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Form, symmetry and packing of biomacromolecules. I. Concepts and tutorial examples

A. Janner

Theoretical Physics, FNWI, Radboud University, Heyendaalseweg 135, NL-6525 AJ Nijmegen, The Netherlands. Correspondence e-mail: a.janner@science.ru.nl

The aim of this paper is to relate morphological properties of single biomacromolecules based on molecular enclosing forms indexed by an appropriate *form lattice* to the symmetry of the crystal where the molecules are periodically packed. Similar to the way in which the 'molécule intégrante' of Haüy permitted a molecular interpretation of the law of rational indices of crystal growth forms, alternative molecular enclosing forms, indexed by a so-called *packing lattice*, allow one to bridge the gap between form and crystal lattices. In this first part, selected tutorial examples illustrate the validity of the approach and the crystallographic compatibility between molecular and crystal structures. In particular, integral molecular lattices are shown to imply the observed axial ratios between crystal lattice parameters, leading sometimes to surprising results, like a cubic crystal lattice with a unit cell having a trigonal molecular filling with hexagonal enclosing form.

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1. Introduction

The RCSB Protein Data Bank (PDB) is the classical portal to biological macromolecular structures, and proteins in particular (visit http://www.pdb.org). In the corresponding PDB files one finds the Cartesian coordinates of the atoms in the crystal, together with the corresponding transformations generating the space group and the biomolecule.

Also indicated are the polypeptide chains of the multimer defining the oligomerization state of the active biomolecule and its point-group symmetry. The molecular symmetry is, possibly, larger than the point group of the crystal and involves additional *non-crystallographic symmetry* transformations, a phenomenon usually denoted as *pseudosymmetry* (Giacovazzo *et al.*, 1992).

The difference in point-group symmetry of the crystal and of the biomolecule is as such not surprising. What is surprising is the observation that the crystal lattice may have a point-group symmetry larger than that implied by the filling of the unit cell. In the PDB files these properties are given in an implicit way only, and are sometimes mentioned in the primary reference.

The two phenomena can be considered hints to a larger class of structural relations not taken into account by classical crystallography. The non-accidental character of these relations has been discussed by the author in a number of papers devoted to about 30 different protein structures and ten nucleic acid compounds. These numbers are ridiculously small in comparison with the more than 50 000 known structures reported in the PDB. This does not imply that they represent a few exceptional cases only. The main, new additional property is the structural relevance of enclosing molecular forms with vertices at points of a lattice: the *molecular form lattice*. This lattice is left invariant by the molecular symmetry group and it typically allows the external envelope to be connected by an invertible integral scaling transformation with a central hole. This transformation often relates C_α positions at extremal distances with respect to a rotational axis. In general, however, a form lattice allows one to describe symmetry-adapted morphological properties and not atomic positions. Quite unexpectedly, the form lattices of axial-symmetric multimers explored so far are integral. This means that the metric tensor of the lattice basis vectors has integral entries (up to a constant factor relating structure with geometry). For example, the lattice parameter relation $a_0 = c_0$ is observed in a number of three-dimensional hexagonal lattices, where a_0 and c_0 are the length of lattice basis vectors in the radial and in the axial direction, respectively (Janner, 2005).

In crystals of any kind, integral lattices show up in peaked statistical distributions of lattice parameter ratios (in the case mentioned above the ratio is one) (Janner, 2004; de Gelder & Janner, 2005*a*,*b*). This feature, not explained by the known crystallographic laws, is not at all or only very badly understood except in the case of close packing of equal spheres.

The question then arises as to the nature of the connection between the global crystal lattice and the local molecular form lattice, at least for biomacromolecules where the form lattice plays a structural role. One can reformulate this problem as the relation between crystal and molecular morphology, both cases obeying the law of rational indices, in the reciprocal and the direct space, respectively. The aim of the present work is to learn more about these aspects through a number of specific examples, analysed in a similar way. It requires new concepts which reflect a working hypothesis more than basic

elements of a theory challenging the foundations of crystallography.

The approach is geometric, phenomenological and morphological. It corresponds, so to say, to crystallography as it was in the past before the use of space groups, when the crystal growth forms were analysed by means of lattices and of crystal classes, and when students were warned against excessive illusions raised by the theory of Schoenflies, still considered in 1911 as a crystallographic speculation (Friedel, 1911).

