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ORIGINAL PAPER

Hydrodynamic multibead modeling: problems, pitfalls, and solutions. 1. Ellipsoid models

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Abstract The shape of macromolecules can be approximated by filling models, if both hydrodynamic and scattering properties should be predicted. Modeling of complex biological macromolecules, such as oligomeric proteins, or of molecule details calls for usage of many beads to preserve the original features. However, the calculation of precise values for structural and hydrodynamic parameters has to consider many problems and pitfalls. Among these, the huge number of beads required for modeling details and the choice of appropriate volume corrections for the calculation of intrinsic viscosities are pestering problems to date. As a first step to tackle these problems, various tests with multibead models (ellipsoids of different axial ratios) were performed. The agreement of the predicted molecular properties with those derived from whole-body approaches can be used as evaluation criteria. Modification of previously available versions of García de la Torre's program HYDRO allows hydrodynamic modeling of macromolecules composed of a maximum of about 11,000 beads. Moreover, application of our recently suggested "reduced volume correction" enables a fast and efficient anticipation of intrinsic viscosities. Correct parameter predictions were obtained for all models analyzed. The data obtained were compared to the results of calculations based on HYDRO programs available to the public. The calculations revealed some unexpected results and allowed founded conclusions of general importance for precise calculations on multibead models (e.g., the requirement of calculations in the double-precision mode).

Keywords Multibead models · Modeling problems · Volume corrections · Parameter predictions · Hydrodynamics

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Introduction

Size, shape, and other solution properties of simple and complex macromolecules or particles of arbitrary shape can be calculated using bead modeling procedures. These approaches, in general, are applied in context with the use of hydrodynamic, scattering, and other solution techniques (García de la Torre et al. 2000; Zipper et al. 2005; Byron 2008). Size and shape of the particle under analysis are approximated by an assembly of spherical elements ("beads") for which profiles and molecular parameters can be computed. Frequently, the results obtained are compared with data obtained by applying simple whole-body (WB) approaches (spheres, prolate and oblate ellipsoids, triaxial ellipsoids etc.).

In the case of hydrodynamic modeling, several bead modeling strategies have been developed (Carrasco and García de la Torre 1999; García de la Torre et al. 2000; Byron 2008). The only condition which must be fulfilled is that the overall size and shape of the bead model must be as close as possible to that of the original particle. This condition is generally already realized (i) by using a relatively small number (N) of beads of quite different size ("bead

modeling in a strict sense") or, more sophisticated, (ii) by a shell characterizing the particle surface by many small beads ("minibead modeling", "shell modeling"). The computer programs HYDRO (García de la Torre et al. 1994) and the more recent version HYDRO++ (García de la Torre et al. 2007) calculate bead models in the strict sense and allow the prediction of several structural and hydrodynamic parameters. The program HYDROPRO (García de la Torre et al. 2000) calculates parameters on the basis of a shell model and, again, allows the anticipation of parameters. The latter approach is preferably used when the information on the atomic-level structure is exploited. In the case of proteins, the atomic coordinates are stored in several data bases such as the Protein Data Bank (Berman et al. 2000), usually in the form of PDB files.

When striving concurrently for both hydrodynamic and scattering parameters, a "filling model" strategy (using all beads, equal- or unequal-sized) has to be used (Zipper et al. 2005), since the prediction of scattering properties requires usage of all constitutive elements (electrons in the case of X-rays). On the other hand, if only hydrodynamic quantities are desired, from the theoretical point of view, shell modeling approaches are more rigorous, e.g., by avoiding overlapping of beads (García de la Torre et al. 2000).

Progress in many fields of science now yields novel modeling challenges: accessibility of precise atomic coordinates of thousands of biomacromolecules (preferably of proteins), development of computation approaches even for large complex particles from the atomic coordinates, existence of high-tech computers, availability of new theoretical approaches and computer programs including automated modeling procedures, etc. The combination of these new possibilities provides the opportunity of comparing and testing the information from high-resolution techniques (crystallography, NMR, 3D reconstructions from cryo-electron microscopy) and low-resolution techniques (small-angle scattering, analytical ultracentrifugation, viscometry etc.), on the one hand, and predicting molecular parameters not accessible to experimental investigation, on the other.

However, leaving the field of classical hydrodynamic bead modeling (in general, N not exceeding 100), and applying the above-mentioned promising tools for modern hydrodynamic modeling to many-bead problems (e.g., retrieval of models from the atomic coordinates of complex proteins), we are confronted with several serious problems (Zipper et al. 2005; Zipper and Durchschlag 2007), because precise and realistic models may consist of several thousands of minibeads of unequal size, and overlapping to some degree. Currently, calculations with standard personal computers are restricted to models consisting of less than about 11,000 beads.

Application of these advanced modeling possibilities, however, requires consideration of several precautions and pitfalls and development of adaptations and improvements of the existing evaluation programs. Recently, we have tested the reduction approaches developed earlier in a systematic manner with two selected proteins, the small protein lysozyme and the huge capsid of the phage fr and its trimeric building block (Zipper and Durchschlag 2007). A factor of about 100 turned out to be the maximum factor for the reduction of multibead (MB) models. Furthermore, a new type of volume correction, a reduced volume correction (RVC), was tentatively suggested for the calculation of intrinsic viscosities of the two proteins under analysis. The most intriguing problem which remained was concerned with the question if the suggested approaches, in particular those for the RVC, may be applied to anisometric particles equally well.

The present study is concerned with a critical assessment of the accuracy of parameter predictions with respect of structural and hydrodynamic properties of two- and three-axial model ellipsoids of different axial ratios. For this purpose, the data obtained by means of HYDRO and/or HYDRO++ for multibead models of the ellipsoids were compared to the outcome of whole-body approaches based on in-house programs (for spheres and ellipsoids of revolution; Durchschlag and Zipper 1997) or ELLIPS2 (Harding et al. 1997). In the following paper, the findings of this study were applied to several selected proteins exhibiting quite different molecular features.

