Chapter 9
Introduction: Calculation of Hydrodynamic Parameters

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Abstract This introduction considers the approaches to the calculation of hydrodynamic (and related) parameters described in detail in the following Chaps. 10, 11 and 12 (Chap. 10, US-SOMO; Chap. 11, the HYDRO suite; and Chap. 12, BEST). Starting with a description of what hydrodynamic modelling is and why it is useful, the first part of this chapter then presents 12 equations as a very basic tutorial in the hydrodynamic computations underlying the majority of the methodology that is then summarised in the subsequent section on current approaches in both rigid body and flexible modelling. The pros and cons of these approaches are then given before a few concluding remarks and an outlook.

Keywords Hydrodynamic modelling • Bead modelling • Boundary element modelling • Electrostatic-hydrodynamic analogy modelling • Sedimentation coefficient • Diffusion coefficient • Macromolecular hydration • Rigid body modelling • Flexible body modelling

9.1 Introduction

One might expect that because this chapter and the subsequent three chapters it seeks to introduce are in a book about analytical ultracentrifugation, the reader would be a paid-up member of the AUC and hydrodynamics club, with corresponding data for their macromolecular system and a clear idea of the utility of hydrodynamic modelling as an interpretative tool. However, in the era of accessing book chapters in isolation, this chapter starts by assuming no such background whatsoever, in the hope and expectation of encouraging new members to sign up!

Most macromolecular systems function in (aqueous or otherwise) solution, but much structural understanding of these systems is based on high-resolution coordinates determined crystallographically or as homology models. And yet it is well recognised that many macromolecules have so far failed to crystallise at all or

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in such a way as to yield diffraction data at a satisfactory resolution, although this is expected to be much less of a limitation to high-resolution structure determination with the advent of free electron lasers and the possibility of determining atomic structures for macromolecules that form micro- (Boutet et al. 2012) or nanocrystals at best. Additionally, many molecules and complexes are too large for routine structural determination via NMR spectroscopy (often a viable alternative to X-ray crystallography), and there are entire classes of macromolecules that are poorly suited to high-resolution structural study with one or other method (e.g. polymers or intrinsically disordered proteins). Low- or meso-resolution solution methods offer valuable insights to macromolecular structure in these instances *and* for systems that *have* been successfully characterised with X-ray crystallography, since it is now accepted that the dilute solution conformation can be significantly different from that adopted in the crystal lattice (Nakasako et al. 2001; Smolle et al. 2006; Trewhella et al. 1988; Vestergaard et al. 2005).

Hydrodynamic modelling entails computation of experimentally determinable hydrodynamic and related parameters (hereafter HARPs) for some form of macromolecular model. If the computed and experimental parameters agree to within some acceptable limit, the model is consistent with the solution macromolecular state. The information content of the single-value parameters concerned (e.g. translational diffusion coefficient, intrinsic viscosity, radius of gyration, etc.) is insufficient to permit *determination* of solution structures *de novo* but, especially when used in combination, can lend strong support to (or rule out) the proposed model.

What kinds of measureable parameters can be computed by these methods? This depends on the particular software that is used. In addition to the sedimentation coefficient (s) and translational diffusion coefficient (D_t), two of the principal experimentally determined parameters at the heart of this book, are the many other complementary parameters, a comprehensive (if slightly outdated) list of which is given in Table 1 of Byron (2008).

In the next three sections of this chapter, the most widely used approaches for macromolecular hydrodynamic computation will be described in some detail. These are summarised in Table 9.1. Emre Brookes and Mattia Rocco's chapter on US-SOMO (Brookes et al. 2010a, b) (Chap. 10) describes in detail its use for computing HARPs for models constructed directly from atomic resolution structures (or homology models) via either (1) the *SoMo* (Rai et al. 2005) or *AtoB* (Byron 1997) methods combined with the algorithms for HARP computation developed by José García de la Torre and collaborators over many years (Chap. 11) or the newer *Zeno* (Kang et al. 2004) algorithm (Brookes and Rocco, Sect. 10.3) or (2) the BEST algorithm of Sergio Aragon (2004, 2011) (Chap. 12 where the theoretical basis for this approach is fully described). US-SOMO has, since its inception, developed to embrace the hugely complementary modelling possibilities afforded by small-angle X-ray and neutron scattering (SAXS and SANS, respectively); this is not covered in any detail by Brookes and Rocco in Chap. 10, but clear reference is made to complementary published work in which it is described. The chapter by José García de la Torre (Chap. 11) on the HYDROxxx suite of programs (for rigid

Table 9.1 Summary of freely available software for the computation of HARPs for macromolecular models

Software	References	URL	Overview of capability
HYDRO++	García de la Torre et al. (2007)	http://leonardo.inf.um.es/macromol/programs . . . . . ./hydro++/hydro++.htm	Computation of HARPs from multi-sphere models that have been generated externally
US-SOMO	Brookes et al. (2010a, b)	http://www.somo.uthscsa.edu/	Constructs bead models from PDB files using either the *SoMo* or *AtoB* algorithm. HARPs are computed for these models using either the García de la Torre and Bloomfield "supermatrix inversion" method or the *Zeno* method. An interface to BEST computation on a cluster (below) is also offered. Molecular flexibility can be evaluated with the DMD tool
Zeno	Kang et al. (2004)	http://web.stevens.edu/zeno/	Computes selected HARPs for any molecule by enclosing it in a sphere from whose internal surface a series of random walks is launched. The fraction of these walks that reaches the molecule determines the HARP value. *Zeno* is also available in US-SOMO
HYDROPRO	Ortega et al. (2011b)	. . ./hydropro/hydropro.htm	Constructs shell models from PDB files and computes HARPs for shell models of decreasing sphere size, extrapolating values to the case of infinitely small spheres to arrive at the final outputs. Also can construct and compute HARPs for bead-per-atom/residue models
BEST	Aragon (2004, 2011)	http://esmeralda.sfsu.edu/	Constructs triangular patchwork surface models from PDB files and computes HARPs for models of decreasing triangle size, extrapolating values to the case of infinitely small triangles to arrive at the final outputs

