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ORIGINAL PAPER

Multi-scale calculation and global-fit analysis of hydrodynamic properties of biological macromolecules: determination of the overall conformation of antibody IgG molecules

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Abstract We present a scheme, based on existing and newly developed computational tools, for the determination of the overall conformation of biological macromolecules composed by domains or subunits, using from such structural determination easily available solution properties. In a multi-scale approach, atomic-level structures are used to provide simple shapes for the subunits, which are put together in a coarse grained model, with a few parameters that determine the overall shape of the macromolecule. Computer programs, like those in the HYDRO suite that evaluate the properties of either atomic or coarse-grained models. In this paper we present a new scheme for a global fit of multiple properties, implemented in a new computer program, HYDROFIT, which interfaces with the programs of the HYDRO suite to find an optimum, best-fitting structure in a robust but simple way. The determination of the overall structure of the native antibody IgG3, bearing a long hinge, and that of the hingeless mutant m15 is presented to test and confirm the validity of this simple, systematic and efficient scheme.

Keywords Multisubunit structure · HYDROSUB · Global fit analysis · HYDROFIT · Antibodies

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Introduction

Multiscale calculations for multisubunit structures

Solution properties, like hydrodynamic coefficients or radiation scattering, are essential sources of information on the overall conformation of biological macromolecules in solution. Historically, flexible chain molecules were treated by means of simplified or approximate theories (Yamakawa 1971) while rigid particles were modelled by geometrically simple models like ellipsoids (Tanford 1961; Harding 1995) or cylinders (Fernandes et al. 2002; Ortega and García de la Torre 2003).

Since the pioneering work of Bloomfield et al. (1967), bead models have overcome the limitations of the simplest models, becoming the usual choice for the prediction of solution properties or structural determination of rigid macromolecules of arbitrary shape (for reviews, see: Byron 2000, 2008; García de la Torre et al. 2005; Carrasco and García de la Torre 1999; Harvey and García de la Torre 1980). Nowadays, bead modelling has reached the atomic scale, with models constructed from atomic coordinates or high-resolution electron density maps (Bernado et al. 2002; García de la Torre et al. 2000, 2001; Ortega and García de la Torre 2005; Zipper and Durchschlag 2003). This approach is remarkably helpful to study the influence of such structural details on the solution properties of, for instance, small proteins and oligonucleotides. Also, the variety of computer programs for hydrodynamic properties that proceed directly from atomic coordinates construct internally the bead/shell model, thus freeing the user of this task.

However, there are instances, usually corresponding to large proteins and macromolecular complexes, where atomic-level calculations are not possible because of the lack of crystallographic or NMR determinations at that

level. Or, simply, atomic details of such structures may be relatively unimportant for their hydrodynamic behaviour. Common cases are those of multisubunit structures, and immunoglobulin antibodies are paradigmatic examples. The overall size and shape or the subunits (more than the atomic detail) and, essentially their spatial arrangements, will be the aspects that determine hydrodynamic properties or solution scattering.

With those situations in mind, we have proposed a procedure in which simple ellipsoidal or cylindrical models are used to represent the (often globular or rodlike) subunits, thus putting the emphasis on their spatial arrangements. This is a "meso-scale" structural modelling. In the "meso-scale" approach, that is becoming increasingly popular in physical and biological sciences, unnecessary details are quoted in the model in order to concentrate in the essential aspects. The computer programs HYDROSUB (García de la Torre and Carrasco 2002) and MULTISUB (García de la Torre et al. 2003) are available for the calculation of the solution properties, and we have applied the procedure to antibody molecules (Carrasco et al. 1999; Harding et al. 2003; Longman et al. 2003; Lu et al. 2007 and references cited therein). In the first step, one has to determine the dimensions of the subunits (axes of revolution ellipsoid, or length and diameter of cylinders). This could be done from experimental data of solution properties of the isolated subunits (Carrasco et al. 1999) but it may happen that data are insufficient, imprecise or simply unavailable. However, the high-resolution, atomic-level structure of the individual subunits may be available, mainly when they are globular proteins. Then such information is employed to determine the dimensions of the equivalent ellipsoid. Initially, this was done by equalizing the moments of inertia of the ellipsoid to those calculated from the atomic coordinates. Now, this criterion is replaced—or complemented—by a fit of a whole set of solution properties predicted from the atomic structure (García de la Torre et al. 2000) and thus the resulting ellipsoidal of cylindrical subunit can be safely regarded as a hydrodynamically equivalent particle.

Once the dimensions of the individual subunits have been determined from atomic-level calculations, they are joined to construct the coarse-grained, or model of the whole particle. This scheme belongs to what is now called the multiscale modelling paradigm, in which the whole system or particle is represented by a coarse-grained, meso-scale model, constituted by simple elements whose parameters have been derived in a previous fine-grained (usually atomistic) simulation of their properties.