In the present work (Part I), the basic concepts are introduced and illustrated by tutorial examples. In Part II (Janner, 2010), polymorphic packings are considered of the same icosahedral form of the various *Rhinovirus* serotypes and which depend on the space-group symmetry. Even in the case of crystal growth forms, morphology also depends on the underlying space-group symmetry, despite what has been said above. A planned future part deals with the rhodopsin–retinal complex which crystallizes together with lipids, observed by X-ray diffraction as fragments, to whom only collectively an indexed enclosing form can be attached. One of these cases is reported here as a tutorial example (the last one).

The results obtained so far, besides providing a solution to the problem of the connection between crystal and molecular structures, should help to formulate the observed relations between form, symmetry and packing in a more precise way.

2. Basic concepts

The starting point is a *biomolecule* as generated from the atomic coordinates of polypeptide chains (and possibly additional atoms) by a set of transformations ('biomt') as given in a PDB file, leading to the molecular symmetry group K_M of the multimer representing the biologically significant state of the molecule.

A *molecular form* is then considered with planar facets and point-group symmetry K_M enclosing the biomolecule and having vertices at points of a lattice: the *form lattice* Λ_F. Note that the enclosing form is not unique, and so neither is the form lattice, and may depend on the accuracy adopted. The form lattice allows one to assign *rational indices* (or equivalently integral ones) to the vertices of the molecular form. These indices are nothing other than the lattice coordinates of the corresponding points. In the usual way, by means of the reciprocal form lattice Λ_F^*, rational indices can also be assigned to the facets of the form. In addition to the facets of the *external envelope*, one often also has internal indexed facets delimiting *molecular holes* and, in particular, a *central channel*.

The key point is that a single form lattice should allow all the vertices involved (internal and external ones) to be indexed without the indices being too high and with a reasonable characterization of the molecular form. As has already been pointed out, the form adopted is a compromise between simplicity of the description and fitting to the atomic structure of the molecule, so that there are cases where one has to accept protruding chain segments.

In general, the properties of the indexable molecular forms and the corresponding form lattices are far from trivial and justify their investigation, even if they are not taken into account by the known crystallographic laws, and not (yet) understood in terms of physical or chemical laws. For example, in the case of the axial-symmetric proteins investigated so far, the central channel and external envelope have vertices related by a *crystallographic scaling* leaving the form lattice invariant and expressible by invertible integral (or rational) matrices. Moreover, the form lattices of proteins are often *integral*, as mentioned in §1. All these aspects involve single molecules.

Considered here are biomolecules periodically packed in a crystal. No quasi-crystals of biomolecules have been observed in nature, even when the point-group symmetry is non-crystallographic in three dimensions (as in quasi-crystals). The molecular enclosing form to be considered for biomolecules packed in a crystal has to be a *crystal symmetry-adapted indexable form*. This implies that the form, and the corresponding lattice, the *packing lattice* Λ_P, have to be invariant with respect to the space group of the crystal. Accordingly, Λ_P contains the crystal lattice Λ as a sublattice, has at least the same holohedry and allows a symmorphic space-group characterization of the crystal.

To conclude, the relation between molecular symmetry and crystal packing is discussed on the basis of the mutual relations between three situations:

(i) the symmetry of the isolated biomolecule, its molecular enclosing form and the corresponding form lattice Λ_F,

(ii) the packing unit of the crystal in terms of a symmetry-adapted molecular packing form, which recalls the historical concept of *molécule intégrante* considered by Haüy, and the packing lattice Λ_P, and finally

(iii) the symmetry of the atomic arrangement, the crystal lattice Λ and the space group G.

3. Tutorial examples

Typical situations are illustrated for a number of biomolecules whose structural data, obtained by X-ray diffraction, have been deposited as a PDB file. The examples are human mitochondrial ferritin [cubic packing of octahedral ferritin cages, PDB code 1r03 (Langlois d'Estaintot *et al.*, 2004)]; hemocyanin (subunit II) from *Limulus* [cubic close packing of hexagonal prismatic forms, PDB code 1ll1 (Liu *et al.*, unpublished)]; vanillyl-alcohol oxidase [close-packed tetragonal forms, PDB code 1vao (Mattevi *et al.*, 1997)]; octameric SAP-like pentraxin from *Limulus polyphemus* [octagonal biomolecule oriented in the crystal according to the 422 subgroup of the molecular 822 symmetry point group, PDB code 3flr (Shrive *et al.*, 2009)]; human Dmc1 protein [layers of octagonal tilings of slightly twisted double octagonal rings, PDB code 1v5w (Kinebuchi *et al.*, 2004)]; heptameric SAP-like pentraxin from *Limulus polyphemus* [monoclinic packing of double-stacked heptameric cylindrical rings, PDB code 3flp (Shrive *et al.*, 2009)]; light-harvesting protein LH2 [face-centred cubic (f.c.c.) packing of cylindrical enclosing forms of double-

stacked nonamers, PDB code 1kzu (Prince *et al.*, 1997)]; rhodopsin–retinal complex [hexagonal form, symmetry and packing, PDB code 1qko (Edman *et al.*, 1999)].