(continued)

Table 9.1 (continued)

Software	References	URL	Overview of capability
HYDRONMR	García de la Torre et al. (2000)	.../hydronmr/hydronmr.htm	Shell modelling as for HYDROPRO but additionally computes the rotational correlation time (τ_c) and NMR relaxation times (T_1 and T_2) and for each residue
HYDROMIC	García de la Torre et al. (2001)	.../hydromic/hydromic.htm	Shell modelling as for HYDROPRO but constructs models from three-dimensional reconstruction (e.g. SPIDER or MRC) files
HYDROSUB	García de la Torre and Carrasco (2002)	.../hydrosub/hydrosub.htm	Shell modelling as for HYDROPRO but shell models are constructed from ellipsoids and/or cylinders that are positioned to represent the modelled system
HYDROPIX	García de la Torre (2001a)	.../hydropix/hydropix.htm	Shell modelling as for HYDROPRO but the shell model is based on a solid geometrical shape that is generated by an ancillary program MAKEPIXB
MULTIHYDRO	García de la Torre et al. (2005)	.../multihydro/multihydro1c.htm	Generates a number of conformations of a given bead model for which solution parameters are then computed with HYDRO++
HYDFIT	Ortega et al. (2011a)	.../hydfit/hydfit.htm	Searches for the best fit structure generated by MULTIHYDRO by comparing the computed solution parameter landscape with experimentally determined values
MONTEHYDRO	García de la Torre et al. (2005)	.../montehydro-4/montehydro-4.htm	A Monte Carlo method is used to generate different conformations of models comprising beads joined by conceptually flexible connectors for which solution parameters are computed and reported as the MC conformational average
SIMUFLEX	García de la Torre et al. (2009)	.../simuflex-4/simuflex-4.htm	Directly simulates the internal dynamics of a bead-and-connector model using a Brownian dynamics simulation engine

body modelling), MONTEHYDRO (García de la Torre et al. 2005) and SIMUFLEX (García de la Torre et al. 2009) (respectively, for Monte Carlo and Brownian dynamics modelling of flexible systems) and HYDFIT (Ortega et al. 2011a) for rigid body modelling of multiple conformations of a given model follows next. While the discrete molecular dynamics (DMD (Dokholyan et al. 1998; Ding and Dokholyan 2006)) module of US-SOMO permits the application of a type of MD to gain some understanding about the conformers that are consistent with HARPs, flexibility is best modelled with the Brownian dynamics approach in SIMUFLEX. The final chapter, by Sergio Aragon (Chap. 12), describes the boundary element (BE) algorithm for computation of HARPs for models whose surface comprises a triangular patchwork constructed from atomic coordinates (e.g. PDB files). HARPs are computed for models of decreasing triangle size, extrapolating values to the case of infinitely small triangles to arrive at the final outputs, in a manner analogous to the bead/shell-model concept implemented in the HYDROPRO (Ortega et al. 2011b) method and other programs in the HYDRO suite of García de la Torre and colleagues.

9.2   Hydrodynamics 101: A Simple Tutorial

Imagine having determined a sedimentation coefficient (*s*) and/or translational diffusion coefficient (D_t) for a macromolecule for which you have very limited other structural data apart from the molecular weight (*M*, determined via, e.g., sedimentation equilibrium or known from the primary structure). What can these parameters tell you? Immediately, you can assess them in terms of what they would tell you about your macromolecule if it was a sphere and was "anhydrous". As described by Uchiyama and Arisaka in Chap. 1, the Svedberg equation

$$s = \frac{M\,(1 - \bar{v}\rho)}{N_A f} \tag{9.1}$$

relates *s* to the frictional coefficient *f* via *M*, the buoyancy factor ($(1 - \bar{v}\rho)$ where $\bar{v}$ is the partial specific volume of the molecule and ρ the solvent density), and Avogadro's number (N_A). Stokes' law

$$f_0 = 6\pi\eta_0\sigma \tag{9.2}$$

describes the dependence of the frictional coefficient of a sphere (f_0) on its radius σ and the solvent viscosity η_0. Knowing that the volume of a sphere with the same *M* and $\bar{v}$ as the molecule is

$$V_a = \frac{M\bar{v}}{N_A} \tag{9.3}$$

an expression for σ can be introduced to Eq. (9.2), and the resultant expression for f_0 can be substituted into Eq. (9.1), i.e.

$$s = \frac{M(1-\bar{v}\rho)}{N_A 6\pi\eta_0}\left(\frac{4\pi N_A}{3M\bar{v}}\right)^{\frac{1}{3}} \tag{9.4}$$

This is the maximum possible sedimentation coefficient a molecule could have – any contribution to the frictional behaviour of the molecule arising from the deviation of its shape from a sphere (which is the most hydrodynamically efficient shape) is neglected, as are any hydration effects. What is meant by hydration in the context of hydrodynamic modelling is discussed briefly in Sect. 9.3.1 and more fully by Aragon in Sect. 12.3. Simplistically, in order to obtain agreement between computed (anhydrous) values for s and their (solvated) experimental counterparts, 0.3–0.4 g water/g protein is included in hydrodynamic models for proteins. A very full account of the various ways in which hydrodynamic hydration is modelled is given in section 5 of Byron (2008).