Global-fitting procedures

Crucial steps in the multiscale approach are those in which geometric parameters are determined, either for the individual subunits, or for the whole particle, so that they fit available or predicted data of various solution properties. Classically (Tanford 1961), this was done for revolution ellipsoids in terms of dimensionless quantities that combined two solution properties, like translational friction and volume (Perrin function), intrinsic viscosity and volume (Einstein coefficient), translational friction or viscosity and radius of gyration (Flory parameters), etc. The idea was later generalized to triaxial ellipsoids (Harding 1995) and finally for arbitrarily shaped particles (García de la Torre et al. 1997). The concept of structural determination by means of a global, simultaneous fits of various properties has been put forward by Nöllmann et al. (2005). Very recently, we have devised a conceptually similar but formally very different approach, based on equivalent radii and ratios of radii, that allow for a robust, unbiased and systematic global fit of a collection of properties to ellipsoidal, cylindrical or worm-like models (Ortega and García de la Torre 2007). One of the contributions of the present work is a generalization of the procedure to particles of arbitrary shape, whose hydrodynamic properties are calculated using the programs in the HYDRO Suite. We have devised a new computational tool, HYDROFIT, that performs the structural optimization in such cases as it will be described below.

Overall conformation of antibody molecules

The problem that we choose to illustrate and confirm the applicability of our scheme is the determination of the overall conformation of antibody molecules. Apart from the antigen-binding capabilities in the subunits, their arrangement mediated by the hinge region is as essential determination of the function of antibodies (Burton 1987; Furtado et al. 2004; Perkins and Bonner 2008). Unfortunately, the difficulties for obtaining crystallographic structures of antibodies, a particularly those with large hinges, are well known (Harris et al. 1998a, b). Thus, the procedure for structural determination of antibodies based on solution properties seems especially valuable. A pioneering study in this direction, using the primitive version of the HYDRO program (García de la Torre et al. 1994) was done by Burton and coworkers (Gregory et al. 1987). Then, we have considered this problem in a series of publications (Carrasco et al. 1999; García de la Torre and Carrasco 2002; Lu et al. 2007), which were all based in the HYDROSUB calculation for models with ellipsoidal and cylindrical subunits. However, our previous approaches relied in some aspects, like non-systematic "universal" shape functions, an ad hoc introduction of hydration, manual and rather cumbersome procedures for structure optimization, etc. We shall show how the present approach makes the structural determination of the antibody global

structure feasible, in a simple and systematic way, making an adequate treatment of experimental values of solution properties. In this way, we confirm that the difference between wild type and a mutant of Ig3 is the much shorter hinge in the case of the mutant.

Methodology

As mentioned above, the present work makes use of existing methodology for the mere calculation of properties from model structures, and proposes a new multi-scale protocol and a new computer program that should be considered as methodological results from this investigation.

Use of existing tools

The procedure employed in this work for the analysis of solution properties of multi-subunit structures, makes extensive use of the existing, public-domain programs of the HYDRO Suite. The prediction of properties for the individual subunits from atomic coordinates (PDB files) is easily accomplished using HYDROPRO (García de la Torre et al. 2000) (we also recall the availability of HYDROMIC (García de la Torre et al. 2001), which works with structures expressed as density maps derived from electron microscopy). The determination of the dimensions of the ellipsoids or cylinders from the solution properties of the subunits is done using Single-HYDFIT (Ortega and García de la Torre 2007), based on the global-fit concept described in next section. For the search of an equivalent ellipsoid, our program ORIEL (Fernandes et al. 2001) can also be useful. The final intention of ORIEL is the prediction of NMR residual dipolar coupling of atomic-level structures but, as an intermediate result, the program finds the revolution ellipsoid that best fits the inertia tensor of the atomic structure.

HYDROSUB (García de la Torre and Carrasco 2002) is available for the shell-model calculation of properties of the whole particle composed by such simply represented subunits. The multi-case execution of HYDROSUB, particularly the preparation of input files and compilation and analysis for the successive cases, is facilitated by MULTISUB (García de la Torre et al. 2003). All the programs mentioned in this work are in public domain, and can be downloaded from our Web site, http://leonardo.inf.um.es/macromol/.

HYDROFIT: a new tool for global-fitting with arbitrarily shaped particles

As mentioned above, we have developed a framework for the joint analysis of a set of various (conformational and hydrodynamic) observable properties in terms of structural features of the particle (Ortega and García de la Torre 2007). Each of the various solution properties is handled in the form of an equivalent radius, a_x, defined as the radius of a sphere that would have the same value for that property. Various, different equivalent radii can be defined for translational friction a_T, intrinsic viscosity, a_I, radius of gyration a_G, etc. These radii depend on size and shape in a more uniform manner than the properties themselves. Any pair of radii can be combined in a ratio of radii, $XY = a_X/a_Y$. For a spherical particle, all the equivalent radii are obviously equal to the sphere radius, and all the ratios are equal to unity. For any other structure, the various radii are not the same, but do no differ too much, and the ratios depart slightly from unity, being dependent on the overall conformation but independent of the absolute size.