Methods

Models

As appropriate whole-body models, the sphere (S), prolate, and oblate ellipsoids of revolution (PE, OE), and triaxial ellipsoids (POE) were chosen. The axial ratios (a:b:c) of the ellipsoids were varied systematically to obtain different particle symmetries. The selected geometrical bodies were modeled by assemblies of equal-sized densely packed beads. The coordinates of the beads of the respective models were generated by an in-house program. Thereby, the radii of the beads (r_b) were varied systematically, in order to generate models of different bead numbers (N), all smaller than the maximum value (N_{max}) allowed by the program HYDRO; the appropriate range of r_b values was chosen separately for each particle geometry. This approach represents a special type of model reduction. The models obtained were visualized by RASMOL (Sayle and Milner-White 1995). As a consequence of varying r_b values, the resultant overall volumes, V, differ slightly, whereas the other calculated parameters should coincide,

provided the evaluation procedures are correct. In the following, the most important structural and hydrodynamic parameters (radius of gyration, R_G, translational diffusion coefficient, D, sedimentation coefficient, s, and intrinsic viscosity, $[\eta]$) were calculated and the values obtained for different input variables were critically compared.

Calculation of parameters

The structural parameter R_G and pair-distance distributions, $p(r)$, of the models were calculated from the radii and coordinates of the constituent beads, using algorithms described earlier (Glatter 1980; Glatter and Kratky 1982). Hydrodynamic parameters (D, s, $[\eta]$) were computed by means of modified versions of HYDRO (García de la Torre et al. 1994) or by HYDRO++ (García de la Torre et al. 2007). Precompiled executables of the latter program (hydro++8cmsd.exe and hydro++8cg77.exe) were downloaded from http://leonardo.fcu.um.es/macromol. Our versions of HYDRO are based on the Fortran source code downloaded previously as file hydrod13.f (HYDRO version 13, dated June 1999) from the same web site. We adapted the program for our purposes only by modifying the sections handling the data input and output and the volume correction of intrinsic viscosity, and by enlarging appropriately the parameter NDIM that is related to N_{max} and governs the dimensions of arrays. Executables were created preferably by means of Lahey LF95 compilers. Double-precision versions were generated from the single-precision code by insertion of IMPLICIT statements or by using an appropriate compiler option.

Application of hydrodynamic modeling approaches requires consideration of several problems, pitfalls and precautions:

(i) The interaction of overlapping beads of unequal size necessitates application of our ad hoc expression for the interaction tensor (Zipper and Durchschlag 1997, 1999; Carrasco et al. 1999). This special case applies, e.g., if bead models should reflect exact copies of crystal structures (e.g., of proteins). As appropriate, recent versions of HYDRO or HYDRO++ take into account this tensor.

(ii) Modeling the fine structure of particles or complex macromolecules built up from many constituents demands use of a multitude of beads. This imposes modifications of the program HYDRO to enable handling and processing of huge bead numbers (N up to more than 10,000).

(iii) If necessary, a data reduction of an enormous number of beads can be achieved by special approaches (running mean, cubic grid, hexagonal grid) (Zipper and Durchschlag 2007). For example, in the case of giant proteins such reduction procedures are necessary if realistic models based on crystallographic data are desired.

(iv) Calculation of rotatory quantities and, in particular, of values for intrinsic viscosities, $[\eta]$, turns out to be a crucial problem for hydrodynamic modeling. Several procedures have been suggested to calculate this intricate quantity, applying different extents of so-called volume corrections (García de la Torre and Carrasco 1998; García de la Torre et al. 2007): Application of "no volume correction" (NVC) usually underestimates the true value for $[\eta]$, whereas the "full volume correction" (FVC), which is based on the total volume V of all N beads, generally leads to too high values for $[\eta]$. The inadequacy of this situation led to search for improvements of volume corrections. In this context, an "adjusted volume correction" (AVC) which takes into account the geometry of the bead model (García de la Torre et al. 2007), and the "cubic substitution" (CBS) approach (Wilson and Bloomfield 1979; García Bernal and García de la Torre 1981; García de la Torre and Bloomfield 1981) were suggested; both approaches are implemented in HYDRO++. The CBS approach was claimed to yield the most reliable results for filling models (García de la Torre et al. 2007) but its application is restricted to models of low bead numbers because the approach implies the replacement of each bead of the model by a cubic array of eight smaller beads. In Fig. 1 this is illustrated for a selected example (S). Recently, we have suggested another alternative: in our modified versions of HYDRO we have implemented a "reduced volume correction" (RVC), which is related to the surface rather than to the volume of the bead model (Zipper and Durchschlag 2007):

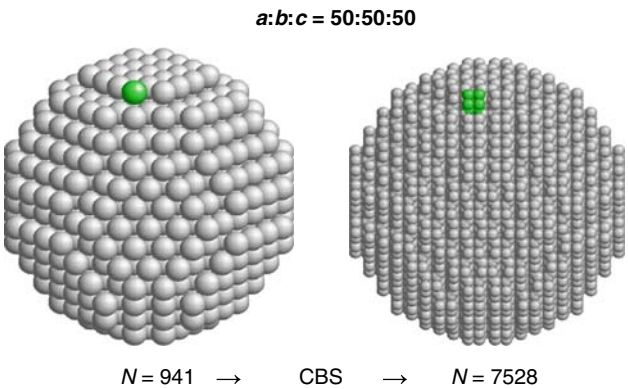

Fig. 1 Exemplification of the CBS approach: each bead in the primary bead model is replaced with a cubic array of eight beads. Consequently, N increases by a factor of eight