Nonsphericity and hydration will both decrease the sedimentation coefficient. Interpreting s in this way depends on a knowledge of $\bar{v}$, which in turn is difficult to measure but can be computed (e.g. from the $\bar{v}$ of constituent residues with, e.g., SEDNTERP (http://sednterp.unh.edu/) (Laue et al. 1992)). But for some macromolecules, the number and type of constituent residues are not well known (e.g. glycoproteins purified from animal serum), making computation of $\bar{v}$ unreliable. The sedimentation coefficient also depends on M, but this can normally be determined with far less uncertainty than $\bar{v}$. The translational diffusion coefficient, on the other hand, does not suffer from this problem since, from the Stokes-Einstein equation for a sphere,

$$D_t = \frac{RT}{N_A f} \tag{9.5}$$

Hence, D_t should be the better parameter of the two for hydrodynamic modelling studies, assuming it can be determined from sedimentation velocity experiments with the same precision that is achievable for the measurement of s.

But we must be able to do better than this. Some molecular systems can be modelled by general triaxial ellipsoids (Harding 1982), for which the exact frictional behaviour is also well known, but many cannot be satisfactorily represented with such regular solid shapes. Instead, it is usual to represent the macromolecule as an assembly of elements for which the solution behaviour is well defined and to derive equations that satisfactorily describe their collective interaction with the solution environment. At this point, it is useful to mention the two extremes of solvent behaviour at the macromolecular surface, termed "stick" and "slip". In the "stick" boundary condition, the solvent at the macromolecular surface has zero velocity and sticks to or moves with the macromolecule. This is in contrast to the alternative "slip" condition at the molecule/solvent boundary where the component of solvent velocity perpendicular to the molecular surface is zero, but the tangential component

is unconstrained and so the solvent slips past the molecular surface. The stick boundary condition is appropriate for macromolecules that are far larger than the solvent molecules, whereas the slip condition better represents the HARPs of much smaller molecules whose size is comparable to that of the solvent (see Aragon, Sect. 12.2).

Considering the case of the frictional interaction of a macromolecule moving in a solution (directly relevant to the translational diffusion coefficient (D_t) and s measured by AUC), the general expression relating the frictional force $\mathbf{F}$ on the molecule to its velocity $\mathbf{u}$ is, for a sphere,

$$\mathbf{F} = -f\mathbf{u} \tag{9.6}$$

Kirkwood (1949, 1954) established the first general theory of "irreversible" (e.g. transport) processes in solutions of macromolecules and devised equations that facilitated the computation of D_t, intrinsic viscosity $[\eta]$ and parameters determined by electric birefringence. Macromolecules were represented by three-dimensional arrays of N spherical beads of radius σ_i ($i = 1$ to N) in a solvent of viscosity η_0. The force exerted on the ith bead by the solvent is the product of the frictional coefficient (f_i) for that bead and the velocity of that bead relative to the solvent:

$$\mathbf{F}_i = -f_i (\mathbf{u}_i - \mathbf{v}_i) \tag{9.7}$$

where $f_i = 6\pi\eta_0\sigma_i$ and $\mathbf{u}_i$ is the velocity of the ith bead while $\mathbf{v}_i$ is the velocity the solvent would have at the centre of that bead were that bead absent from the system. This is an equation of frictional drag but neglects the motion of the other elements in the model (beads $j = 1$ to N; $j \neq i$) that perturbs the solvent flow pattern. Oseen (1927) and Burgers (1938) had already derived a correction for this perturbation for stick boundary conditions, as follows:

$$\mathbf{F}_i = -f_i \left(\mathbf{u}_i - \mathbf{v}_i^0\right) - f_i \sum_{j=1}^{N} \mathbf{T}_{ij}\mathbf{F}_j \tag{9.8}$$

where $\mathbf{v}_i^0$ is the velocity the solvent would have at the centre of the ith bead were *all other* beads to be absent – i.e. the unperturbed velocity of the solvent – and $\mathbf{T}_{ij}$ is the hydrodynamic interaction tensor, which is at the heart of hydrodynamic bead modelling computations. A tensor is a geometric object that describes a relationship between vectors (geometric quantities with magnitude and direction, e.g. $\mathbf{F}$, $\mathbf{u}$, $\mathbf{v}$), scalars (real numbers with magnitude only, e.g. f) and other tensors (e.g. $\mathbf{I}$ below). When a vector force is applied to a material system and the response of that system to the force is a vector that does not point in the same direction as the original force, then that response is described by a matrix of numbers instead of a scalar. This matrix is a tensor. Oseen (1927) described the hydrodynamic interaction tensor as

follows:

$$\mathbf{T}_{ij} = \frac{1}{8\pi\eta_0 R_{ij}}\left(\mathbf{I} + \frac{\mathbf{R}_{ij}\mathbf{R}_{ij}}{R_{ij}^2}\right) \tag{9.9}$$

where $\mathbf{R}_{ij}$ is the distance vector between the centres of beads i and j, R_{ij} is the distance and $\mathbf{I}$ is the unit tensor:

$$\mathbf{I} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \tag{9.10}$$

Equation (9.9) is equivalent to Eq. (9.2) in Aragon, Chap. 12, and Eq. (9.8) is an approximate form of the exact integral formulation of hydrodynamics for infinitesimal surface elements (Eq. 9.1 in Aragon, Chap. 12), one of the unique features of which is that either stick, slip or mixed boundary conditions can be addressed, while bead modelling is currently limited to stick boundary conditions.