These concepts have been implemented in a scheme that measures the agreement between an assumed structure and the observed properties of a macromolecule. Such agreement can be quantified in terms of the equivalent radius by means of the quantity:

$$\Delta^2 = \left(\sum_x w_x\right)^{-1} \sum_x w_x \left[\frac{a_x(\text{cal}) - a_x(\text{exp})}{a_x(\text{exp})}\right]^2 \qquad (1)$$

Note that Δ^2 is the mean square relative deviation between the calculated and experimental equivalent radii. If there is a special reason to do so, the average can include different weights w_x for each property; otherwise, they can be the same for all the properties or, in other words, neglected. Note also that $100\sqrt{\Delta^2}$ is the percent deviation of the calculated equivalent radii from the experimental value.

The goodness of fit of the assumed structure can also be determined from the ratios of radii, as measured by the quantity:

$$\nabla^2 = \left(\sum_{XY} w_{XY}\right)^{-1} \sum_{XY} w_{XY}[XY(\text{cal}) - XY(\text{exp})]^2 \qquad (2)$$

which ∇^2 is the mean square difference between the calculated and experimental ratios of radii. Again, the weights w_{XY} are optional. Now, $100\sqrt{\nabla^2}$ is the percent difference of the calculated ratios of radii from the experimental values. In a recent work (Ortega and García de la Torre 2007), we proposed to use Δ and ∇ as the target functions to be minimized in the search of a structure that fits best a set of solution properties. This global-fit idea is implemented in the computer programs Single-HYDFIT and Multi-HYDFIT (the latter, specific for cases comprising a homologous series of samples, which is not the case here). The macromolecular models specifically considered in those programs were revolution ellipsoids, cylinders and wormlike chains. Thus, as anticipated above, Single-HYDFIT is useful in the

multi-scale modelling of multi-subunit structures for determining the optimum parameters of the ellipsoids or cylinders that represent the subunits.

In this work we present for the first time an extension of the global fit concept for the structural search in the case of arbitrary shapes whose solution properties can be calculated with the programs in the HYDRO Suite (HYDRO++, HYDROPRO, HYDROSUB, etc.). All these programs are able to process, in a single run, a number of different structures, provided that the corresponding structural files have been previously constructed. This task can be accomplished in some cases with our ancillary programs, like MULTI-HYDRO or MULTISUB, or with user-written programs. A modification has been introduced in all the programs of the HYDRO Suite, so that they now produce a file, named *hydfitdat.txt*, which collects the result for every structure in the run. As a sequel to Single-HYDFIT and Multi-HYDFIT, we have produced a new computer tool, HYDROFIT, that takes, on one hand, experimental values of the solution properties, and, on the other one, the *hydfitdat.txt* file. Then, HYDROFIT determines for each structure the Δ indicator (Eq. 1), determining the best-fitting one and reporting the calculated radii and properties compared to the experimental ones.

In the present multi-scale strategy, HYDROFIT is employed to evaluate the various conformations considered for the whole particle, comparing the HYDROSUB results with the experimental one in order to find the optimum structure.

Scheme of the multi-scale approach

Figure 1 displays the scheme of the multi-scale approach. Summarizing, the primary data consists of the high-resolution structure of the domains, or, eventually, their solution properties. The intermediate results are the optimum parameters of their simplified models, that are finally used for building the coarse-grained model of the whole molecule. The scheme shows the computational tools involved in each step, for both hydrodynamic calculations and global fits, including the new HYDROFIT program.

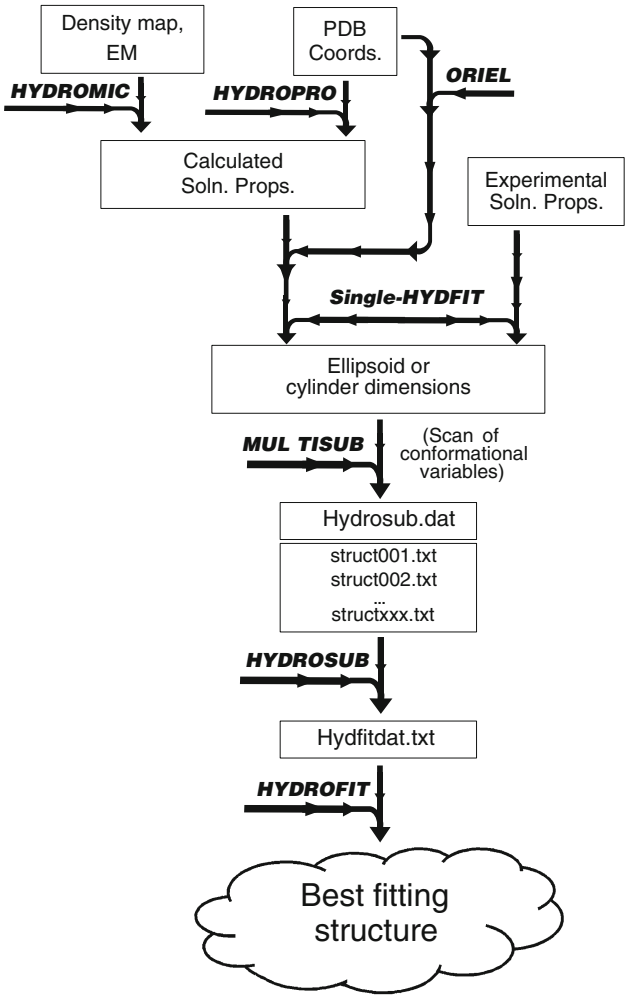

Fig. 1 Scheme of the procedure to determine the overall conformation of multisubunit structures. The names of computational tools involved in each step are in *italics*

