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S.E. Harding
E. Longman
A. Ortega
K. Kreusel
S.B. Tendler
K. King
J. García de la Torre

Use of the sedimentation coefficient for modelling antibodies. Refinements to the crystallohydrodynamics approach

S.E. Harding (✉) · E. Longman
National Centre for Macromolecular Hydrodynamics, School of Biosciences, University of Nottingham, Sutton Bonington LE12 5RD, UK
e-mail: steve.harding@nottingham.ac.uk
Tel.: +44-115-9516148

A. Ortega
J. García de la Torre
Departamento de Quimica Fisica, Facultad de Quimica, Universidad de Murcia, 30071 Murcia, Spain

K. Kreusel
S.B. Tendler
Pharmaceutical Sciences, University of Nottingham, Nottingham NG7 2GG, UK

K. King
Pfizer Limited, Sittingbourne, Kent ME9 8AG, UK

Abstract In a previous paper (Carrasco B, García de la Torre J, Davis KG, Jones S, Athwal D, Walters C, Burton DR, Harding SE (2001) *Biophysical Chemistry* 93:181–196) we introduced the concept of "Crystallohydrodynamics" for modelling the conformation of antibodies in solution and allowing for the so-called hydration problem. We now make some further improvements to allow for the hydrated dimensions of the domains before assembling models for the intact antibody using the algorithms HYDROSUB and SOLPRO. The procedure is illustrated by application to a human immunoglobulin construct of the subclass IgG4 from the mouse IgG1 (γ4P).

Keywords Bead-shell · Uniqueness · Hydration

Introduction

Although high-resolution crystal structures are readily available for the "fragment crystallisable" (Fc) and "fragment antigen-binding" (Fab) (also crystallisable) domains of the IgG and now IgE classes of immunoglobulins, the structure determination for intact, immunologically active antibodies has proved elusive, except for a few cases: hinge deleted mutants or antibodies with a short hinge region [1, 2, 3]. A further drawback is that the upper limit of molar mass in high-resolution NMR studies is around 50,000 Da, thus ruling this approach out for the study of the high-resolution structure of the intact molecule for molar masses of more than 146,000 Da.

Hydrodynamic methods can give low-resolution structural information in terms of orientation of domains. An early successful attempt was the first demonstration, using hydrodynamic bead modelling and the algorithm TRV [4], that IgE was cusp shaped [5]. The classical hydration problem (namely hydrodynamic parameters like the frictional ratio also depend on the time-averaged hydration as well as the conformation) was dealt with by comparison of the hydrodynamic properties of the IgE molecule with those for the hingeless mutant IgGMcg molecule, whose crystal structure was known.

An improved method of dealing with the hydration problem was given for modelling the IgG subclasses in the "crystallohydrodynamics" approach introduced earlier [6], which employed

1. The latest bead–shell approach (HYDRO/SOLPRO) for surface modelling the domains as surface ellipsoids.
2. The known crystal structures of the IgG Fab and Fc domains, which, when combined with measured

hydrodynamic properties of these domains, allowed a better estimate of the apparent time-averaged hydration of the domains (and, hence, from a weighted average, the intact antibody) to be made.

δ_{app} is referred to as "time-averaged" in the sense that hydration is a dynamic rather than a static process. It is referred to as an "apparent" hydration because besides volume effects it also includes the contribution of all other nonconformational and non-mass factors to the frictional coefficient. The value calculated for δ_{app} depends not only on the true hydration but is also affected by the fact that the domains are not true ellipsoid structures, the domains have considerable surface rugosity, and small imperfections in the bead model approximation exist (the hydrodynamic parameters for a bead and bead–shell model cannot be calculated exactly, as they can for ellipsoids). The δ_{app} for intact IgG antibody molecules was thus evaluated and this value, when combined with the experimentally measured translational frictional ratio of the intact antibody molecule, provided a route to obtaining the solution conformation of the intact molecule.

We now present a further refined approach which takes into account the hydrated dimensions of the domains.