In order to compute a measureable parameter such as D_t or s for a hydrodynamic model, the hydrodynamic interaction equation (Eq. 9.8), which is actually a system of N linear equations with $3N$ unknowns, has to be solved. This is usually done by inversion of a coefficient supermatrix of N^2 blocks with dimension 3×3. The computational time taken for this supermatrix inversion follows an approximate cubic dependence on the number of elements comprising the model (i.e. N^3). Since the advent of hydrodynamic modelling, computers have become unimaginably fast so that this dependence on N^3 (and its associated memory requirement) is a consideration only for very large molecules or complexes comprising many elements.

There are limitations to the expression for the hydrodynamic interaction tensor (Eq. 9.9) in the case of bead modelling: it does not take into account the finite volume (and thus the radius) of the beads; it is restricted to beads of equal radius and the beads cannot overlap. Since this tensor does not take into account the bead radius, it is not possible for it to "sense" any bead overlap. This instead becomes an issue when the tensor does take into account the finite bead size, as is the case for the hydrodynamic interaction tensor devised by Rotne and Prager (1969) and Yamakawa (1970) for *overlapping* beads of *equal* radius σ:

$$\mathbf{T}_{ij} = \frac{1}{6\pi\eta_0\sigma}\left(\left(1 - \frac{9R_{ij}}{32\sigma}\right)\mathbf{I} + \frac{3\mathbf{R}_{ij}\mathbf{R}_{ij}}{32\sigma R_{ij}}\right) \tag{9.11}$$

whereas the Oseen tensor (Eq. 9.9) was extended by García de la Torre and Bloomfield (1977) to account for *non-overlapping* beads of *differing* radii:

$$\mathbf{T}_{ij} = \frac{1}{8\pi\eta_0 R_{ij}}\left(\mathbf{I} + \frac{\mathbf{R}_{ij}\mathbf{R}_{ij}}{R_{ij}^2} + \frac{\sigma_i^2 + \sigma_j^2}{R_{ij}^2}\left(\frac{\mathbf{I}}{3} - \frac{\mathbf{R}_{ij}\mathbf{R}_{ij}}{R_{ij}^2}\right)\right) \tag{9.12}$$

There remains no tensor to describe the hydrodynamic interaction between *overlapping* beads of *differing* radii. There have been successive incremental developments of the theory underlying and formulation of the hydrodynamic interaction tensor. One notable correction to it is known as the "volume correction" (García de la Torre and Rodes 1983) which overcomes a deficiency that becomes obvious when hydrodynamic bead models are dominated by a small number of beads whose volume is comparable to that of the entire macromolecule. The correction term (important in the computation of $[\eta]$ and rotational diffusion coefficient (D_r), only) is proportional to the volume of the constituent beads. Regardless of any of the corrections mentioned here, bead modelling hydrodynamic interaction tensors usually assume stick boundary conditions and are approximations to what is an infinite series of powers in the inverse of R_{ij}.

The Oseen and Burgers tensor (Eq. 9.9), however, gives the exact hydrodynamic interaction between two points on a molecular surface and is the starting point for the computation of HARPs by boundary element (BE) modelling (Aragon, Chap. 12) under stick boundary conditions.

9.3 Current Approaches

9.3.1 Rigid Body Modelling

The HYDRO suite of programs (see García de la Torre, Chap. 11) for the computation of HARPs follows on from the pioneering work by García de la Torre and colleagues from the late 1970s onwards (see, e.g., García de la Torre and Bloomfield (1981)). The first in the suite was HYDRO (García de la Torre et al. 1994), the forerunner of the currently used version HYDRO++ (García de la Torre et al. 2007), a general-purpose program that enables the calculation of HARPs for rigid macromolecules and colloidal particles that can be represented as bead models. Hydrodynamic parameters are computed by HYDRO++ by solving the equation for frictional drag with hydrodynamic interaction (Eq. 9.8) as described above for any particle that can be represented as a three-dimensional bead array. The user generates and supplies to HYDRO++ the Cartesian coordinates and radii of the composite beads which must include some volume to account for hydrodynamic hydration (see below for more on hydration and section 5 of Byron (2008) for a very full consideration of the topic).

Bead model coordinates are easily derived when (1) the model is based on atomic coordinates from a crystal or NMR structure (in which case the use of HYDROPRO ((Ortega et al. 2011b), below), US-SOMO ((Brookes et al. 2010a, b), below) or BEST ((Aragon 2004; 2011), below) is more appropriate) or (2) electron microscopy density maps (when HYDROMIC ((García de la Torre et al. 2001), below) can be used) or (3) the particle can be reliably represented by a geometric shape which can, in turn, be defined by an equation and populated with spheres by

HYDROPIX ((García de la Torre 2001a), below) or (4) *AtoB* (Byron 1997) is used to construct a bead model *de novo* (see, e.g., Byron (2008)).