Determination of the overall conformation of antibodies

As indicated in the introduction, the elucidation of the overall structure of antibodies is the example chosen to illustrate the multi-scale approach. The determination of the effect of the hinge and the relative position of the subunits provides a good demonstration and test of the methodology.

In the first stage we look for the dimensions of the hydrodynamic ellipsoidal models that will represent the Fab and Fc fragments in the coarse-grained model of the whole molecule. In the second stage, the arrangement of the subunits in the whole molecule is determined.

Modelling the Fab and Fc domains

Experimental data for the Fab and Fc fragments are scarce; the only solution properties of the Fab and Fc subunits for which experimental data are available are the sedimentation coefficient s, and the radius of gyration, R_G. Values are listed in Table 1. This pair of properties provide limited information for the determination of the two parameters of the ellipsoids, the semiaxes a and b. Then, we decide to fit the parameters of the hydrodynamic ellipsoidal model to the predictions of the hydrodynamic model with atomic resolution, constructed and evaluated with the program HYDROPRO. Indeed, this is the primary step in the multi-scale approach, in which the parameterization of the coarse-

Table 1 Results for the ellipsoidal models of Fab and Fc, and comparison of the calculated properties with the experimental values

	Fab	Fc
PDB accession codes	1BBJ	1FC1
HYDROPRO: *a, b* (Å); *p*/(Δ)	43.7, 24.9; 1.80/0.043	14.5, 43.0; 0.32/0.027
ORIEL: *a, b* (Å); *p*	42.3, 23.1; 1.83	20.0, 38.2; 0.52
Ellipsoid: *a, b* (Å); *p*/(Δ)	43.7, 24.9; 1.76/0.043	17.1, 41.9; 0.41/0.035
Experimental: M (g/mol), $\bar{v}$ (cm^3/g)	47,500[a], 0.725[a,b,c]	51,000[c], 0.732[c]
s (S): ellipsoid/experimental	3.58/3.6[a], 3.92[b,c]	3.63/3.85[c]
R_G (Å): ellipsoid/experimental	25.1/26.9[a], 28.2[d]	26.9/29.3[a]

Obtained from [a]Byron (2000), [b]Carrasco et al. (1999), [c]Carrasco et al. (2001), and [d]Lu et al. (2007)

grained models is made from atomic-level calculations. From the crystal structure of the subunits, obtained from the Protein Data Bank under ID codes 1BBJ (Brady et al. 1992) and 1FC1 (Deisenhofer 1981), and using the standard value of 3.5 Å for the equivalent hydrodynamic radius of the atoms, HYDROPRO gives a comprehensive list of solution properties. In addition to s and R_G, we pick several other properties: the translational diffusion coefficient D_t, the intrinsic viscosity $[\eta]$, and the longest relaxation time τ_1, the covolume u and the longest distance, D_{max}. The set of properties from HYDROPRO is the input for Single-HYDFIT, which is first run in the mode that considers $p = a/b$ as the only parameter, in an optimization of the size-independent, shape(p)-dependent ratios of radii, based on the minimization of the ∇ target function (Eq. 2). Single-HYDFIT reports the absolute minimum, but it may also provide the full variation of ∇ with p. Inspection of the ∇ versus p curves (Fig. 2) reveals that, in addition to the absolute minimum, there is another secondary minimum that is nearly as deep. This circumstance is related to an ambiguity of the revolution ellipsoid in the representation of globular proteins that is known from the pioneering studies of this model (Tanford 1961): two ellipsoids, one prolate and another oblate reproduce the experimental data. In our previous work we already described this problem with lysozyme as example (Ortega and García de la Torre 2007), and we proposed the use of a complementary, purely geometric approach that seeks the revolution ellipsoid that fits best the moments of inertia of the atomic structure. The procedure is implemented in the computer tool ORIEL (Fernandes et al. 2001). For Fab, the HYDROPRO data produce an absolute minimum with $p = 1.8$ and a secondary minimum at $p = 0.3$. The ORIEL result is $p = 1.8$, coincident with the former 1.8. For Fc, the absolute minimum is at $p = 1.5$ and the secondary one, with a very similar depth is at $p = 0.32$. The ORIEL estimation favours clearly the oblate shape, although with a somewhat different axial ratio, $p = 0.52$. Then we take finally the mean of the HYDROPRO and ORIEL results, $p = 0.42$.