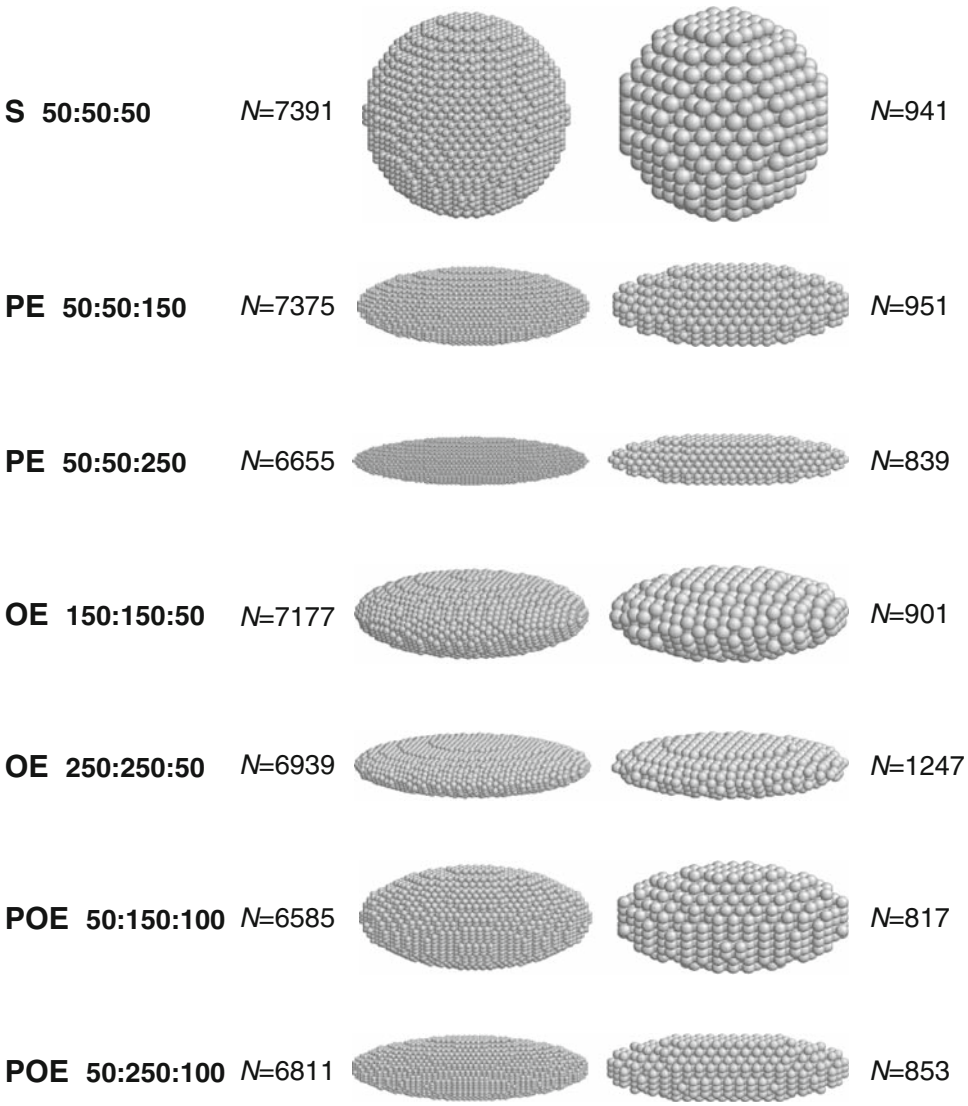

Fig. 2 Selected space-filling multibead models for ellipsoids of semiaxes *a*:*b*:*c* (in Å). The models (*S*, *PE*, *OE*, *POE*) were generated by means of an in-house program. By varying the radius of the beads systematically (usually in steps of 0.25 and 0.5 Å) each ellipsoid was represented by a set of models of different bead numbers *N*. The models with the highest *N*, not exceeding the limiting value N_{max} for double-precision versions of HYDRO, are shown on the left. The right column contains the models with the highest *N* that may be used as starting points for CBS and subsequent application of double-precision versions of HYDRO, except the case of $N = 1{,}247$ where only single-precision versions of HYDRO are applicable to the output of CBS

$$[\eta]_{RVC} = [\eta]_{NVC} + \frac{2.5 N_A V}{N^{1/3} M}$$

where N_A is Avogadro's number, N the number of beads, and V and M their total volume and mass. Obviously, this type of volume correction is not impaired by a diminution of N_{max}.

(v) Use of a plethora of beads for the Fortran program HYDRO poses several questions concerning computation mode (single or double-precision), the platform (Windows, Linux) and the compilation mode. In particular, the accuracy of predicted data and the value of N_{max} are crucial points.

(vi) In the case of proteins or other classes of macromolecules, further problems are a matter of concern. Inter alia, appropriate values for partial specific volume and hydration of macromolecule and bound ligand(s), appropriate substitutes for constituents missing in the available data bases (e.g., missing amino acid residues or linkers in the Protein Data Bank), and efficient docking approaches for molecules composed of building blocks (e.g. subunits of oligomeric proteins) have to be used, and several other cruxes of matter such as rugosity, electrostriction, mobility of chains have to be considered (Zipper et al. 2005; Durchschlag and Zipper 2005, 2008; Durchschlag et al. 2007). For the calculation of structural parameters of proteins, the statistical weight of the beads has to be included (see Part 2).

Results and discussion

For the following hydrodynamic computations, several models of quite different axial ratios were applied. A few representative examples for S, PE, OE, and POE models are displayed in Fig. 2 and the results of a great variety of

Table 1 Comparison of structural and hydrodynamic parameters of whole-body and multibead models of ellipsoids of semiaxes *a*, *b*, and *c*