US-SOMO (Brookes and Rocco, Chap. 10) offers a choice of utilising the same hydrodynamic interaction tensors as HYDRO++ (as originally implemented in the BEAMS suite (Spotorno et al. 1997)) or the alternative *Zeno* (Kang et al. 2004) method with two alternative methods for bead model construction. The so far more frequently utilised of these is the *SoMo* algorithm (Rai et al. 2005) that generates medium-resolution bead models from the atomic coordinates of biomacromolecules by placing a bead of volume equal to the sum of the constituent atom volumes at the centre of mass of the main-chain segment of each residue (or equivalent) and a second bead at a defined position for the side-chain segment depending on its chemical characteristic. The resultant models comprise about one-quarter the number of atoms in the original Protein Data Bank (PDB, (Berman et al. 2000)) file. The volumes of the beads are increased in order to include water of hydration for particular residues according to the data of Kuntz and Kauzmann (1974) for proteins (and other data for different kinds of biomacromolecules; see Brookes and Rocco, Chap. 10). Overlaps between beads are removed in either a hierarchical or a synchronous process that tries to maintain the topography of the original outer surface and so maximises the reliability of the subsequently computed HARPs (Brookes et al. 2010a, b; Rai et al. 2005). Because *SoMo* also determines which beads of the resultant model are solvent exposed (and thus contribute to the frictional interaction with solvent), buried beads can be excluded from core hydrodynamic calculations, increasing the size limit and speed with which hydrodynamic computations can be completed. US-SOMO also accepts as input models of the type generated from SAXS or SANS data by the *ab initio* dummy atom or residue modelling programs DAMMIF (Franke and Svergun 2009) and GASBOR (Svergun et al. 2001). This is particularly useful in applying, e.g., *s* as a restraint on *ab initio* modelling: if *s* and/or D_t computed for a (hydrated) dummy atom/residue model disagrees with the experimentally determined value, the model is likely to be incorrect.

Less frequently utilised is the *AtoB* (Byron 1997) algorithm that conceptually superimposes a three-dimensional grid of user-defined resolution onto the molecular structure and places one bead at the centre (or centre of mass) of each cubic element of the grid with a volume corresponding to the atoms contained within that cube. *AtoB* is useful for the construction of (appropriately hydrated) bead models for very large molecular complexes in order to economise on CPU time in subsequent hydrodynamics computations – especially if many conformations of the molecule are to be assessed after, e.g., discrete molecular dynamics (DMD) calculations (see below and Brookes and Rocco, Chap. 10).

Probably the most utilised of the HYDRO suite of programs is HYDROPRO (Ortega et al. 2011b) which (in one mode) constructs shell models, composed of very small beads, from atomic coordinates (from PDB files) and computes HARPs for shell models of decreasing sphere size (and increasing sphere number), extrapolating the resultant values to the case of infinitely small spheres to generate the finally reported values for subsequent comparison with, e.g., experimentally

determined parameters. The primary hydrodynamic model on which the shell model is built is characterised by an adjustable parameter, the radius of its constituent beads (the atomic element radius (AER)). The choice of this radius was important in providing a basis for empirically adjusting the anhydrous HARPs computed by HYDROPRO to values that agreed with those experimentally observed. The currently recommended value for globular macromolecules is 2.9 Å. This adjustment is historically and usually referred to as hydration, but, as pointed out by Halle and Davidovic (2003) and expounded at length by Aragon in Sect. 12.3, the residence time of water molecules at the surface of a protein is of the order of 50 ps, regardless of the chemical nature of the residue side chain. There is, thus, unlikely to be a layer of water that associates or moves with a sedimenting (or tumbling) protein. And the increase in experimental f (or decrease in s) compared with that computed for a hydrodynamic model is likely to arise instead from a difference in the viscosity of the first solvation layer, compared with the bulk solvent. This in turn is the result of the rough and dynamic nature of the protein surface where the side-chain atoms "jostle" water molecules in this solvation layer. It is a fortunate coincidence that assuming a number of "bound" waters nearly exactly compensates for this local viscosity change effect (Halle and Davidovic 2003). Interestingly, the value of 2.9 Å, currently recommended as the AER in HYDROPRO, is equal to the sum of a typical protein constituent atom van der Waals radius (see, e.g., Tsai et al. (1999)) and 1.1 Å, which happens to be the thickness of the "hydration layer" utilised by BEST (see next paragraph).

While surface modelling by HYDROPRO with default settings that limit the maximum number of shell beads used offers a computational time advantage over programs that convert the entire atomic resolution structure to beads (e.g. US-SOMO, albeit recognising the exclusion from *SoMo* models of non-surface beads that do not contribute to the frictional interaction with the solvent), this advantage is lost in the need to repeat the HARP computations for a series of surfaces comprising increasingly small beads prior to the extrapolation to zero bead size. For macromolecules that can be represented in *SoMo* or *AtoB* with up to 2000 exposed beads (the default maximum number of shell beads in HYDROPRO), HYDROPRO is slower than *SoMo* or *AtoB* followed by supermatrix inversion computation or *Zeno* computation, although HYDROPRO can be operated in a "one-bead-per-residue" mode which is extremely fast for moderately sized proteins for which this number of beads is not too large. For extremely large models, the computing time in US-SOMO can be reduced by using *AtoB* with a suitably large grid size to decrease the number of beads comprising a given model.