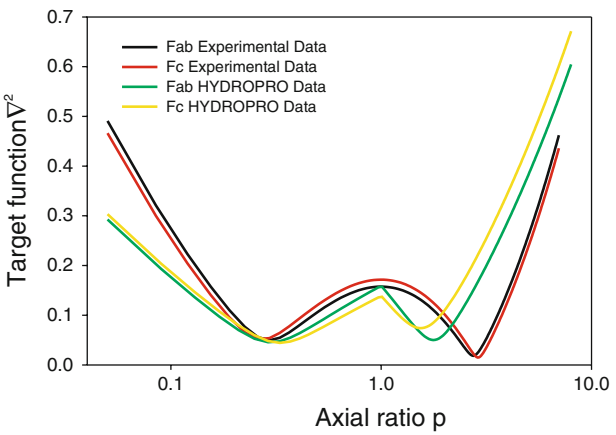

Fig. 2 Variation of the target function ∇ with the ellipsoidal shape parameter (axial ratio $p = a/b$)

Next, Single-HYDFIT is run in the two-parameter model, that minimizes Δ (Eq. 1) as a function of p and a, giving their best values, and that of $b = a/p$. Figure 3 depicts the contour plots of Δ versus p and a, showing the absolute minimum and the secondary one. The resulting dimensions of the ellipsoids are listed in Table 1 (entry "ellipsoid"), and are those to be used in the coarse-grained model. It is noteworthy that these results agree qualitatively (prolate Fab and oblate Fc), and semiquantitatively (similar axial ratios and dimensions) with those obtained by Carrasco et al. (1999, 2001). The present results are, likely, more precise, and have been obtained in a rather robust and systematic way. Finally, we recall the available values of experimental properties. As mentioned above, such values have not been used in the determination of the ellipsoid dimensions, but we should at least check a posteriori whether the deduced dimensions are compatible with them. So, in Table 1 we give the values of R_G and s for the ellipsoids that resulted from our analysis, along with the experimental one. The typical difference is about 5%, similar to the expected experimental uncertainties of these properties.

Modelling the whole IgG

The model for whole IgG molecules consists of: (a) the two prolate ellipsoids that represent Fab's, with semiaxes $a_{ab} = 43.7$ Å and $b_{ab} = 24.9$ Å, (b) the oblate ellipsoid for

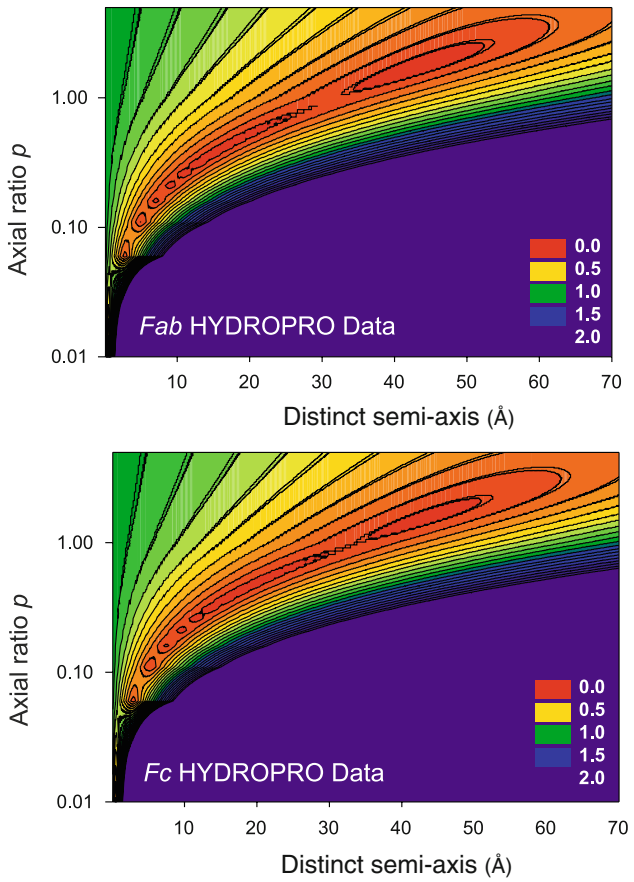

Fig. 3 Contour plot of the target function Δ versus p and a. Note that the scale for p is logarithmic, in order to display well both the prolate and the oblate region. **a** Fab fragment. **b** Fc fragment