3.1. Human mitochondrial ferritin

The first example is a very simple one. The *biomolecule* is a 24-mer forming an octahedral cage with point symmetry $K_M = 432$. The molecular form has six tetragonal central channels with square edge $L_0 = 2a_0$ and height $H = 20a_0$ equal to the cubic edge L_e of the external envelope. The fitting value a_0 = 5.9 Å is the lattice parameter of the cubic form lattice $\Lambda_F(a_0)$. These properties have already been derived in a paper devoted to octahedral protein cages (Janner, 2008).

The *packing form* is the same as the molecular form. The sphere inscribed in the cubic envelope has radius $R_0 = 10a_0$ and represents an alternative packing form.

The *packing lattice* $\Lambda_P(u)$ is cubic as is the crystal lattice $\Lambda(a)$. The corresponding lattice parameters are $a = 30u$ = 181.41 Å, so that u = 6.047 Å, which has practically the same value as a_0. Accordingly one has here the special case: $\Lambda_F = \Lambda_P \supset \Lambda$, as shown in Fig. 1.

The *crystal space group* is *F*432. The centres of the packing forms are at the Wyckoff positions 4(*a*) 000, $0\frac{1}{2}\frac{1}{2}$, $\frac{1}{2}0\frac{1}{2}$, $\frac{1}{2}\frac{1}{2}0$,

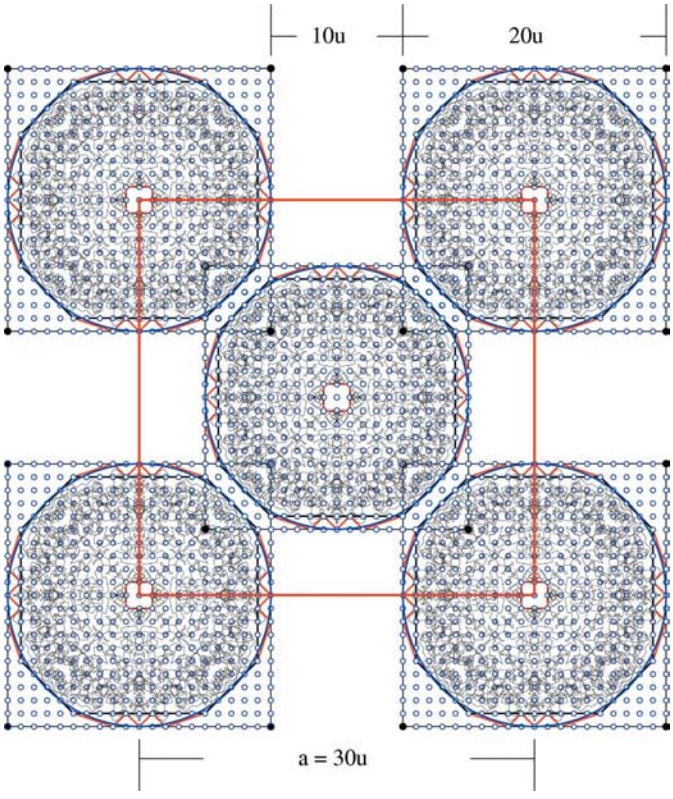

Figure 1
The packing of the human mitochondrial ferritin in the crystal with space-group symmetry *F*432 and lattice parameter *a* is shown along a fourfold axis, together with three enclosing forms: a cubic one with edge $2a/3$ and vertices at points of an f.c.c. lattice with parameter $u = a/30$, the refined form discussed in Janner (2008) and a sphere inscribed in the cubic form. The tetragonal central channel has the square edge 2*a*. The centres of the packing forms are at the Wyckoff position 4(*a*).

leading to an f.c.c. packing with inter-spherical distance $D = a/2^{1/2} - 2R_e = 1.21u \simeq 2^{1/2}u$. Note that for the close-packed case D would be zero.

3.2. Hemocyanin (subunit II) from *Limulus*

The second example illustrates the intriguing case of a cubic crystal lattice, which appears as an hexagonal integral lattice with axial ratio $c/a = 6^{1/2}$, simply because the content of the unit cell has trigonal symmetry.