BEST (BE modelling under stick boundary conditions) (Aragon 2004, 2011) is conceptually similar to HYDROPRO in that the surface of the macromolecule is discretised, in order to facilitate the solution of the integral form of Eq. (9.8), not by dividing its volume into beads but instead by covering it with a patchwork of N very small triangles. From this, it computes HARPs, for surfaces comprising triangles of increasing number and decreasing size, and extrapolates the HARPs to the case of infinitely small triangles in order to obtain values for comparison with experimentally determined counterparts. Any differences in approaches

to discretisation used by BE and the bead/shell-model concept implemented in HYDROPRO and related programs should largely vanish upon extrapolation to zero element size. In order to match computed HARPs with experimental values, BEST applies a default 1.1 Å uniform layer of conceptual hydration (see Aragon, Sect. 12.3) for globular monomeric proteins. This 1.1 Å does not reflect the actual thickness of the solvation layer that is perturbed by the residue side chains but is simply the magnitude required to adjust the HARPs computed with stick boundary conditions to a level that agrees with a set of experimentally determined values. Some multimeric proteins may require a higher level of "hydration" (for reasons proposed by Aragon, Chap. 12), and HARPs for small rigid molecules, whose size is comparable to that of the solvent molecules, are best computed with slip boundary conditions since their atoms perturb the solvent very little and it is free to simply "slip" past. An important difference between BE and bead modelling is that BE modelling requires no approximations to account for bead overlaps or the "volume correction". Therefore, computed HARPs are very precise. But BEST is computationally more intensive, and, while US-SOMO offers an interface to BEST, it currently does this only via cluster access. However, a Windows OS 64-bit command line version of BEST that runs on a single fast processor with 8 GB RAM is available separately (see Aragon, Chap. 12).

In HYDRONMR (García de la Torre et al. 2000), the rotational diffusion tensor ($\mathbf{D}_{rr}$) and the coordinates of the centre of diffusion are combined with the atomic coordinates of the experimental particle to compute the rotational correlation time (τ_c) and the NMR relaxation times (T_1 and T_2) for each residue. As for HYDROPRO (Ortega et al. 2011b), calculations in HYDRONMR are based on a shell model and the assumption is made that relaxation stems only from the modulation of dipolar couplings and chemical shift anisotropy by global tumbling. Bernadó and colleagues (2002) noticed that if the AER was optimised to maximise the agreement between calculated and experimental T_1/T_2 ratios, it became diagnostic of "problems" with the molecular system: larger values indicative of oligomerisation or aggregation and smaller values stemming from models that do not adequately describe solution molecular conformation.

In the absence of atomic resolution coordinates, HYDROMIC (García de la Torre et al. 2001) can construct bead models from 3D reconstruction (e.g. SPIDER or MRC) files generated from cryo-electron microscopy data. It assigns constituent voxels to the particle according to a selected threshold and calculates their Cartesian coordinates. The voxels are then converted to beads, yielding a primary hydrodynamic model for subsequent shell modelling for the computation of hydrodynamic and related parameters. If even cryo-EM data are lacking for a system of interest, HYDROSUB (García de la Torre and Carrasco 2002) can be used to generate shell models of ellipsoids and/or cylinders from which models based on user-defined parameters such as subunit dimensions and coordinates of subunit centres of mass and polar angles that define the orientation of the major symmetry axis can be composed and for which solution parameters can be computed. Last in this sequence of decreasing model "resolution" is HYDROPIX (García de la Torre 2001a) wherein the shell model, for which solution parameters are computed, is generated from a

solid geometrical shape which is constructed according to a user-supplied formula by the ancillary program MAKEPIXB. In its original inception, *AtoB* could also be used to construct *de novo* a bead model to represent *any* three-dimensional shape via a combination of geometric operations (including, e.g., add a new bead; delete a bead; move a bead or a subset of beads by a defined distance in x-, y- and/or z-space; rotate a bead or a subset of beads about a defined origin or axis; create a circular array of defined radius of touching beads; expand a bead or a subset of beads). This functionality will shortly be reintroduced to *AtoB* together with an alternative "drag-and-drop" tool in the GUI (E. Brookes, personal communication). The coordinates of the finalised model can then be introduced to US-SOMO or HYDRO++ for hydrodynamic computation.

A number of different conformations of a given bead model can be evaluated in a single run of the program MULTIHYDRO (García de la Torre et al. 2005) which produces the conformers and ports them to whichever of the HYDRO suite of programs is to be used to compute the solution parameters. The results are then evaluated by the program HYDFIT (Ortega et al. 2011a) which, like an earlier program *Rayuela* by Nöllmann and colleagues (2004, 2005), searches for the best fit structure by comparing the computed solution parameter landscape with the experimentally determined values. A similar tool (model classifier; see Brookes and Rocco, Sect. 10.4) is incorporated into US-SOMO for selection of the best-fitting models when HARPs for, e.g., a range of conformations have been computed.

The *Zeno* (Kang et al. 2004) method can be used to compute f, electrostatic capacity, $[\eta]$, intrinsic conductivity and electrical polarisability of arbitrarily shaped objects. The electrostatic analogy used by *Zeno* to compute HARPs does not generate tensor values of translational diffusion and is an approximate method but a good one. From f, D_t can be directly computed utilising the Stokes-Einstein relation ($D_t = kT/f$). *Zeno* encloses the test object (i.e. a macromolecule, presented to the program in its properly hydrated form, e.g., as a *SoMo* or *AtoB* model) in a sphere from whose internal surface it launches a series of random walks which eventually (after a number of steps) either reach the molecular surface or return to the sphere surface at which point the walk is either terminated or restarted. Computed parameters are determined from the fraction of random walk trajectories that reach the molecule surface. The procedure computes the electrostatic capacity and electrostatic polarisability of a perfect conductor having the same size and shape as the model. From the electrostatic capacity, f can be computed and has been shown to be accurate within 1 %. From the electrostatic polarisability, $[\eta]$ can be computed to within 2–3 % (Mansfield and Douglas 2008). The method can be used on bead models or atomic structures defined as bead models (e.g. utilising van der Waals radii). Importantly, the bead models may contain overlaps, and the individual beads can be arbitrarily sized, allowing high-resolution structures to be processed. Additionally, the required computation time scales *linearly* with the number of random walk trajectories, the number of beads (N) or the molecular volume (Kang et al. 2004), as opposed to cubically (N^3) as in methods solving the system of equations for frictional drag with hydrodynamic interaction (e.g. the HYDRO programs, US-SOMO or BEST) making the *Zeno* computation of f

for high-resolution bead models relatively fast and feasible. The *Zeno* method is available in US-SOMO (see Brookes and Rocco, Sect. 10.3).