Shape	*a*:*b*:*c* (Å)	*N*	r_b (Å)	$V \times 10^{-6}$ (Å^3)	R_G (Å)	$D \times 10^7$ (cm^2/s)	$[\eta]_{NVC}$ (cm^3/g)	$[\eta]_{FVC}$ (cm^3/g)	$[\eta]_{RVC}$ (cm^3/g)	$[\eta]_{CBS}$ (cm^3/g)	$[\eta]^a_{AVC}$ (cm^3/g)
S	50:50:50, WB			0.5236	38.73	4.286	2.639[b]				
	–, MB	10,777–65	2.0–10.0	0.357 ± 0.027	39.0 ± 1.0	4.46 ± 0.35[c]	2.58 ± 0.22[c]	4.38 ± 0.36[c]	2.81 ± 0.23[c]		
		7,391–65	2.25–10.0			4.32 ± 0.15[d]	2.58 ± 0.23[d]	4.38 ± 0.37[d]	2.83 ± 0.23[d]		
		1,363–65	4.0–10.0							2.82 ± 0.26[c]	
		941–65	4.5–10.0							2.84 ± 0.27[d]	
		1,989–65	3.5–10.0								3.04 ± 0.50
PE	50:50:150, WB			1.5708	74.16	2.671	3.889[e]				
	–, MB	9,395–233	3.0–10.0	1.065 ± 0.030	74.2 ± 0.9	2.75 ± 0.10[c]	3.81 ± 0.12[c]	5.60 ± 0.17[c]	3.99 ± 0.11[c]		
		7,375–233	3.25–10.0			2.69 ± 0.03[d]	3.82 ± 0.13[d]	5.61 ± 0.17[d]	4.00 ± 0.11[d]		
		1,185–233	6.0–10.0							4.06 ± 0.12[c]	
		951–233	6.5–10.0							4.06 ± 0.13[d]	
		1,555–233	5.5–10.0								4.69 ± 0.21
PE	50:50:250, WB			2.6180	116.19	2.006	6.128[f]				
	–, MB	9,965–409	3.5–10.0	1.782 ± 0.029	115.9 ± 1.2	2.05 ± 0.03[c]	5.99 ± 0.14[c]	7.79 ± 0.16[c]	6.15 ± 0.11[c]		
		6,655–409	4.0–10.0			2.02 ± 0.02[d]	5.99 ± 0.15[d]	7.78 ± 0.18[d]	6.15 ± 0.13[d]		
		1,235–409	7.0–10.0							6.23 ± 0.13[c]	
		839–409	8.0–10.0							6.24 ± 0.16[d]	
		1,985–409	6.0–10.0								7.04 ± 0.20
OE	150:150:50, WB			4.7124	97.47	1.865	3.620[g]				
	–, MB	10,007–757	4.25–10.0	3.213 ± 0.040	97.3 ± 1.6	1.91 ± 0.05[c]	3.59 ± 0.05[c]	5.39 ± 0.07[c]	3.72 ± 0.06[c]		
		7,177–757	4.75–10.0			1.87 ± 0.01[d]	3.60 ± 0.06[d]	5.40 ± 0.08[d]	3.74 ± 0.06[d]		
		1,213–757	8.5–10.0							3.74 ± 0.07[c]	
		901–757	9.5–10.0							3.77 ± 0.06[d]	
		1,837–757	7.5–10.0								4.55 ± 0.20
OE	250:250:50, WB			13.090	159.69	1.198	4.969 [h]				
	–, MB	9,887–1,247	6.0–12.0	8.892 ± 0.110	159.7 ± 0.5	1.21 ± 0.01[c]	4.90 ± 0.06[c]	6.70 ± 0.08[c]	5.03 ± 0.06[c]		
		6,939–1,247	6.75–12.0			1.20 ± 0.01[d]	4.90 ± 0.07[d]	6.69 ± 0.09[d]	5.03 ± 0.07[d]		
		1,247	12.0							5.19[c]	
		1,833–1,247	10.5–12.0								5.89 ± 0.13

Table 1 continued

Shape	*a*:*b*:*c* (Å)	*N*	r_b (Å)	$V \times 10^{-6}$ (Å^3)	R_G (Å)	$D \times 10^7$ (cm^2/s)	$[\eta]_{NVC}$ (cm^3/g)	$[\eta]_{FVC}$ (cm^3/g)	$[\eta]_{RVC}$ (cm^3/g)	$[\eta]_{CBS}$ (cm^3/g)	$[\eta]^a_{AVC}$ (cm^3/g)
POE	50:150:100, WB			3.1416	83.67	2.184	3.425[i]				
	–, MB	9,663–505	3.75–10.0	2.135 ± 0.019	83.8 ± 0.4	2.26 ± 0.10[c]	3.37 ± 0.05[c]	5.16 ± 0.06[c]	3.52 ± 0.06[c]		
		6,585–505	4.25–10.0			2.19 ± 0.01[d]	3.37 ± 0.05[d]	5.17 ± 0.06[d]	3.54 ± 0.06[d]		
		1,215–505	7.5–10.0							3.57 ± 0.08[c]	
		817–505	8.5–10.0							3.61 ± 0.09[d]	
		1,861–505	6.5–10.0								4.29 ± 0.19
POE	50:250:100, WB			5.2360	122.47	1.690	4.716[j]				
	–, MB	9,339–749	4.5–10.5	3.567 ± 0.038	122.5 ± 0.7	1.72 ± 0.04[c]	4.65 ± 0.07[c]	6.45 ± 0.09[c]	4.79 ± 0.08[c]		
		6,811–749	5.0–10.5			1.70 ± 0.01[d]	4.66 ± 0.08[d]	6.45 ± 0.09[d]	4.80 ± 0.08[d]		
		1,165–749	9.0–10.5							4.88 ± 0.15[c]	
		853–749	10.0–10.5							4.95 ± 0.23[d]	
		1,669–749	8.0–10.5								5.68 ± 0.13

S sphere, *PE* prolate ellipsoid of revolution, *OE* oblate ellipsoid of revolution, *POE* triaxial ellipsoid, *WB* whole-body, *MB* multibead, *N* number of beads, r_b radius of beads, *V* total volume, R_G radius of gyration, *D* translational diffusion coefficient, $[\eta]$ intrinsic viscosity, *NVC* no volume correction, *FVC* full volume correction, *RVC* reduced volume correction, *CBS* cubic substitution, *AVC* adjusted volume correction, *M* molar mass

[a] All values were computed by HYDRO++ (single-precision)