Low-resolution information obtained

As before we would like to stress at the outset that although high-resolution information can be entered into hydrodynamic analysis, high-resolution information is not returned, a limitation which unfortunately is not always appreciated. Furthermore, an important drawback that limits the application of hydrodynamic methods is the influence of nonconformational parameters on the hydrodynamic properties – the most important of these is the time-averaged effect of water association with the protein or "hydration" – which is very difficult to estimate with any precision. Another drawback is that the most sophisticated shape for which hydrodynamic parameters can be calculated exactly is still the ellipsoid [7, 8]: although the overall conformation of antibody domains may be represented by equivalent ellipsoids, the intact immunologically active structure cannot be represented by either axially symmetric (prolate/oblate "ellipsoids of revolution") or centrally symmetric ("general triaxial ellipsoid") shapes. Good approximations are available in terms of multiple-sphere array or "bead models", and have been thoroughly checked against ellipsoids, yielding errors of no worse than around 2% for parameters based on the translational frictional property. Bead modelling strategies can therefore be applied with considerable confidence for representing domain orientations of antibodies.

For a typical globular protein, fine structural details (crevices, pockets, protrusions, etc.) can make a relatively large contribution to the hydrodynamics. However, for multisubunit structures (antibodies are a paradigmatic example) it seems evident that the main aspect is the arrangement of the subunits: whether or not there is a hinge (which may be flexible), and whether the conformation is more open or closed. As mentioned before, there is the complicating effect of hydration; therefore, it is really justified to reduce the complexity of the problem, by making structural approximations for the subunits, thus allowing us to concentrate on their spatial arrangement. This approach also greatly facilitates the modelling of the flexibility between domains [9].

Background to modelling methods

For the last 2 decades, bead modelling approaches have been popular for modelling the hydrodynamic properties of macromolecules, with the overall shape of the macromolecule being represented as an array of spheres of either uniform or nonuniform size [4, 10, 11, 12, 13, 14]. An alternative approach is to represent only the surface of the particle with identical "minibeads", in what is known as a bead–shell model. Such bead–shell models are not only valuable for calculating hydrodynamic parameters, but a decrease in the size of the beads enables a closer representation of the surface [15]. The hydrodynamic properties of multiple sphere arrays – beads or shells, although approximate, can be calculated to a high but finite degree of accuracy (normally better than around 2%).

In the studies of Carrasco and coworkers [6, 16] the main parameter used for representing the solution conformation for the human IgG antibodies was the Perrin function, P, or "translational frictional ratio due to shape". The Perrin function is a universal shape parameter, meaning it is independent of the size of the particle, and therefore the shape of the particle alone determines the value. In order to obtain the Perrin function of a particle, its sedimentation coefficient must first of all be combined with the partial specific volume and molar mass (molecular weight), in order to gain the translational frictional ratio, f/f_o. The translational frictional ratio is defined as "the ratio of the frictional coefficient of the macromolecule to that of a sphere of the same mass and anhydrous volume". In terms of experimental parameters

$$f/f_o = [M(1 - \bar{v}\rho_o)/N_A s^o_{20,w}]/[6\pi\eta_0(3M\bar{v}/4\pi N_A)^{1/3}], \quad (1)$$

in which M is the molar mass (grams per mol), $\bar{v}$ is the partial specific volume (millilitres per gram), N_A is Avogadro's constant (6.0221×10^{23} mol^{-1}) and $s^{\circ}_{20,w}$ is the sedimentation coefficient (seconds or Svedbergs) corrected to the standard conditions of density (ρ_0, grams per millilitre) and viscosity (η_0, poise) of water at 20.0 °C, and extrapolated to infinite dilution.

In order to find the Perrin function value from the translational frictional ratio, the time-averaged apparent hydration value (δ_{app}, in grams of water per gram of protein) for the particle must be known:

$$P = (f/f_o)[1 + (\delta_{app}/\bar{v}\rho_o)]^{-1/3}. \quad (2)$$

With the hydration value already being available for the Fab domain [16], Carrasco et al. [6] used the same approach to estimate the hydration value for the Fc domain. This approach was to fit an inertial triaxial ellipsoid to the surface of the crystal structure of the Fc domain. The two axial ratios a/b and b/c [6, 16] from this ellipsoid are then entered into the routine ELLIPS2 [8], which specifies the value of P. When piecing the molecule together, ellipsoids cannot be used directly, but have to be converted to surface bead–shell models for the hydrodynamically equivalent ellipsoids of revolution. Carrasco and coworkers [6, 16] showed that the calculated P values for the bead–shell models were in excellent agreement with those for ellipsoid models. Combining the frictional ratio of the particle with its Perrin function gives the apparent hydration value, δ_{app} (Eq. 2). The apparent hydration value for the intact antibody can therefore be found once the apparent hydration values for the Fc and Fab domains are known. P, and the experimental f/f_o for each domain then yield δ_{app}. δ_{app} for the whole antibody is approximately $[2\delta_{app}(\text{Fab}) + \delta_{app}(\text{Fc})]/3$. This value, combined with the experimentally measured f/f_o for the intact antibody then yields an experimental P, which can then be compared with the P values calculated for the various bead models.