9.3.2   Flexible Body Modelling

The methods so far summarised compute solution parameters for rigid models. What about flexible molecules? There are (at least) four approaches to calculating HARPs for flexible systems. In one, Monte Carlo rigid body (MCRB) modelling (with the program MONTEHYDRO (García de la Torre et al. 2005)), the flexibility is approximated by using an MC method to generate many different (random) conformations of models comprising beads joined by conceptually flexible connectors (characterised by potentials), and solution parameters for the MC sample are computed. This sample comprises models whose averaged solution properties agree with the experimentally determined values. Because the result is an average over conformations, the MCRB approach is appropriate for the evaluation of overall properties like s, D_t, $[\eta]$, R_g and scattering form factors, but it does not model the *internal* dynamics of the system.

An alternative exploration of conformational space is afforded by the discrete molecular dynamics (DMD) approach offered within US-SOMO (see Brookes and Rocco, Sect. 10.5) that, like the MCRB approach, also does not model the internal dynamics of the system but instead allows the generation of numerous conformers of a starting model, delivered by changing the conformation of model segments presented to the program as having the potential to be flexible. The starting model is a PDB file, for which residues that are to remain static (i.e. are non-flexible) are identified. Control parameters for the DMD simulation that generates different conformations of the flexible regions of the model include the Andersen thermostat temperatures, durations, time intervals and number of models to be generated for the relax and run phases of the simulation. The recommended Andersen thermostat temperature is 0.5 kcal/mol/kB where most proteins will not unfold or deviate much from native state. This temperature corresponds to 251 K, although the temperature in these simulations generally does not correspond to the physical temperature at which, e.g., hydrodynamics measurements are made. Since water is not explicitly defined in DMD simulations, the system will not freeze, and there will be sufficient sampling of conformational dynamics near the native states. At higher thermostat temperatures, fluctuations will have larger amplitudes, and the protein might unfold. This is useful in the search for atomic resolution models that are consistent with HARPs for partially disordered proteins. A starting (perhaps fully folded model or actual structure) can be presented to the DMD interface and successively more unfolded versions of the putative partially disordered region generated. These can then be evaluated by computation of their HARPs.

BEST has also been used to model HARPs for flexible macromolecules, again by using carefully selected MD force fields, volumes, temperatures, pressures, salt concentrations and time frames. The precise simulations performed were fully

atomistic with discrete water molecules using the AMBER (Perlman et al. 1995) MD package. HARPs averaged over typically 3000 structures captured from an MD simulation have been shown to provide excellent agreement with experimental data for the case of a monoclonal antibody (see Aragon, Sect. 12.5.3 and references cited therein). MONTEHYDRO, US-SOMO-DMD and BEST-MD share a common approach to model and HARP generation: they generate a set of conformations from a starting model and compute HARPs for these, reporting averaged HARPS according to user preference.

The fourth method considered here is a Brownian dynamics (BD) approach delivered by the program SIMUFLEX (García de la Torre et al. 2009) which directly simulates the internal dynamics of a bead-and-connector model by generating the macromolecular trajectory using Brownian dynamics simulation (with full consideration of hydrodynamic interactions) and then analysing this trajectory to extract the macromolecular HARPs. Unlike the MD simulations used in combination with BEST, the more approximate BD method of SIMUFLEX is not fully atomistic, nor does it use discrete water molecules. It has the advantage, however, of much shorter computation times.

All four approaches are relatively new in the field of hydrodynamic modelling and so examples of their use are few. There is an excellent tutorial mini-review on MONTEHYDRO and also SIMUFLEX that serves as a good starting point (García de la Torre et al. 2010), and the DMD and BEST tools within US-SOMO are explained in the on-line manual.

9.4 Pros and Cons of Current Approaches

Each of the programs previewed in this introduction and described much more fully in the following three chapters has their strengths and weaknesses. There is no such thing as the perfect hydrodynamic modelling program since different molecular systems are better suited by different programs.

For instance, HYDRO++ (García de la Torre et al. 2007), the descendant of the first freely available hydrodynamic bead modelling program HYDRO, appears not to be used as much now as it was prior to the release of programs such as HYDROPRO and US-SOMO for modelling directly from PDB files. However, there remain systems that can best (or only) be described by very simple bead models, for example, dumbbell-shaped polyelectrolyte brush particles (Hoffmann et al. 2008). That said, the same authors (Hoffmann et al. 2009) later chose to replace very simple (four-)bead models for colloidal clusters with shell models generated by and evaluated by HYDROPRO, taking advantage of the concomitant increase in precision of the HARP computations and the more reliable procedure for mimicking hydration.