[b] Based on $M = 298.8$ kg/mol

[c] Obtained by executing HYDRO in single-precision mode

[d] Obtained by executing HYDRO in double-precision mode

[e] Based on $M = 896.3$ kg/mol

[f] Based on $M = 1{,}493.8$ kg/mol

[g] Based on $M = 2{,}688.8$ kg/mol

[h] Based on $M = 7{,}468.8$ kg/mol

[i] Based on $M = 1{,}792.5$ kg/mol

[j] Based on $M = 2{,}987.5$ kg/mol

Table 2 Comparison of mean ratios of $[\eta]$ values of multibead models of ellipsoids of semiaxes a, b, and c

Shape	a:b:c (Å)	N	r_b (Å)	$\langle[\eta]_{NVC}\rangle/[\eta]_{WB}$	$\langle[\eta]_{RVC}\rangle/[\eta]_{WB}$	$\langle[\eta]_{NVC}/[\eta]_{RVC}\rangle$	$\langle[\eta]_{FVC}/[\eta]_{RVC}\rangle$	$\langle[\eta]_{CBS}/[\eta]_{RVC}\rangle$	$\langle[\eta]_{AVC}/[\eta]_{RVC}\rangle$[a]
S	50:50:50, MB	10,777–65	2.0–10.0	0.977 ± 0.085[b]	1.066 ± 0.088[b]	0.916 ± 0.037[b]	1.557 ± 0.056[b]		
		7,391–65	2.25–10.0	0.979 ± 0.088[c]	1.072 ± 0.088[c]	0.913 ± 0.036[c]	1.550 ± 0.050[c]		
		1,363–65	4.0–10.0					0.990 ± 0.006[b]	
		941–65	4.5–10.0					0.994 ± 0.007[c]	
		1,989–65	3.5–10.0						1.070 ± 0.162
PE	50:50:150, MB	9,395–233	3.0–10.0	0.981 ± 0.031[b]	1.026 ± 0.028[b]	0.956 ± 0.016[b]	1.404 ± 0.022[b]		
		7,375–233	3.25–10.0	0.982 ± 0.032[c]	1.030 ± 0.027[c]	0.954 ± 0.016[c]	1.401 ± 0.020[c]		
		1,185–233	6.0–10.0					1.012 ± 0.005[b]	
		951–233	6.5–10.0					1.015 ± 0.004[c]	
		1,555–233	5.5–10.0						1.169 ± 0.024
PE	50:50:250, MB	9,965–409	3.5–10.0	0.978 ± 0.023[b]	1.004 ± 0.019[b]	0.974 ± 0.008[b]	1.267 ± 0.010[b]		
		6,655–409	4.0–10.0	0.977 ± 0.025[c]	1.004 ± 0.021[c]	0.973 ± 0.008[c]	1.265 ± 0.007[c]		
		1,235–409	7.0–10.0					1.023 ± 0.005[b]	
		839–409	8.0–10.0					1.027 ± 0.004[c]	
		1,985–409	6.0–10.0						1.150 ± 0.011
OE	150:150:50, MB	10,007–757	4.25–10.0	0.991 ± 0.015[b]	1.028 ± 0.017[b]	0.964 ± 0.010[b]	1.447 ± 0.015[b]		
		7,177–757	4.75–10.0	0.994 ± 0.017[c]	1.034 ± 0.016[c]	0.962 ± 0.009[c]	1.443 ± 0.012[c]		
		1,213–757	8.5–10.0					1.004 ± 0.003[b]	
		901–757	9.5–10.0					1.012 ± 0.000[c]	
		1,837–757	7.5–10.0						1.211 ± 0.036
OE	250:250:50, MB	9,887–1,247	6.0–12.0	0.987 ± 0.013[b]	1.011 ± 0.013[b]	0.976 ± 0.005[b]	1.333 ± 0.008[b]		
		6,939–1,247	6.75–12.0	0.987 ± 0.014[c]	1.013 ± 0.014[c]	0.974 ± 0.005[c]	1.330 ± 0.007[c]		
		1,247	12.0					1.011[b]	
		1,833–1,247	10.5–12.0						1.170 ± 0.007
POE	50:150:100, MB	9,663–505	3.75–10.0	0.984 ± 0.013[b]	1.027 ± 0.019[b]	0.958 ± 0.013[b]	1.468 ± 0.019[b]		
		6,585–505	4.25–10.0	0.986 ± 0.015[c]	1.032 ± 0.016[c]	0.955 ± 0.012[c]	1.463 ± 0.015[c]		
		1,215–505	7.5–10.0					1.004 ± 0.005[b]	
		817–505	8.5–10.0					1.011 ± 0.002[c]	
		1,861–505	6.5–10.0						1.208 ± 0.058
POE	50:250:100, MB	9,339–749	4.5–10.5	0.986 ± 0.016[b]	1.015 ± 0.017[b]	0.971 ± 0.008[b]	1.348 ± 0.012[b]		
		6,811–749	5.0–10.5	0.987 ± 0.017[c]	1.019 ± 0.018[c]	0.969 ± 0.007[c]	1.344 ± 0.009[c]		
		1,165–749	9.0–10.5					1.013 ± 0.003[b]	
		853–749	10.0–10.5					1.020 ± 0.001[c]	
		1,669–749	8.0–10.5						1.181 ± 0.015

Abbreviations are explained in Table 1

[a] All values were obtained by executing HYDRO++ and HYDRO in single-precision mode