Using this procedure Carrasco et al. [6] found, for example, IgG4 to be effectively T-shaped and hingeless, resembling the hingeless mutant antibody IgGMcg, shown to be in agreement with X-ray crystallography [1].

The refined crystallohydrodynamics approach

The same approach as introduced before [6] is utilised to estimate the apparent time-averaged hydration of an IgG antibody, i.e., from representing the crystallographic structure of the individual domains as a smooth ellipsoidal surface shell–bead model, calculating the Perrin translational frictional ratio due to shape, P, and comparing this with the experimentally obtained translational frictional ratio, f/f_o, to give the apparent time-averaged hydration, δ_{app}, for each domain. Hence δ_{app} of the intact IgG can be estimated on the basis of IgG having two Fabs and one Fc. This value is necessary to calculate the experimental P from f/f_o for the intact antibodies, whose crystal structures are generally not known. The refinement we make here is that when modelling the assembled domains, even though P is size-independent, we take into account the effect of different hydrations on the relative dimensions of the domains, i.e., we find the dimensions of the ellipsoids of revolution, whose hydrodynamic properties mirror those of the antibody fragments they are representing, and the algorithm HYDROSUB is used to specify the surface bead coordinates of the ellipsoidal surfaces used for the Fc and Fab domains.

Finding the relative dimensions of the hydrated domains

For an ellipsoid of revolution of axial ratio $p = a/b$ ($a > b$), with a prolate ellipsoid having semiaxial dimensions (a,b,b), and an oblate ellipsoid having the dimensions (a,a,b), a and b can be obtained provided the axial ratio and the hydrated volume of the particle, V_{hyd}, are known:

$$V_{anh} = M\bar{v}/N_A, \quad (3)$$

$$V_{hyd} = (M\bar{v}/N_A)(1 + \delta/\bar{v}\rho). \quad (4)$$

For a prolate ellipsoid,

$$V_{hyd} = (4/3)\pi b^3 p. \quad (5)$$

For an oblate ellipsoid,

$$V_{hyd} = (4/3)\pi a^3/p. \quad (6)$$

The axial ratio p is not to be confused with the Perrin function P. We have to assume the effect of hydration does not change the axial ratio significantly of course, but if we are using the value for p obtained from the Perrin function and δ (approximated as δ_{app}) both according to the crystallohydrodynamic procedure we can obtain an estimate for a and b for both the Fab domain (represented as a prolate model) and the Fc domain (represented as an oblate model).

Method for construction of bead models: HYDROSUB

Another improvement to our earlier [6] approach is that we can now take advantage of using the computer program HYDROSUB [15], for the construction of the bead models. HYDROSUB has the capacity to build models of multisubunit macromolecules, by modelling

them as structures composed of ellipsoids of revolution and cylinders: the coordinate data can then be interfaced directly into the algorithm SOLPRO for the calculation of the hydrodynamic properties and shape functions – including the Perrin P parameter, corresponding to the bead model and domain arrangement.

To build the bead–shell model, the program requires the input of four pieces of information. For each ellipsoid, HYDROSUB requires (1) the dimensions a and b, (2) the three Cartesian coordinates that define the position of the centre, and (3) the two polar angles θ and ϕ that define the orientation of the main axis of the ellipsoid. There is another improvement on the earlier approach where we used three Euler angles [6]; we have realized (and this is implemented in HYDROSUB) that for an axisymmetric particle the orientation is defined by just two spherico-polar angles, θ and ϕ (Fig. 1).