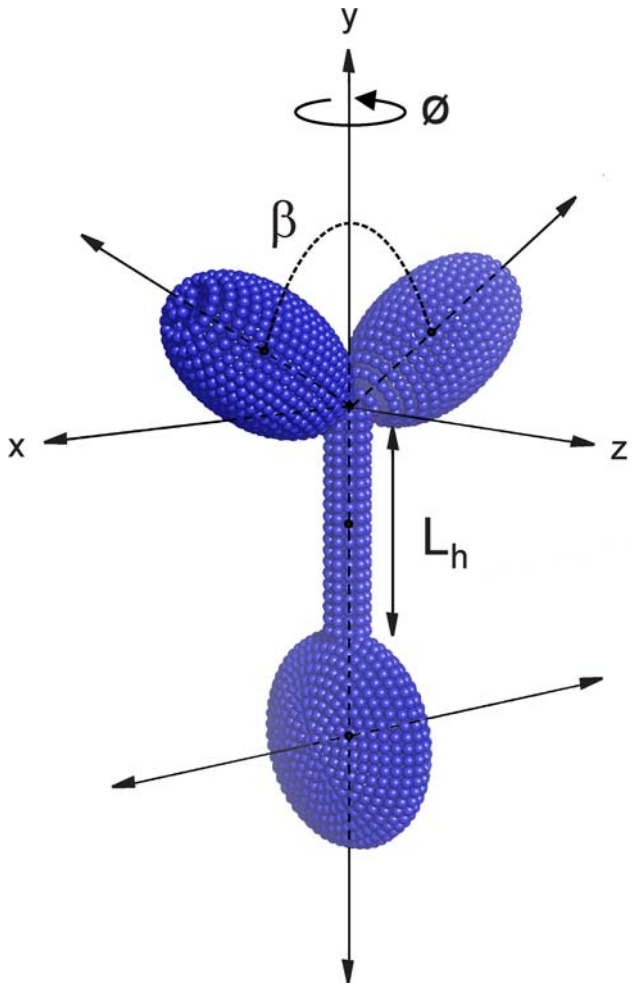

Fig. 4 Geometry of the coarse-grained model for whole IgG. *Dots* are subunit centers, and *dotted lines* correspond to the main (revolution) axis of the subunits

Fc with the semiaxes $a_c = 14.5$ Å and $b_c = 43.0$ Å, and (c) a hinge that is represented by a rod whose length L_h, is going to be a primary parameter to be determined in the conformational search. However the diameter of the rod has a negligible influence in the computed properties, so that it is fixed at a value, $d_h = 7.5$ Å, which seems reasonable for the hydrated intertwined polypeptide chains.

The properties for this array of four subunits will be calculated using HYDROSUB, for which the structural information is a set of values, for each ellipsoidal or cylindrical subunit, of the coordinates x_i (x_i, y_i, z_i)b of their centers, and the polar and azimuthal angles, θ_i and ϕ_i that specify the orientation of the main (revolution) axis of the ellipsoid or cylinder, all referred to an arbitrary, conveniently chosen system of axes.

As shown in Fig. 4, the rodlike hinge is placed on the negative side of the z axis, so that its center is placed at coordinates (x_h, y_h, z_h) = (0, 0, L_h/2), and its orientation is that of $\theta_h = \phi_h = 0$. The end of the rod touches the oblate Fc, which will be centered at (x_c, y_c, z_c) = (0, 0, L/2 + a_c), and its equatorial plane lies on the (x, y) plane, so that $\theta_c = 90°$ and $\phi_h = 0°$. The two Fab prolate ellipsoids are joined to the other end of the rod, which is the center of coordinates. We are going to restrict the search to a symmetric arrangement of the Fab subunits, with the aim of reducing the number of parameters. Thus, we assume that the angle between the long axes of the two Fab's, β, is bisected by axis z, so that the polar angles are $\theta_{ab} = \beta/2$ for the two subunits, and the azimuthal angles are of the form $\phi_{ab1} = \phi$ and $\phi_{ab2} = \phi + 180°$. Then, the coordinates of the centers of the prolate ellipsoid are (x_{ab}, y_{ab}, z_{ab}) = ($\pm a_{ab}\sin\theta_{ab}\cos\phi$, $\pm a_{ab}\sin\theta_{ab}\sin\phi$, $\pm a_{ab}\cos\theta_{ab}$). Note that ϕ is the angle between the equatorial plane of Fc and the plane defined by the long axes of the Fab's. In summary, our coarse-grained model for the overall structure of antibody molecules contains up to three parameters: the hinge length, L_h, and the angles β and ϕ that define the orientation of the Fab fragments.

We intend to determine the overall structure of two distinct IgG3 molecules, the wild type antibody IgG3wt, and mutant IgGm15, their structures already solved by

previous, more complex approaches. Intensive experimental work on these and other antibodies has provided data for various solution properties: sedimentation coefficient, s, intrinsic viscosity, $[\eta]$, and X-ray scattering results for the radius of gyration R_G and the longest distance, D_{max}. Experimental data are listed in Table 2.

According to our multi-scale calculation and global-fit structural search (Fig. 1), the best-fitting structure is determined by a HYDROFIT analysis of the experimental properties along with HYDROSUB calculations (García de la Torre and Carrasco 2002) for a number of structures, with varying parameters, whose data files are generated with MULTISUB (García de la Torre et al. 2003). It is quite straightforward to implement the simple geometrical conditions that give the centers and orientations of the subunits within the MULTISUB code, whose output files are the input files for a multi-case HYDROSUB calculation.