[b] Obtained by executing HYDRO in single-precision mode

[c] Obtained by executing HYDRO in double-precision mode

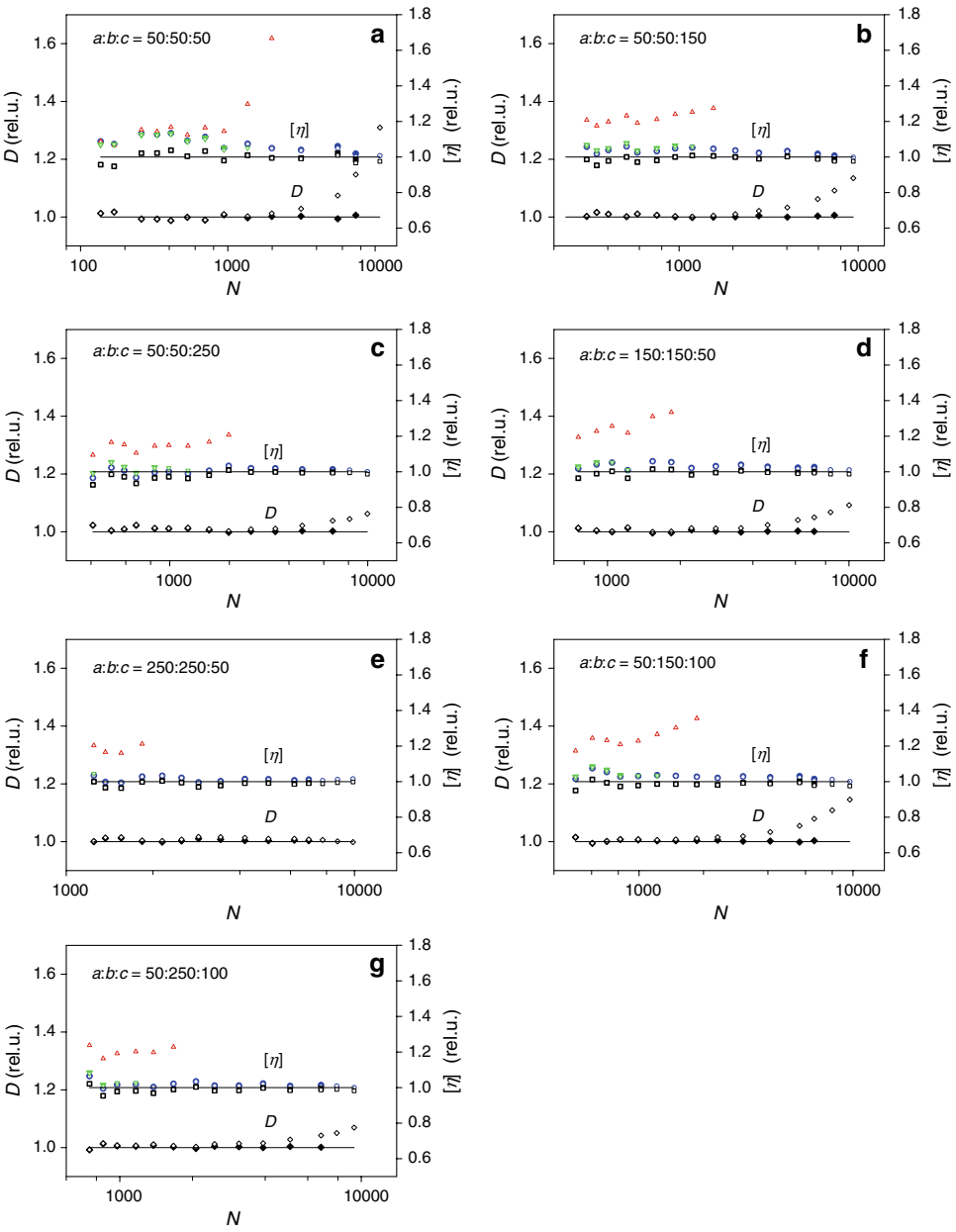

Fig. 3 Comparison of the accuracy of different computational approaches used for predicting the translational diffusion coefficient D and the intrinsic viscosity $[\eta]$ of selected space-filling multibead models for ellipsoids of different axial ratio (**a** S; **b**, **c** PE; **d**, **e** OE; **f**, **g** POE) by means of HYDRO and HYDRO++. For each ellipsoid a set of bead models differing in the number of beads, N, was created and analyzed. The results obtained from HYDRO or HYDRO++ for D and $[\eta]$ of the bead models were normalized by dividing them by the theoretical values for the underlying ellipsoids. *Open symbols* signify single-precision computation and *closed symbols* refer to double-precision computation. For calculation of $[\eta]$, different types of volume corrections were applied: NVC (*black*). AVC (*red*), CBS (*green*), and RVC (*blue*)

hydrodynamic calculations are summarized in Table 1. For the multibead calculations by HYDRO, several input parameters such as r_b, and thus also N, were varied, and, in addition to the usual prediction of structural and hydrodynamic parameters, several computation modes for $[\eta]$ were tested (NVC, FVC, RVC, CBS, AVC). The number of beads, N, applied spans a wide range (65–10,777); the models constructed do not require bead reduction and avoid bead overlapping. As far as possible, computations with both single-precision and double-precision were performed. The results obtained by these multi-body approaches were compared to the data obtained by simple whole-body approaches, based on the axial dimensions, which may be used as reference systems.

As may be taken from Table 1, for all model types and axial ratios applied, the data for D (and therefore also for s) are anticipated correctly, however, more precisely when using the double-precision mode. The prediction of $[\eta]$ values clearly shows that the FVC and AVC approaches fail; however, the data obtained by the AVC being slightly better than those by the FVC. In all cases, the CBS and RVC approaches coincide nearly quantitatively and yield quite correct values, similar to the NVC values.

Quantifying the results obtained for $[\eta]$ by different calculative approaches in terms of mean ratios of viscosities renders quotients (Table 2), which underline the above statements. Dividing the averaged $[\eta]$ values of the various MB models (Table 1) by the values of $[\eta]_{WB}$ obtained for

