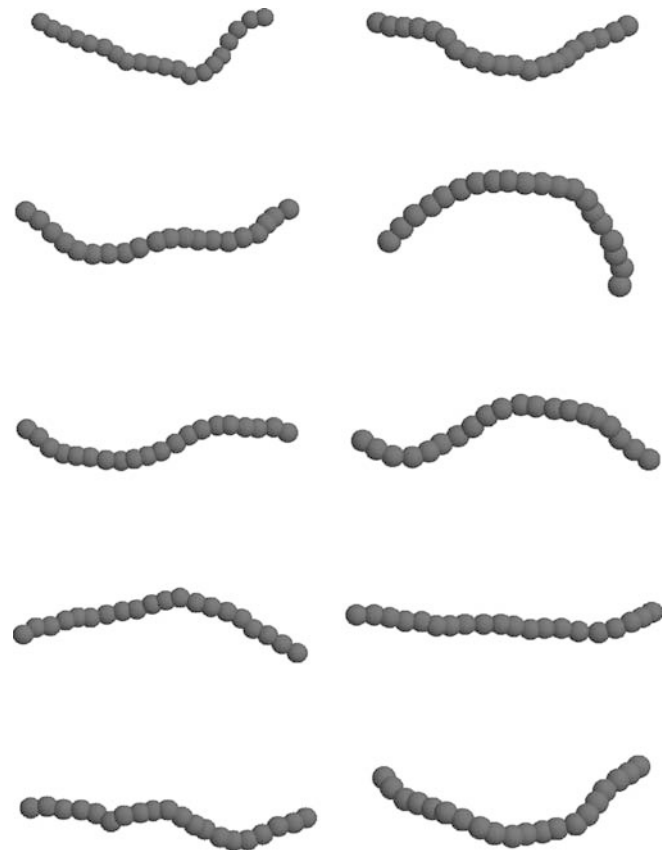


Fig. 5 Snapshots, taken at regular times, of the 20-bead chain that represents the 144-bp DNA. The viewpoint is moved so as to show the maximum extension of the molecule or some features like sharp bends

trajectory are shown in Fig. 5. We note the typical wormlike aspect of the chain, with an appreciable curvature, and even with some sharp bends. Primary information that can be extracted from the trajectory is that corresponding to the macromolecular dimensions. Thus we find the mean-square end-to-end distance and radius of gyration, $\langle r^2 \rangle = 1.69 \times 10^{-11} \text{ cm}^2$ and $\langle s^2 \rangle = 1.72 \times 10^{-12} \text{ cm}^2$. These results can be compared with the exact ones, available from simple formulas for the wormlike chain in terms of L and P (Bloomfield et al. 1974, 1999), and the agreement is within a few percent. Note that the dimensions deviate appreciably from those of a straight rod of the same L , which would be $\langle r^2 \rangle = L^2 = 2.50 \times 10^{-11} \text{ cm}^2$ and $\langle s^2 \rangle = L^2/12 = 2.09 \times 10^{-12} \text{ cm}^2$.

As an example of the dynamic information that can be obtained from the BD-HI trajectory (the above dimensions could have been obtained in a BD-noHI run), we first obtain the correlation function for the r.m.s. displacement of the center of mass using Eq. 4. The results are shown in Fig. 6A. From the slope of the right-hand-side of Eq. 4 versus time, the translational diffusion coefficient, D_t , is obtained from the slope, $6D_t$, which gives $D_t^{20,w} = 3.25 \times 10^{-7} \text{ cm}^2/\text{s}$. This value agrees well with the experimental values for sedimentation coefficients (Kovacic and Van Holde 1977) of DNAs in this range of contour length.

Transient electric birefringence (TEB) decays at low field strengths, as well as all the properties related to the rotational and internal mobility of the semiflexible molecule, can be extracted from the Brownian trajectory. Another relevant quantity is the correlation function for

depolarized dynamic light scattering (DDLS) (Berne and Pecora 1976). It is given by:

$$C_d(t) = \left\langle \frac{1}{(N-1)^2} \sum_{i=1}^N \sum_{j=1}^N P_2(\mathbf{u}_i(t_0) \cdot \mathbf{u}_j(t_0 + t)) \right\rangle_{t_0} \quad (6)$$

where \mathbf{u}_i is a unitary vector along the bond from i to $i+1$.

