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HYDROMIC: prediction of hydrodynamic properties of rigid macromolecular structures obtained from electron microscopy images

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Abstract We have developed a procedure for the prediction of hydrodynamic coefficients and other solution properties of macromolecules and macromolecular complexes whose volumes have been generated from electron microscopy images. Starting from the structural files generated in the three-dimensional reconstructions of such molecules, it is possible to construct a hydrodynamic model for which the solution properties can be calculated. We have written a computer program, HYDROMIC, that implements all the stages of the calculation. The use of this procedure is illustrated with a calculation of the solution properties of the volume of the cytosolic chaperonin CCT, obtained from cryoelectron microscopy images.

Keywords HYDROMIC · Hydrodynamic coefficients · Solution properties · Macromolecules · Electron microscopy

Introduction

Hydrodynamic coefficients and other solution properties are useful sources of information on the solution structure of macromolecules and macromolecular complexes. Their utility has been classically appreciated (Tanford 1961; Daune 1998; van Holde 1998), and it is presently enhanced after the advent of modern instrumentation for analytical ultracentrifugation (Harding et al. 1992) and a variety of dynamic spectroscopic techniques. The theoretical and computational procedures, obviously required to relate structure and properties, and particularly the bead models pioneered by Bloomfield et al. (1967), have been developed (García de la Torre and Bloomfield 1981; García de la Torre 1989; Carrasco and García de la Torre 1999), benefiting from advances in hydrodynamic theory and the unceasing increase in computing power.

Along the years, our knowledge of macromolecular structure has become more extensive and accurate. This has motivated further efforts in hydrodynamic theory and computation, which was initially intended for low-resolution modelling (García de la Torre et al. 1994). Recently, implementations of the hydrodynamic bead modelling procedures have reached the atomic level. New computational tools like HYDROPRO (García de la Torre et al. 2000a) and HYDRONMR (García de la Torre et al. 2000b) allow the prediction of hydrodynamic coefficients and NMR relaxation times, and the program SOLPRO (García de la Torre et al. 1999) is also available for calculation of scattering and other solution properties. These tools are intended for the prediction of properties from the atomic structures of proteins and nucleic acids as specified by atomic coordinates from crystallographic or NMR structures deposited in the PDB (Protein Data Bank) or similarly formatted files.

During the last decade, electron microscopy has become a major tool in macromolecular structure determination. The intensive use of cryoelectron microscopy and the development of more powerful image methods of quantitative image analysis have extended the use of electron microscopy towards high resolution. Presently, electron microscopy is capable of providing not only information of proteins at atomic resolution (Henderson et al. 1990; Kühlbrandt and Wang 1991; Nogales et al. 1998) but also of generating medium-high resolution information of large macromolecular complexes that
are difficult to crystallize, and which can be later used to
dock the atomic structure of their basic constituents
(Kalko et al. 2000) to obtain very useful biological
information in this way.

In this work we have developed the methodology that
enables the prediction of solution properties from the
3D reconstructed images derived from electron micro-
scopy and related techniques, using bead-model calcula-
tions. Different varieties of bead modelling have been
described (Carrasco and García de la Torre 1999), so it
has been necessary to find the proper way of doing such
modelling in a way that starts directly from the 3D im-
age formats that are usually produced by those tech-
niques.

As a test for the methodology, we have used the 3D
reconstruction of the cytosolic chaperonin CCT, ob-
tained using cryo-electron microscopy and image pro-
cessing. Chaperonins are a group of proteins that are
involved in the assistance to the folding of other pro-
teins. They form large macromolecular complexes built
by two rings, each one having 7–9 subunits, depending
on the chaperonin, and with a total molecular weight
ranging from 840 to 1080 kDa. CCT is the only
chaperonin found in the eukaryotic cytosol and is a very
unique chaperonin in the sense that is much more
complex than any other chaperonin known, and that it
functions in the folding of a very small number of pro-
teins, especially actin and tubulin, whose importance
in the cell function is paramount (Llorca et al. 1999a,
1999b, 2000).