Of all the currently available approaches to hydrodynamic modelling, HYDROPRO is the least demanding and will accept any properly formatted PDB file as an input, while US-SOMO is more demanding, needing an internal "coding"

of each residue comprising a biomacromolecule to properly translate it into a bead model. While codes are provided for a large number of commonly encountered residues for proteins, nucleic acids, carbohydrates and some lipids, detergents and prosthetic groups, the list is far from being exhaustive, and coding for new residues can be demanding. Furthermore, based on this coding, US-SOMO will check any given input model for missing atoms in coded residues or breaks in the sequence, warning the user and requiring remedial action. However, approximate methods are available in US-SOMO to cope with either non-coded residues or missing atoms within coded residues. This level of scrutiny is not exerted by HYDROPRO, which can therefore generate HARPs that lack precision if the user has not realised that the input PDB is incomplete in some way compared with the experimental macromolecule.

Both HYDROPRO and BEST require extrapolations of parameters computed for shell models with spheres of decreasing radius (HYDROPRO) or for surfaces with triangles of decreasing area (BEST). On occasion, the computed parameters can lie surprisingly far from the line of best fit, and the extrapolation can require the application of statistical treatments in order to gain validity (see, e.g., Brookes and Rocco, Chap. 10). For this reason, it is important to visually inspect the extrapolation prior to finalisation of the result; this is implemented in the US-SOMO BEST interface (see Brookes and Rocco, Sect. 10.6).

Both HYDROPRO and BEST treat hydration water as a uniform layer, while US-SOMO uses a differential hydration scheme that reflects in some way the chemical and hydrodynamic interaction between the macromolecular surface and the solvent. While the uniform layer of HYDROPRO and BEST is a *mimic* of hydration, the layer thickness is a parameter that can be adjusted to render the computed HARPs comparable with the experimentally determined values for a particular category of molecule (e.g. in the case of BEST: monomeric compared with multimeric). This adjustment is not required for the hydration apportioned by US-SOMO where it is as effective for monomeric or multimeric models. That said, if a correct "hydration" level could be determined for BEST modelling, it should provide unparalleled precision thereafter. Hydration in the context of hydrodynamic modelling is currently a topic of intense discussion amongst the main protagonists, and those of us who benefit from their efforts can expect some form of agreement to be reached in the near future.

BEST definitely offers the most precise computation of D_r and $[\eta]$, since it does not require the "volume correction" that plagues bead modelling (see Sect. 9.2 above and García de la Torre and Carrasco (1998)). But BEST is computationally more intensive and therefore currently less practical for the evaluation of many conformations or very large molecules. In this instance, *Zeno* becomes attractive since the time taken for its computations depends linearly in the number of beads used in an input model.

Interestingly, a recent comparison of the hydrodynamic modelling methods available in US-SOMO and HYDROPRO shows that *SoMo* slightly overestimates *s* and D_t, whereas BEST slightly underestimates them and HYDROPRO even more so (Rocco and Byron 2015). But *AtoB* with a 5 Å grid size performed better,

giving computed values that more closely matched the experimentally determined parameters. This notwithstanding, the overall best performance was delivered by bead models generated with *SoMo without* the removal of bead overlaps, for which HARPs were computed using *Zeno*.

Whereas MONTEHYDRO, US-SOMO DMD and BEST-MD provide an exploration of conformational space and essentially deal in conformational averages, flexibility can also be modelled for an individual bead-and-connector model with SIMUFLEX (García de la Torre et al. 2009) using its explicit Brownian dynamics engine.

9.5 Concluding Comments and Outlook

Some questions remain: what is the sensitivity of solution parameters to the conformations on which they report? How much can an individual parameter tell us about molecular conformation? A rapid answer to this is that within limits a single parameter can be used to support or discard a potential molecular model. But a far more satisfactory outcome is arrived at when a model has to reproduce a combination of solution parameters (e.g. MULTIHYDRO or the model classifier in US-SOMO).

How much detail in a model is reasonable or necessary? All of the programs described in this chapter generate s in the form of the ratio (or inverse thereof) of f/η_0. In Sect. 9.2, the dependence of s on other experimental parameters (such as η_0, M, T, $\bar{v}$ and ρ) is explored briefly. Each of these parameters has an error associated with it that will contribute to uncertainty in s. The same will apply to other HARPs. Therefore, any comparison between the various computational procedures in terms of the numerical difference in their predictions should be done with this accumulated uncertainty in mind (see García de la Torre (2001b) for a thorough analysis of error propagation in HARPs). In Chap. 11 (García de la Torre), an important conclusion is reported: the difference in precision in the computation of HARPs for bead models generated from atomic coordinates and those generated on the basis of one bead per residue is comparable with the experimental errors. And in Chap. 12 (Aragon), it is reported that the precision in transport coefficients computed with BE methods (compared with exact results for shapes for which exact or very precise values are available (e.g. ellipsoids of revolution, toroids, etc.)) is 1 %, whereas it is 2 % for rotational parameters and [η] (comparable with the precision reported for *Zeno*). This precision is better than the accuracy with which most HARPs can be experimentally determined.

In the field of small-angle X-ray and neutron scattering (SAXS, SANS), a publicly accessible repository (called SASBDB, http://www.sasbdb.org/) of experimental data and low-resolution models derived therefrom has been established. This is in response to requests by the SAXS/SANS community and a recommendation made by the wwPDB Small-Angle Scattering Task Force (Trewhella et al. 2013). Given the complementary nature of SAXS/SANS and hydrodynamics, the AUC

community should consider whether it could usefully contribute to a project such as SASBDB so that models consistent with hydrodynamic calculation can be made available to the wider community for further evaluation.

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