We now provide an example of how the IgG model for HYDROSUB can be constructed (Fig. 1). Fc is represented as an oblate ellipsoid, and then we place its equatorial circle on the (y,z)-plane, with its centre on the negative part of the z-axis, at a distance, d_3, from the origin equal to (or slightly greater than, if we wish to consider some spacing, hinge, etc.) the longest semiaxis. Thus, its coordinates are $x_{c3}=0$, $y_{c3}=0$, $z_{c3}=-d_3$. With this placement, the main axis of the oblate Fc is along the x-axis, and therefore its polar angles are $\theta_3=90$, $\phi_3=0$. Now we have to situate the two Fabs (represented as prolate ellipsoids), which will be situated above the (x,y) horizontal plane. The essential choice is that of the angles. For one of the Fab ellipsoids we adopt certain values of θ_1 and ϕ_1. The distance from the centre of the ellipsoid, d_1, to the origin will be equal to or greater than the longest semiaxis of the prolate ellipsoid, and the coordinates of the centre are $x_{c1}=d_1\sin\theta_1\cos\phi_1$, $y_{c1}=d_1\sin\theta_1\sin\phi_1$ and $z_{c1}=d_1\cos\theta_1$. For the other Fab, d_2 is the same, and in principle there will be a variability in the choices of θ_2 and ϕ_2. However, we restrict ourselves to symmetric configurations, having a Y or a T shape, where the essential parameter is the angle, β, between the two arms. Then with the values of θ_1 and ϕ_1, the value of β, and the condition that Fc is on the plane of the angle or bisecting the angle, then the polar angles θ_2 and ϕ_2 can be deduced from geometric arguments. Of course, any other choice of axes, and any other initial placement of the first subunit, can be made. The details of this description would change, but the procedure would be equivalent.

The program then builds the bead–shell model by stacking rings of minibeads, in the direction of the symmetry axis, varying the radius of the rings to form an ellipsoidal shape (or not varying this radius in the case of constructing a cylinder). Thus, the result is a structure in which the ellipsoidal surface is formed by the central points of the minibeads.

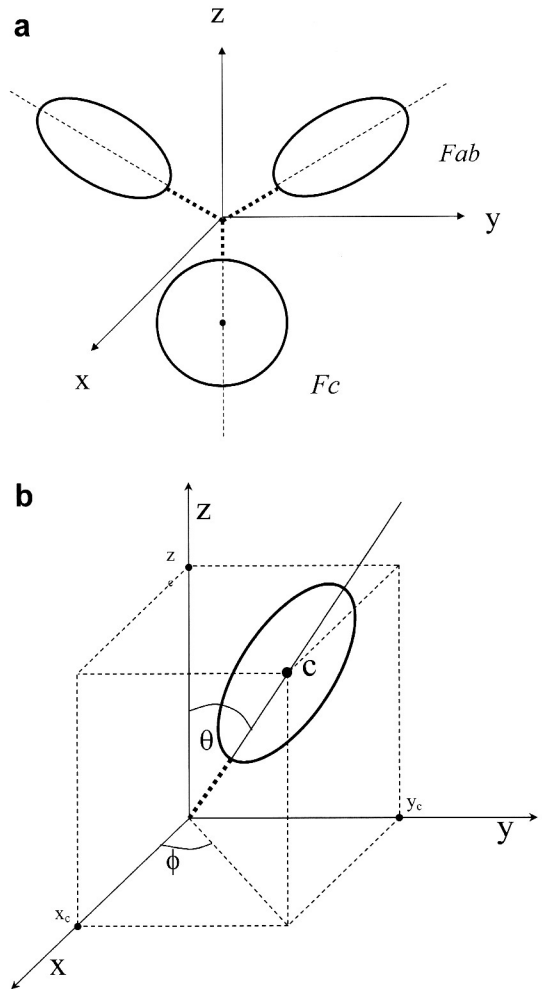

Fig. 1a, b Scheme for the construction of IgG HYDROSUB models. **a** A view along the x-axis. The *circle* represents the oblate fragment crystallisable (*Fc*), lying in the (y,z) plane, and the ellipses represent the prolate fragment antigen-binding (*Fab*) domains, which in this example are above the (x,y) plane. The *dotted lines* indicate an optional separation of the tips of the subunits from the centre of the coordinates (or a connector of negligible friction). **b** Definition of the Cartesian coordinates of the centre, C, of an ellipsoid, and the spherical-polar angles θ and ϕ

The number of beads, N, is clearly dependent upon the number of minibeads, and this affects the shape of the ellipsoidal surface produced and therefore the resulting properties produced by the program. In order to overcome this problem, the program calculates the value of each property, for a range of bead sizes, and then extrapolates forward to an infinite number of beads. One restriction is that the time taken to run a program to construct a shell model is proportional to N^3, and as a result the program works with a maximum of 2,000 minibeads. Once the bead–shell model of the antibody is constructed, an output file produced by HYDROSUB can be run with SOLPRO