Method

HYDROMIC

The first part of the methodology has the purpose of building a
suitable hydrodynamic model from the 3D shape of the particle,
and this has to be done according to the contents and format of the
computer files that contain the shape information. A common
feature of some of these files is that they are based on a cubic grid,
which is superimposed on the particle. In the file, the nodes of the
grid, usually named pixels, are assigned numerical values. The
number of segments in which the x, y and z dimensions are divided
are, respectively, $N_x$ (number of rows), $N_y$ (number of columns)
and $N_z$ (number of slices). The spacing, $\delta$, is the same for the three
axes. The various file formats differ in how this and other informa-
tion is organized and coded. Here we consider the so-called
spider format (Frank et al. 1996), which is one of the most fre-
quently employed in the electron microscopy field.

Files in the spider format (see the web sites http://
html and http://www.wadsworth.org/spider_doc/spider/docs/
image.doc.html, and Frank et al. 1996) contain a header within
which the values of $N_x$, $N_y$ and $N_z$ are given. The total number of
pixels in the file is $N_{tot} = N_x N_y N_z$. Then there is a list of values,
one for each pixel, whose values correspond to their level of density.
For our present purpose, we consider that a pixel belongs to the particle
if the value, $v$, assigned to it exceeds some threshold, $v_{min}$. The values
of $\delta$ and $v_{min}$ have to be supplied separately. Our HYDROMIC
program reads the spider format, selects the pixels that belong to the
particle and calculates their Cartesian coordinates.

After processing the structural file, HYDROMIC has a list of
coordinates of $N_p$ points, or pixels, which fills the particle contour.
Some basic geometric information is determined at this stage; thus,
the particle’s volume can be estimated as $V = \frac{b^3 N_p}{N_{tot}}$ and the
radius of gyration, $R_g$, can be readily calculated from the pixel
coordinates. From this list of coordinates, a primary hydrodynamic
model (PHM), composed of spherical elements, is obtained by re-
placing each pixel by a sphere. The radius of the spheres, $a$, whose
precise value is not essential for the final results, is selected in such a
way that each sphere overlaps appreciably with its neighbours, so
that there will be no interstitial voids in the model. A proper choice
is $a = b$.

Once HYDROMIC has built the PHM, the calculation of its
hydrodynamic properties is carried out following the shell-limit
strategy (Carrasco and García de la Torre 1999). The PHM is first
replaced by a hexagonal, closest-packed array of non-overlapping
spheres (“minibeads”) of radius $\sigma$, thus obtaining a filling model,
and then all the internal beads are removed to obtain the shell
model, which is the one on which the hydrodynamic calculations
are done using the HYDRO subroutine (García de la Torre et al.
1994). Such calculations are repeated for several values of $\sigma$, and
for each property the results are extrapolated to $\sigma = 0$. Other sol-
solution properties can be calculated from the bead models using
procedures coded in our SOLPRO program (García de la Torre et al.
1997, 1999). The basic procedures developed to deal with
electron microscopy data can be applied also to X-ray, neutron or
light scattering. Therefore, we have included in HYDROMIC the
calculation of the scattering form factor and the distribution of
distances.

Some elementary physical data of solute and solvent are re-
quired for the calculation of solution properties: temperature $T$
(e.g. $T = 293$ K); solvent viscosity $\eta$ (e.g. $\eta = 0.01$ poise for aqueous
solution); solution density, $\rho$ (approximately $\rho = 1.0$ for dilute
aqueous solution); solute specific volume, $\tau_s$; and molecular weight
of the particle, $M$. The radius of gyration and scattering-related
quantities are essentially independent of these data. Diffusion co-
efficients and relaxation times depend only on $T$ and $\eta$. The in-
trinsic viscosity, $[\eta]$, requires the value of $M$, and for the
sedimentation coefficient, $s$, the values of $M$ and the buoyancy
factor $(1 - \tau_p)$ are required. Values of properties calculated by
HYDROMIC for some given values of these physical constants can
be recalculated manually for other values. Formulas for the changes
produced by $\eta$ and $T$ can be found in textbooks. If the
molecular weight and/or the buoyancy factors are changed to, say,$\ M'$
and $(1 - \tau_{p'})$, then the sedimentation coefficient and the
intrinsic viscosity change as $s' = s M' (1 - \tau_{p'}) / M (1 - \tau_p)$ and
$[\eta]' = [\eta] M' / M'$.