It has been demonstrated that the normalized decay of DDLS is identical to that of low-field TEB when the electrical orientation mechanisms is of a certain type. We avoid here any discussion of that complex problem, recalling that one expects a common set of relaxation times that is a characteristic, inherent property of the molecule, for all of these related properties (Roitman and Zimm 1984a, 1984b; Díaz et al. 2000; Pérez Sánchez et al. 2002). Other interesting functions are of the type:

$$C_b(t) = \langle P_2(\cos \theta_b(t_0, t_0 + t)) \rangle_{t_0} \quad (7)$$

where θ_b is the angle subtended by two successive orientations of a characteristic vector, for which we choose the central bond in the chain (between beads 9 and 10). This function is related to the fluorescence anisotropy decay. Although the calculation of the successive relaxation times from the time-dependent functions is practically impossible, one may try to extract at least the longest relaxation time τ_1 that governs the long-time behavior or the time dependence: the decay is fitted to a multi-exponential using the DISCRETE procedure (Provencher, 1976a, 1976b), which has been implemented in our analysis programs for this purpose. DISCRETE provides the optimum number of exponentials, and we collect the value of τ_1 which should be the same for DDLS, TEB, etc. (García de la Torre 1994). DISCRETE is not the only algorithm for multi-exponential data; other procedures are available, but they all suffer, to a lesser or greater extent, the defect of being somehow uncertain when the number of parameters to be determined is large (say, for three or more exponentials), and even for a bi-exponential when the relaxation times are not widely different. However, in our experience it gives reliable estimates of, at least, the longest relaxation time, which is the most relevant parameter, τ_1 . Furthermore, the DISCRETE code can be easily embodied within our program. In Fig. 6B we display the results for the two correlation functions. The DDLS function $C_d(t)$ is practically monoexponential, while $C_b(t)$ is biexponential, and DISCRETE gives two amplitudes a_1 and a_2 and two relaxation times τ_1 and τ_2 . The fastest time is less important, and, as shown by our results, it would be difficult to detect experimentally. The important quantity is the longest relaxation time, τ_1 , which corresponds to the end-over-end tumbling of the molecule. It is noteworthy that [as predicted in general for semiflexible particles (García de la Torre 1994)] τ_1 is the same for $C_b(t)$ as for $C_d(t)$, as shown in Fig. 6B where we see that the slope of the long-time behavior is

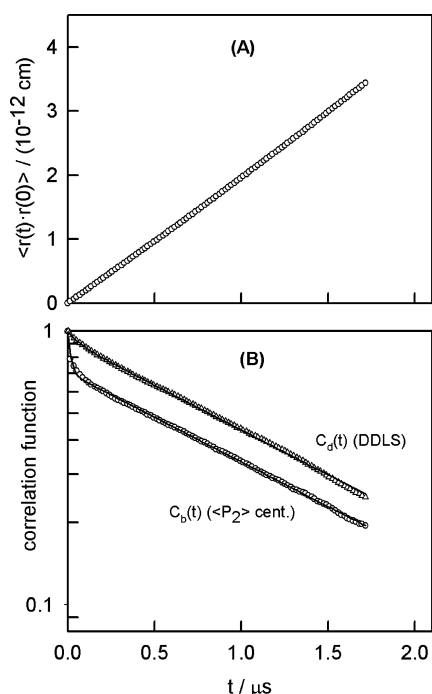


Fig. 6A Einstein-law plot of the r.m.s. displacement of the center of mass of the 144-bp DNA. **B** Time-dependent DDLS correlation function for the 144-bp DNA

the same for the two functions. From the DISCRETE fits we obtain $\tau_1 = 1.32 \mu\text{s}$. This value is in reasonable agreement with the TEB relaxation times determined by Hagerman and Zimm (1981a). Indeed, we have not tuned carefully the model parameters, because, as in the previous application of the Monte Carlo method, we have studied the dynamics of this DNA fragment exemplarily, with the main purpose of illustrating the use of the BD methodology.

Other applications

For the sake of brevity we have illustrated in detail only the two previously described applications. However, it is clear that any other type of partial flexibility can be studied using either the Monte Carlo rigid-body hydrodynamics or Brownian dynamics simulations. Such is the situation for segmentally flexible macromolecules, for which there is a basic theory, developed by Wegener (1980, 1982) and Harvey, García de la Torre and co-workers (Harvey and Cheung 1980; Harvey and García de la Torre 1980, Mellado et al. 1988), and for which some methodologies have been developed (García de la Torre et al. 1994; Spotorno et al. 1997). Programs, perhaps specific for each case under study, can be developed, following the example of MONTESUB, to generate Monte Carlo sample of conformations, with output prepared for the HYDRO suite of programs. Brownian dynamics of a beads-and-springs model can also be employed, representing each rigid subunit as a subset of beads, which would be joined by very stiff linear and angular springs, and joining the subunits with springs of variable flexibility. Examples already described in the literature include a hinged, broken rod (Iniesta et al. 1991) and a four-subunit model of antibodies (Díaz et al. 1990).

Computer programs

The HYDRO suite of computer programs, including the new version of HYDROSUB, is available from our web site <http://leonardo.fcu.um.es/macromol>. The source code of the program MONTESUB, which shows how the Monte Carlo can be implemented, and serves as a template to program similar problems, is also available from the same address. BROWFLEX is still under development and the first version will be released soon.

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