Table 1 Theoretical SOLPRO Perrin function values, P, obtained for antibody bead–shell models with varying angles θ and ϕ. Unless otherwise stated, the position of the fragment crystallisable (*Fc*) domain is represented by the orientation angles $\theta = 90$ and $\phi = 90$. β represents the angle between the main axis of the ellipsoids representing the fragment antigen-binding (*Fab*) domains. The spaces in the models represent the distances between the Fc ellipsoid and each Fab ellipsoid. Entries shown in *bold* correspond to those models which fit the experimental range of values of P between 1.195 and 1.230 for γ4P

Space	Angles between main axis of ellipsoids	P
None	$\beta = 90, \theta = 90, \phi = 45/315$	1.177
	$\beta = 80, \theta = 90, \phi = 40/320$	1.170
	$\beta = 120, \theta = 90, \phi = 60/300$	1.180
	$\beta = 120, \theta = 90, \phi = 60/300$ (oblate, $\theta = 0, \phi = 90$)	1.173
	$\beta = 180, \theta = 90, \phi = 90/270$	1.15
	$\theta = 70, \phi = 45/315$	1.168
	$\theta = 70, \phi = 40/320$	1.165
	$\beta = 90, \theta = 45, \phi = 90/270$	1.107
5 Å	**$\beta = 90, \theta = 90, \phi = 45/315$**	**1.208**
	$\beta = 80, \theta = 90, \phi = 40/320$	**1.200**
	$\beta = 45, \theta = 45, \phi = 57.24/122.77$	1.131
	$\theta = 45, \phi = 90/270$	1.135
	$\beta = 90, \theta = 50, \phi = 22.62/157.38$	1.166
	$\beta = 180, \theta = 90, \phi = 0$	1.160
	$\beta = 90, \theta = 90, \phi = 45/315$ (oblate, $\theta = 0, \phi = 90$)	**1.199**
	$\beta = 120, \theta = 90, \phi = 60/300$	**1.204**
8 Å	**$\beta = 90, \theta = 90, \phi = 45/315$**	**1.225**
10 Å	$\beta = 90, \theta = 90, \phi = 45/315$	1.237
	$\beta = 120, \theta = 90, \phi = 60/300$	1.246
	$\beta = 180, \theta = 90, \phi = 90/270$	**1.208**
	$\beta = 90, \theta = 90, \phi = 45/315$ (oblate, $\theta = 0, \phi = 90$)	**1.229**
20 Å	$\beta = 90, \theta = 90, \phi = 45/315$	1.294
25 Å	$\beta = 90, \theta = 90, \phi = 45/315$	1.320
	$\beta = 120, \theta = 90, \phi = 60/300$	1.330
	$\beta = 180, \theta = 90, \phi = 90/270$	1.289

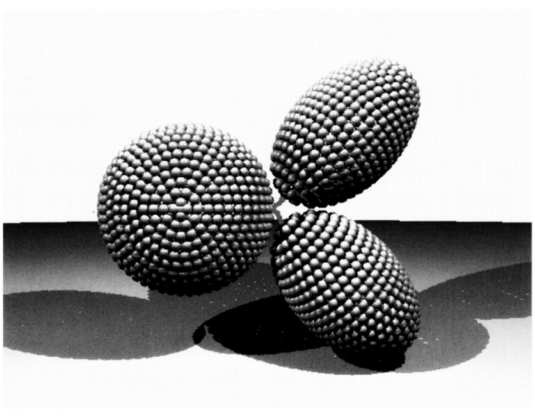

Fig. 2 One of the bead–shell models with a Perrin function value matching that of the experimentally measured value for the IgG4 chimeric antibody γ4P. Domain angles $\theta = 90$, $\phi = 45$, 10 Å between domains (shown as frictionless spacers). Theoretical $P = 1.229$. Experimental range of allowed values $P_{exp} = 1.195$-1.230

[17] in order to obtain the full spectrum of hydrodynamic parameters.

If the calculated Perrin function matches the experimental Perrin function of the antibody, the model represents a potential conformation for the antibody. This procedure may then be repeated many times, to investigate many different domain positions, and identify models fitting the experimental data.