Results

3D reconstruction of the cytosolic chaperonin CCT

Cytosolic chaperonin CCT was chosen to illustrate the
HYDROMIC method because its 3D reconstruction of the
chaperonin, using frozen-hydrated specimens, has been
recently accomplished in our laboratory. For
details, see Llorca et al. (2000).

Application of HYDROMIC to the cytosolic
chaperonin CCT

We have tested HYDROMIC using the volume ob-
tained from the 3D reconstruction of the cytosolic
chaperonin CCT, using images of frozen-hydrated im-
ages of this specimen. The chaperonin used in this study
is the eukaryotic type II cytoplasmic chaperonin con-
taining TCP-1 (CCT). CCT is a heteromeric structure
made of two rings, each one built up by eight different,
albeit homologous, subunits (Willison 1999) and has an overall cylindrical shape (150x160 Å; Llorca et al. 1999a). The 3D structure obtained by cryo-electron microscopy (Fig. 1; Llorca et al. 2000) has a great similarity with the X-ray structure obtained for the thermosome (Ditzen et al. 1998), a homologous chaperonin found in Thermoplasma acidophilum. Binding of ligands and substrates triggers large conformational changes and modifies the architecture of CCT (Llorca et al. 2000). From a hydrodynamic point of view, this structure presents very interesting features, such as a cavity in each of the two rings, and 16 lateral windows that make the central part of the structure accessible to the solvent (Fig. 1). Owing to its size and the characteristic shape, this oligomer is a good candidate for hydrodynamic modelling (Walters et al. 2000).

The structure of apo-CCT (i.e., the chaperonin without any ligand or substrate bound) was coded in a spider format file (apo_cct.spi). The spacing is \( b = 3.9 \text{ Å} \), and the threshold for intensities is at \( r_{\text{min}} = 0.37 \) (threshold value which generates a mass with a molecular weight similar to that of the chaperonin). This file is read by HYDROMIC, which extracts the following parameters: \( N_x = 70 \), \( N_y = 70 \) and \( N_z = 70 \), with \( N_\text{tot} = 343,000 \). Using the above-mentioned threshold, the total number of pixels considered to represent the particle is \( N_p = 20,445 \). Thus, the volume of the structure is estimated to be \( 1.21 \times 10^6 \text{ Å}^3 \). If we consider a specific volume of 0.738 cm\(^3\)/g, the mass of the protein should be approximately 980 kDa, which fits quite well with the theoretical molar mass of CCT, calculated from the sequence of all eight subunits that make each of the two rings. Figure 2 reproduces the input data file for HYDROMIC.

A technical detail that deserves some comment is the replacement of the PHM by a shell model. The number of minibeads in the shell model increases as their radius, \( \sigma \), is decreased, as required by the shell-model limit extrapolation (Carrasco and García de la Torre 1997). The HYDRO calculations are feasible for models with up to \( N_b = 2000–3000 \) minibeads, and this places a lower bound for \( \sigma \); in the example of chaperonin CCT this is about \( \sigma = 5 \text{ Å} \). The value of \( \sigma \) can be regarded as the resolution of the hydrodynamic model, and \( b \) as that of the original image. Then, for large macromolecular structures, determined with high resolution (small \( b \), very large \( N_b \)) as is the case in the example of CCT chaperonin, we notice that the resolution in the hydrodynamic model (larger \( \sigma \), smaller \( N_b \)) is lower, although the main features of the particle are preserved as is clearly shown by the visual comparison of Figs. 1 and 3.