Before proceeding with the final, HYDROFIT step, it is worthwhile to look at the stronger or weaker dependence of the predicted properties on the model parameters, L_h, β and ϕ. For this purpose, we set two of them to a reasonable (intermediate) value, and change the third one. Some results are presented in Fig. 5, where they are presented as values relative to the maximum reached within the range of the varied parameter. We find that, while the properties change appreciably with both L_h and β, all of them are essentially insensitive to ϕ. This was somehow expected since, as ϕ is changed, the centers of the subunits remain at the same points; only their relative orientation is changed. From the theory that gives R_G of multisubunit structures (Sólvez et al. 1988), we know that this property depends on the position of the centers and radii of gyration of each subunit, but not on their orientation, so that R_G must be invariant to ϕ. Then, one may expect that the other properties will also be practically insensitive to this parameter. This finding is helpful for a further model reduction, as only two parameters L_h and β have to be floated in the structural search.

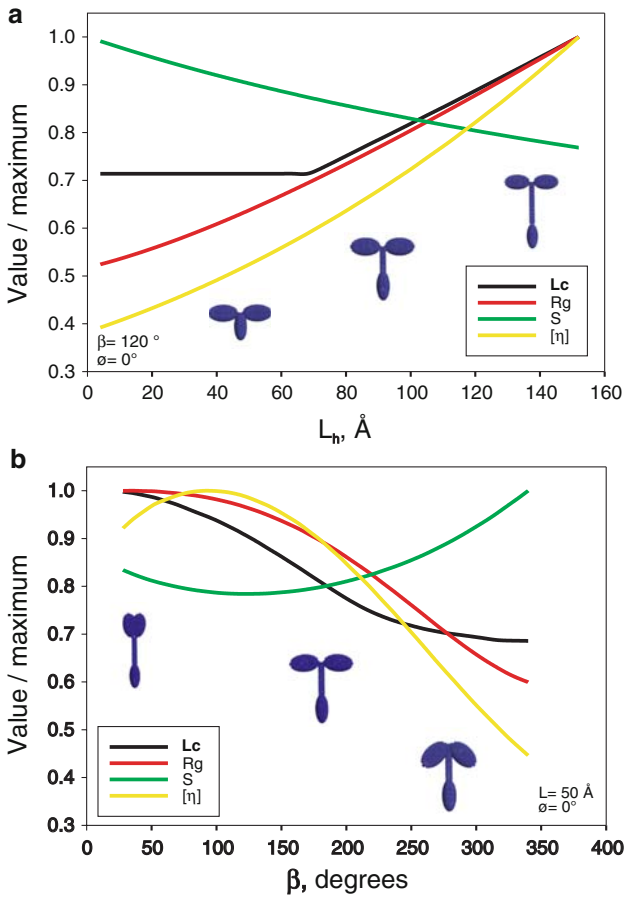

Fig. 5 Dependence of the calculated properties with the IgG3 model parameters. Two of them are fixed values given and the other is varied. Properties are expressed as ratios relative to the maximum value within the range of the varied parameter

Table 2 Results for the IgG3wt and IgGm15 best fitting models, experimental data and calculated properties are shown

	IgG3wt	IgG3m15
M (g/mol)	158,000	150,000
$\bar{v}$, (cm^3/g)	0.73	0.73
$S^\circ_{20,w}$, (S) (exptal)	6.11	6.77
R_G, (Å) (exptal)	77.7	51.5
$[\eta]$, (cm^3/g) (exptal)	9.9	5.7
$D_{max,}$ (Å) (exptal)	195	165
$L_{h,}$ (Å) (exptal)	92	0
β, degrees	212	40
$S^\circ_{20,w,}$ (S/%) (dif)	5.94/2.8	6.73/0.6
R_G (Å/%) (dif)	73.8/5.0	50.8/1.3
$[\eta]$ (cm^3/g/%) (dif)	10.8/−9.0	6.13/−7.5
D_{max} (Å/%) (dif)	201/−3.1	175/−6.1

Results

Using MONTESUB and HYDROSUB, the solution properties of the IgG model in Fig. 5 were evaluated as a function of L_h and β. The set of results is compared to the experimental properties of, first, IgG3wt and next, IgGm15, using HYDROFIT to determine the best-fitting parameters for each antibody. Our program reports also list of values of the target function, Eq. 1, from which we draw the contour plots presented in Fig. 6.

For the wild type IgG3 antibody, Fig. 6a shows the existence of an unique structure that fits the experimental data, whose main features is long hinge with $L_h \approx 80$–100 Å, which confirms the one obtained in our previous work (Lu et al. 2007) and the β angle, close to $\approx 200°$ gives