Case study: an IgG4 construct

We illustrate the refined procedure by application to an IgG4 construct, known as γ4P [18] with the following hydrodynamic properties as reported by Longman et al. [19]: $s^{o}_{20,w} = (6.80 \pm 0.10)$ S, $M = 147$ kDa and $\bar{v} = 0.730$ (millilitres per gram). According to Eq. (1) this gives a range of values (allowing for experimental error) for f/f_0 of between 1.456 and 1.499, and using the estimate for the time-averaged apparent hydration for IgGs found previously of 0.59, we estimate the experimental Perrin function, P_{exp}, as between 1.195 and 1.230. We can then check off from a table of values for P for the various models to see which ones fit the data.

The various combinations of the θ and ϕ angles used for the construction of candidate bead–shell models using HYDROSUB and their resultant Perrin function values obtained from SOLPRO are shown in Table 1. Models representing antibodies with no spacing between the domains all provide P values significantly below the P_{exp} values determined. However, by including frictionless linkers between the domains, the P value is seen to increase, as expected for more extended structures. A bead–shell model with a similar P value to that of the γ4P chimera, and therefore potentially representing the structure of this chimera in solution, is shown in Fig. 2. Other models matching the (γ4P) chimera data contained either 5-Å spaces between the domains and 90–120° between Fab domains or 10 Å between the domains and 90–180° between Fab domains and are specified in bold type in Table 1. The spacing of domains in the final, more open bead–shell model structure shows similarity to those previously produced for murine IgG1 and IgG2a molecules through X-ray crystallography [2, 3].

It will be patently clear from Table 1 that more data are required to be specific about the configuration since a wide range of models will have P values agreeing with the experimental P values: this is the classical "uniqueness problem" [4, 7]. Data from viscosity and low-angle X-ray scattering are required, but this is a subject for a further study.

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References

1. Rajan SS, Ely KR, Abola EE, Wood MK, Colman PM, Athay RJ, Edmundson AB (1983) 3- Mol Immunol 20:787–799
2. Harris LJ, Larson SB, Hasel KW, McPherson A (1997) Biochemistry 36:1581–1597
3. Harris LJ, Skaletsky E, McPherson A (1998) J Mol Biol 275:861–872
4. García de la Torre J (1989) In: Harding SE, Rowe AJ (eds) Dynamic properties of biomolecular assemblies. Royal Society of Chemistry, Cambridge, UK, pp 3–31
5. Davis KG, Glennie M, Harding SE, Burton DR (1990) Biochem Soc Trans 18:935–936
6. Carrasco B, García de la Torre J, Davis KG, Jones S, Athwal D, Walters C, Burton DR, Harding SE (2001) Biophys Chem 93:181–196
7. Harding SE (1989) In: Harding SE, Rowe AJ (eds) Dynamic properties of biomolecular assemblies. Royal Society of Chemistry, Cambridge, UK, pp 32–56
8. Harding SE, Horton JC, Cölfen H (1997) Eur Biophys J Biophys Lett 25:347–359
9. García de la Torre J, Perez Sanchez HE, Ortega A, Hernandez JG, Fernandes MX, Diaz FG, Lopez Martinez MC (2003) Eur Biophys J 32:477–486
10. Byron O (1992) Solution studies on the conformation and assembly of the monoclonal antibody B72.3. University of Nottingham, UK
11. Byron O (2001) Methods Enzymol 321:278–304
12. Carrasco B, Harding SE, García de la Torre J (1998) Biophys Chem 74:127–133
13. Carrasco B (1998) Propiedades de macromoleculas rigidas en disolucion: Modelos, metodos computacionales y analysis de los datos experimentales. Universidad de Murcia, Spain
14. Carrasco B, García de la Torre J (1999) Biophys J 76:3044–3057
15. García de la Torre J, Carrasco B (2002) Biopolymers 63:163–167
16. Carrasco B, García de la Torre J, Byron O, King D, Walters C, Jones S, Harding SE (1999) Biophys J 77:2902–2910
17. García de la Torre J, Carrasco B, Harding SE (1997) Eur Biophys J 25:361–372
18. Kreusel KM, Adair JR, Eeley NRA, Davies MC, Jackson DE, Roberts CJ, Tendler SJB, Williams PM (1994) J Vac Sci Technol B 12:1517–1520
19. Longman EJ, Kreusel KM, Tendler SJB, Fiebrig I, King K, Adair JR, O'Shea P, Ortega A, García de la Torre J, Harding SE (2003) Eur Biophys J 32:503–510