[![Fig. 1](image1.png)](image1.png)

Fig. 1 Three-dimensional structure of the cytosolic chaperonin CCT obtained from image processing of frozen-hydrated specimens, as described in Llorca et al. (2000). The file with the sections of this volume (apo_cct.spi) constitutes the structural input file for HYDROMIC.

[![Fig. 2](image2.png)](image2.png)

Fig. 2 Printout of the input file of the HYDROMIC calculation for apo_cct

[![Fig. 3](image3.png)](image3.png)

Fig. 3 Primary hydrodynamic model (PHM) of the CCT chaperonin
However, this is in part compensated by the calculations for varying \( \sigma \) followed by the shell-limit extrapolations to \( \sigma = 0 \) (we also recall that the finest details of the structure will have practically no effect on the solution properties). An appropriate checking of the performance of this procedure can be done by comparing the value of \( R_g \) of the (filling) hydrodynamic model with that previously obtained for the original structure. In the present example, we even notice (Fig. 4) that the calculated properties are quite insensitive to \( \sigma \), so that the extrapolation is safe.

Figure 5 shows a reproduction of the output file from HYDROMIC, containing the results for the hydrodynamic coefficients and other solution properties. Our program also provides the distribution of intramolecular distances, \( p(r) \), and the scattering function (or form factor), \( P(h) \), with \( h = (4 \pi / \lambda) \sin(\theta / 2) \) being the scattering variable for observation angle \( \theta \) with radiation wavelength \( \lambda \) (Glatter and Krakty 1982). In Fig. 5, the lines corresponding to these functions are removed, but a plot of them is shown in Fig. 6. It is interesting to note the skewness of \( p(r) \) towards the long distances, and the early minimum in \( P(h) \). Both features are probably associated with the hollow structure of the chaperonin.

Little information is available about solution properties of CCT chaperonin. Melki et al. (1997) have reported a sedimentation velocity experiment that was somehow complicated by the presence in the sample of a broad distribution of materials with different sedimentation velocities. Anyhow, these authors assigned a sedimentation coefficient of 25.6 S (water, 20°C) to ADP-bound CCT. The value calculated by HYDROMIC using the volume of CCT obtained in the 3D electron microscopy reconstruction is 24.1 S (Fig. 5), which deviates only 5% from that mentioned above. In addition to range of accuracy of bead-shell model predictions (2–3%), the uncertainty of the experimental data and other minor effects from solvent or temperature could account for the remaining difference. Indeed, the agreement is rather good, especially when electron microscopy studies show that ADP, unlike what happens with ATP, is not able to generate large conformational changes in the chaperonin, and therefore

![Summary of data and results](image)

**Fig. 4** Variation of the calculated properties with the minibead radius, \( s \), showing the shell-limit extrapolations for the radius of gyration and the sedimentation coefficient. *Data points* correspond to several runs of HYDROMIC, with various values of \( s \), and the *straight lines* are the least-squares fit used for extrapolations

![Plot of scattering data](image)

**Fig. 5** Printout of the output file of the HYDROMIC calculation for CCT chaperonin (some lines removed for brevity)
HYDROMIC, a program that predicts the hydrodynamic properties of rigid macromolecular structures obtained from electron microscopy images, has been developed. The program has been tested with a large macromolecular complex, the chaperonin CCT, and the results obtained fit with the theoretical value of the molecular mass. The program opens the possibilities of predicting not only the hydrodynamic properties of a given macromolecule, but also the changes in these properties that certain ligands or substrates induce upon their binding. The experimental test of such predictions, using analytical centrifugation, low-angle X-ray or low-angle neutron scattering, can be useful for the support of the structural transitions defined by indirect experimental methods.

HYDROMIC will be freely available, along with our other computer programs, from our web site: http://leonardo.lcu.um.es/macromol/.

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References


Fig. 6 Top: distribution of intramolecular distances, \( p(r) \); bottom: scattering function, \( P(h) \), obtained for CCT chaperonin

maintains its structure in the same conformation as the apo-CCT used in this study (Llorca et al. 1998).