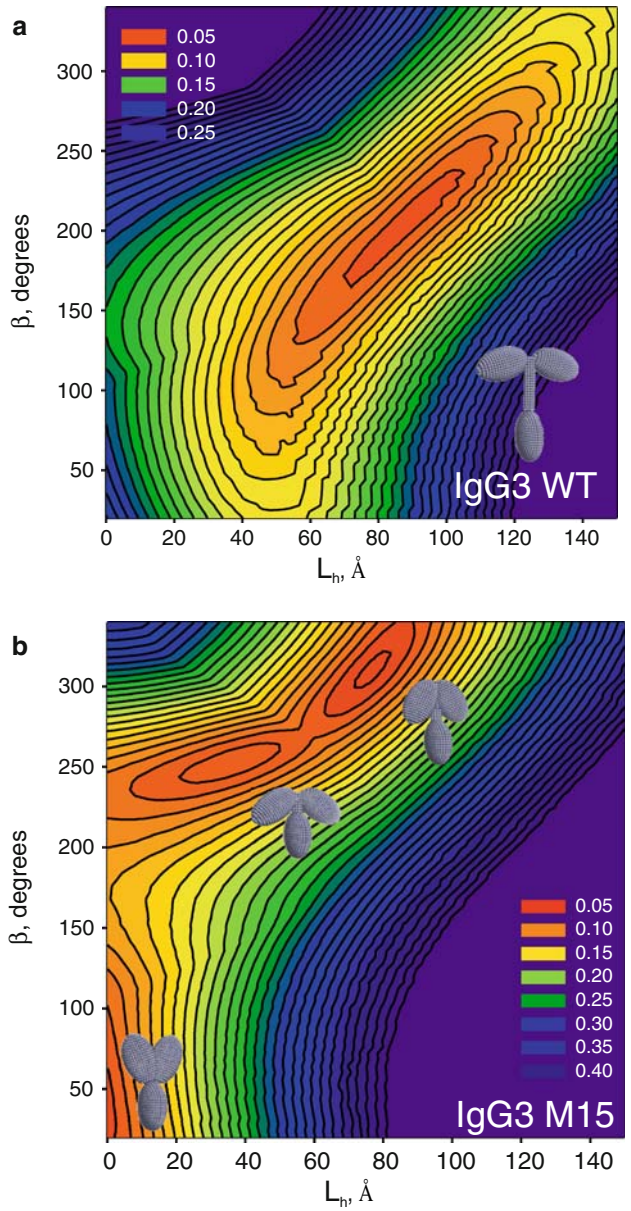

Fig. 6 Contour plot of the target function Δ versus the model parameters for the two IgG3 antibodies. Inserts are views of the best fitting structures. **a** IgG3 wild type. **b** IgG3 m15 mutant

a typical "*T*"-shaped conformation. For this structure, the $\Delta = 0.0353$, indicating the typical difference between experimental and calculated equivalent radii is only 3.53%. Table 2 lists the solution properties calculated for this structure, which differ from the experimental values by only a few percent.

For the hingeless, m15 mutant, the outcome from HYDROFIT has to be regarded carefully. As hinted above, in addition to the main results file that reports the lowest Δ structure, one should also take a look to the list of Δ values as a function of the parameters, which produces the contour plot in Fig. 6b. Inspection of such results reveals that in this case there are several minima with nearly the same depth. One is the expected structure with $L_h \approx 0$, in which the disposition of the Fab subunits gives a "*Y*"-shaped conformation. Again, this structure is essentially the same as that found in our previous work, and the agreement of its calculated properties with the experimental value is a good as for IgG3wt, as shown in Table 2. But the HYDROFIT analysis produces also other unrealistic but "numerically" acceptable structures, with an unexisting hinge and the Fab fragments strangely folded towards Fc. So this study has provided also a good example of the limitations and cautions proper of—not just our specific methodology—but of any global fitting structure.

Concluding remarks

In this work we have demonstrated a scheme, and constructed a new tool, HYDROFIT, for the determination of the overall structures of complex (typically multi-domain) structures. We propose a multi-scale procedure in which, in the spirit of what we have previously named crystallo-hydrodynamic approach, atomic-level information is employed to assign values to some of the parameters of the coarse-grained model. The most important parameters, that determine the overall conformation, are obtained by fitting a collection of experimental data to the results from model calculations.

As in our previous works, we considered antibody molecules, as excellent cases for which the procedures can be tested. For the wild type and mutant IgG antibodies considered here, we obtain essentially the same structures as in our previous studies. However, we can remark that there are a number of new conceptual and methodological contributions.

Thus, in the present procedure do not need an estimate of protein hydration δ (g/g), which is included in the data generated with HYDROPRO from the atomic structure of the subunits. Also, the classical but somewhat cumbersome Perrin, Mittelbach, Scheraga/Mandelkern, etc.—shape functions are not employed; the SOLPRO program is not needed, and instead the optimizations are done directly with the experimental properties. Thus, the subunit dimensions (including hydration) are directly obtained from experimental properties by HYDROFIT global analysis, and the calculations and global fitting are carried out in an automated, efficient way combining multi-case calculations using, for instance, HYDRO and MULTIHYDRO, or HYDROSUB and MULTISUB with the newly developed HYDROSUB for structural optimizations.

It should be mentioned that, like any other hydrodynamic modelling approach, the present one cannot lead to

unique structure determination. This is particularly true when the set of available experimental data contains only a few properties. It may happen that not one but several structures may fit equally well the set of data, and in such a case HYDROFIT could find several minima of the Δ score function if a sufficiently wide range of parameters is scanned, as shown by our results for the m15 mutant. Thus, the program is able to detect such possible ambiguities, letting the user to choose the most plausible one.

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