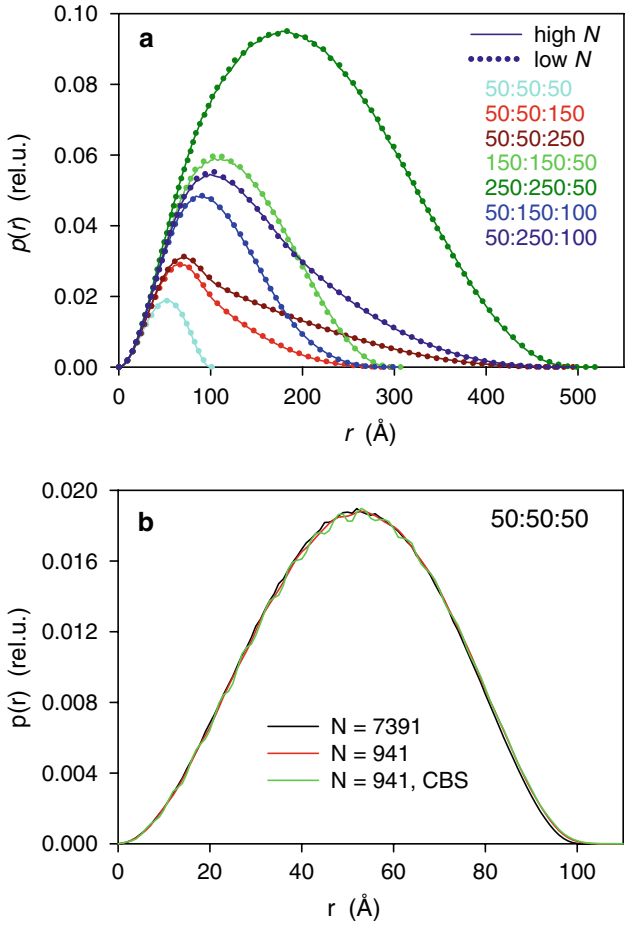

Fig. 4 Comparison of distance-distribution functions $p(r)$ of selected space-filling multibead models for ellipsoids of different axial ratio (S, PE, OE, POE). **a** Profiles for S, PE, OE, and POE models obtained at high or low N, respectively; the integral values of the profiles are proportional to the volumes of the ellipsoids; **b** Profiles for S model obtained at high and low N and after CBS

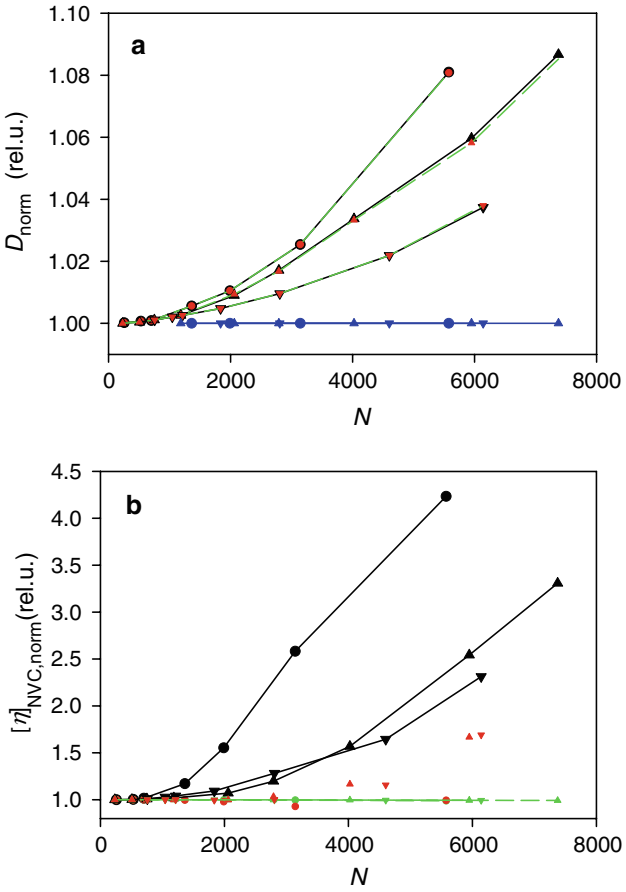

Fig. 5 Plots showing the dependence of predicted values for the translational diffusion coefficient D (**a**) and the uncorrected intrinsic viscosity $[\eta]_{NVC}$ (**b**) of selected ellipsoidal models of different axial ratios (*circle* 1:1:1, *up triangle* 1:1:3, *low triangle* 3:3:1) on the bead number N and the mode of compilation of the Fortran code of HYDRO. For convenience, the predicted data were normalized by dividing them by the results obtained by means of code-optimized double-precision versions of HYDRO. The data displayed in *black* refer to compilations of single-precision code, performed without any optimizations on the Windows platform by the Lahey LF95 compiler and on the Linux platform by the GNU g77 compiler. Enabling optimization of the single-precision code by setting the respective compiler option led to the results displayed in *green* (LF95: -O1) and *red* (g77: -O2). The data displayed in *blue* refer to compilations of double-precision code without optimization. While the optimization of single-precision code is already able to improve the prediction of $[\eta]_{NVC}$ (**b**), especially when using the Lahey LF95 compiler, the prediction of D (**a**) can only be improved by using double-precision versions of HYDRO; these optimizations also provide additional improvements in the prediction of $[\eta]_{NVC}$: the resulting normalized data amount to 1.0, irrespective of N and the compiler used (**b** double-precision data are not displayed in *blue* because they coincide with the *green lines* and *symbols*)

the WB models yields mean ratios like $\langle[\eta]_{NVC}\rangle/[\eta]_{WB}$ or $\langle[\eta]_{RVC}\rangle/[\eta]_{WB}$ (Table 2) which may be used as a basis for a quantitative comparison of the different approaches for the prediction of $[\eta]$. Unfortunately, these ratios suffer from an increasing scatter of $[\eta]_{NVC}$ and other $[\eta]$ values at low N (cf. Fig. 3). This scatter presumably reflects imperfections (e.g., rugosity) of the MB models for the ellipsoids, which become more pronounced at low N, and leads to relatively large standard deviations of the averaged data, particularly in the case of the models for the sphere. Nevertheless, the results indicate that, on average, the $[\eta]_{NVC}$ values slightly underestimate the theoretical values for $[\eta]_{WB}$, while the $[\eta]_{RVC}$ overestimate them a little. A similar conclusion can be drawn if only the $[\eta]$ values obtained at the highest N are considered. To get rid of the influence of model imperfections, we adopted an alternative strategy of relating directly the various $[\eta]$ values obtained from HYDRO or HYDRO++, for convenience using always the values of $[\eta]_{RVC}$ as a reference. While the mean ratios $\langle[\eta]_{NVC}/[\eta]_{RVC}\rangle$ and $\langle[\eta]_{CBS}/[\eta]_{RVC}\rangle$ turn out to be close to unity, the mean ratios $\langle[\eta]_{AVC}/[\eta]_{RVC}\rangle$ and, in particular, $\langle[\eta]_{FVC}/[\eta]_{RVC}\rangle$ are substantially greater. The supposition that these mean ratios are less affected by the model imperfections mentioned is corroborated by the

Table 3 Comparison of maximum bead numbers, N_{max}, allowed in different versions of HYDRO

Program	Option	N_{max}		Calculation of $[\eta]$
		Windows	Linux	
Hydro7c[a]		2,000	2,000	$[\eta]_{FVC}$
Hydro++8c[a]	ICASE = 11	2,000	2,000	$[\eta]_{FVC}$
	ICASE = 12	2,000	2,000[b]	$[\eta]_{AVC}$
	ICASE = 21	250	250	$[\eta]_{CBS}$
Easyhyd (modified version of Hydro13[c], single-precision)		10,529[d]	10,922[e]	$[\eta]_{NVC}$, $[\eta]_{FVC}$, $[\eta]_{RVC}$; $[\eta]^{f}_{CBS}$
Dbleasyhyd (modified version of Hydro13[c], double-precision)		7,445[g]	7,722[e]	$[\eta]_{NVC}$, $[\eta]_{FVC}$, $[\eta]_{RVC}$; $[\eta]^{f}_{CBS}$

[a] The binaries were downloaded from García de la Torre's web site (http://leonardo.fcu.um.es/macromol)

[b] Nominal value; actually a segmentation fault occurs if the number of beads exceeds 310

[c] The Fortran source code of Hydro13 (single-precision) was downloaded from García de la Torre's web site. Binaries were obtained by compiling the modified code by means of Lahey compilers LF95 for 32-bit Windows and Linux, and GNU compilers g77 and gfortran for Linux

[d] This limit is imposed by the operating system; the compilers would allow $N_{max} = 10{,}922$

[e] This limit is imposed by the compilers

[f] To obtain $[\eta]_{CBS}$, a CBS of the model (by means of an auxiliary program) has to precede the execution of this program. The bead number of the original model must not exceed $N_{max}/8$ (i.e., 1,316 or 1,365, and 930 or 965, respectively, depending on the platform and precision selected)

[g] This limit is imposed by the operating system; the compilers would allow $N_{max} = 7{,}722$

generally smaller standard deviations obtained for these ratios. The extreme standard deviation observed for $\langle[\eta]_{AVC}/[\eta]_{RVC}\rangle$ of the spherical models reflects a considerable scatter of the $[\eta]_{AVC}$ values in this case (cf. Fig. 3).

Plots illustrating the accuracy assessment of different computational approaches for the prediction of D and $[\eta]$ by HYDRO and HYDRO++ as a function of N (Fig. 3), again, reveal these findings: more accurate results for D at large N (>2,000) when using double-precision calculations; quite correct predictions for $[\eta]$ when applying CBS or RVC approaches or NVC, and rather insufficient predictions when using AVC, even at the smallest N used.

Distance distribution functions, $p(r)$, exhibit nearly perfect agreement between the profiles obtained at low or high N, and, as shown for the S model, also after applying CBS (Fig. 4). As known per se, the r values where the $p(r)$ functions converge to zero correspond to the maximum particle distances of the respective models.

To check the validity of the above statements concerning precision and N_{max}, we performed several programming attempts. Especially designed single- and double-precision binaries of HYDRO, for appropriately selected values of N_{max}, were obtained by compiling the accordingly modified Fortran source code using different compilers (various versions of Lahey LF95 compilers for Windows and Linux; various versions of GNU compilers for Linux) and applying different levels of code optimization (including no optimization at all). The comparison of the data predicted for D and uncorrected intrinsic viscosity $[\eta]_{NVC}$ obtained by application of these binaries (Fig. 5) shows that the prediction of $[\eta]_{NVC}$ may be improved by optimization of the single-precision code (especially when using the Lahey LF95 compilers), whereas improvements of the prediction of D can only be achieved by using double-precision versions of HYDRO. The latter statement, of course, holds for the prediction of s as well. On the other hand, for predictions of rotatory quantities (e.g., the relaxation times τ_1–τ_5; data not shown) computations in single-precision mode turned out to give quite reliable results, even at high N and regardless of the compiler used. The accuracy of the data is, however, restricted by the neglect of third-order hydrodynamic interactions in the algorithm used (García de la Torre et al. 2007). Usage of double-precision versions of HYDRO yielded nearly the same values for the relaxation times as the single-precision versions.

Finally, a comparison of the N_{max} values attainable with different versions of HYDRO (Table 3) unravels that our modified versions expand the limits of the original versions ($N_{max} = 2{,}000$ or 250 for CBS calculations) to about 10,500 (single-precision) or 7,500 (double-precision) for Windows, and even a bit higher for Linux. Concerning use of the CBS approach, one has to bear in mind that this approach diminishes N_{max} by a factor of eight, while application of RVC allows calculations with the entire number of possible beads.

Conclusions

The results obtained confirm the validity of our RVC approach for predicting correct values for $[\eta]$ of models of quite different shape and axial ratios, and the equivalence

of the results with those obtained by means of the CBS approach. Our modified versions of HYDRO allow hydrodynamic calculations in single-precision mode even for bead numbers N up to nearly 11,000. Usage of single-precision computations turned out to be quite sufficient for predictions not only of $[\eta]$ but also of the relaxation times τ_1–τ_5, even when using large bead numbers. To obtain, however, accurate results for D and s at $N > 2{,}000$, calculations in the double-precision mode are required, at the expense of a reduced value of $N_{\max}$. Using the Linux platform for executing the modified versions of HYDRO has the advantage of higher $N_{\max}$ values; however, GNU compiled single-precision binaries should not be used since they may yield erroneous results for $[\eta]$ at high N. To test our calculations of models against reality more purposively, also hydrodynamic calculations on proteins of different size and shape have to be performed. These issues will be treated in Part 2.

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