The *biomolecule* is the hexameric metcyanin II (a double-stacked trimer) with point-group symmetry $K_M = 32$. The hexagonal enclosing form and a trigonal refinement is shown in Fig. 2. The radius R_0 of the hexagonal central hole defines the radial lattice parameter $r_0 = a_0$ of the hexagonal form lattice Λ_F, the axial lattice parameter being $c_0 = r_0/2^{1/2}$. The hexagonal prismatic envelope has radius $R_e = 10r_0$ and height

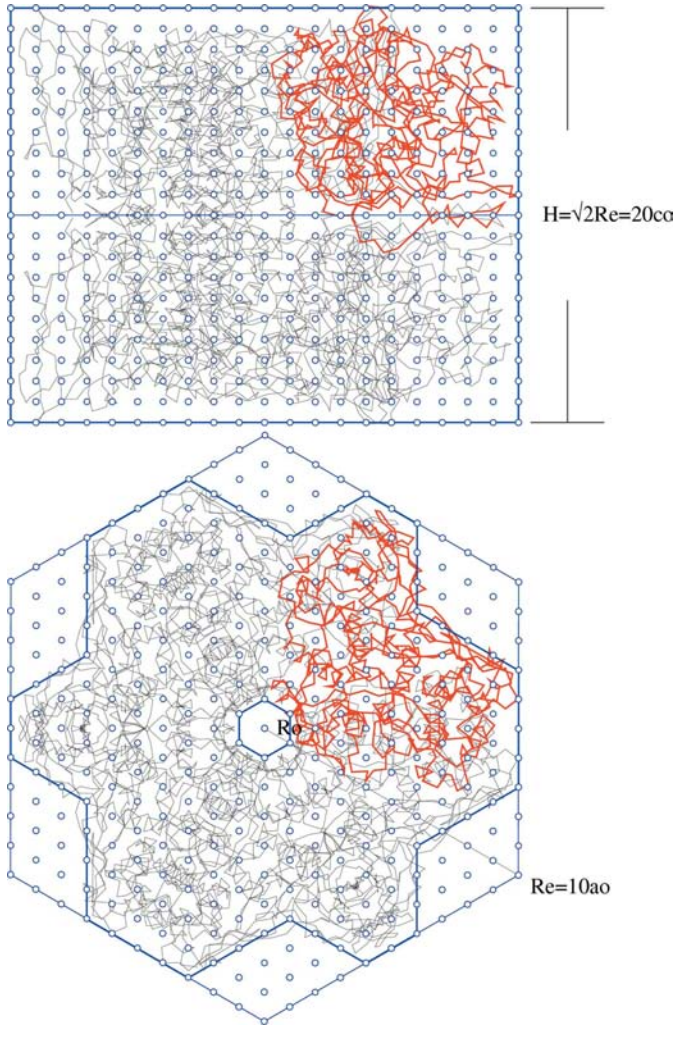

Figure 2
The hexagonal enclosing form of metcyanin II (a double-stacked trimer of the hemocyanin subunit II from *Limulus*) has height H and a central hole with radius R_0, which define the parameters of the hexagonal form lattice $\Lambda_F(a_0, c_0)$ by $R_0 = a_0$ and $H = 20c_0 = 10(2)^{1/2}a_0$. Therefore Λ_F is integral with axial ratio $c_0/a_0 = 1/2^{1/2}$. The radius of the external envelope is $R_e = 10a_0 = H/2^{1/2}$.

$H = 10(2)^{1/2}r_0 = 20c_0$. Accordingly, Λ_F is integral hexagonal with an axial ratio $c_0/a_0 = 1/2^{1/2}$.

The *packing form* is the same as the hexagonal prismatic form of the biomolecule, having inscribed, as alternative packing form, a cylinder with radius R_c and height H:

$$R_c = \frac{3^{1/2}}{2}R_e = 5(3)^{1/2}r_0,\ H = 10(2)^{1/2}r_0,\ H/R_c = \frac{2(6)^{1/2}}{3}. \quad (1)$$

The *crystal packing* is a cubic close packing of hexagonal prismatic (or cylindrical) enclosing forms. Indeed, the interpacking distances along and perpendicular to the threefold axis are zero. The crystal lattice is hexagonal $\Lambda(a, c)$, Bravais type $32H$, with $a = 116.61$ and $c = 285.61$ Å. The packing lattice $\Lambda_P(u, w)$ is also hexagonal, with $u = a/12$ and $w = c/6$ (Fig. 3). The relations with the molecular form parameter r_0 are $u = 5(3)^{1/2}r_0/6$ and $w = H/2 = 5(2)^{1/2}r_0$, implying an axial ratio $c/a = 6w/12u = 6^{1/2} = 2.4495$, which is the value observed experimentally (2.4492).

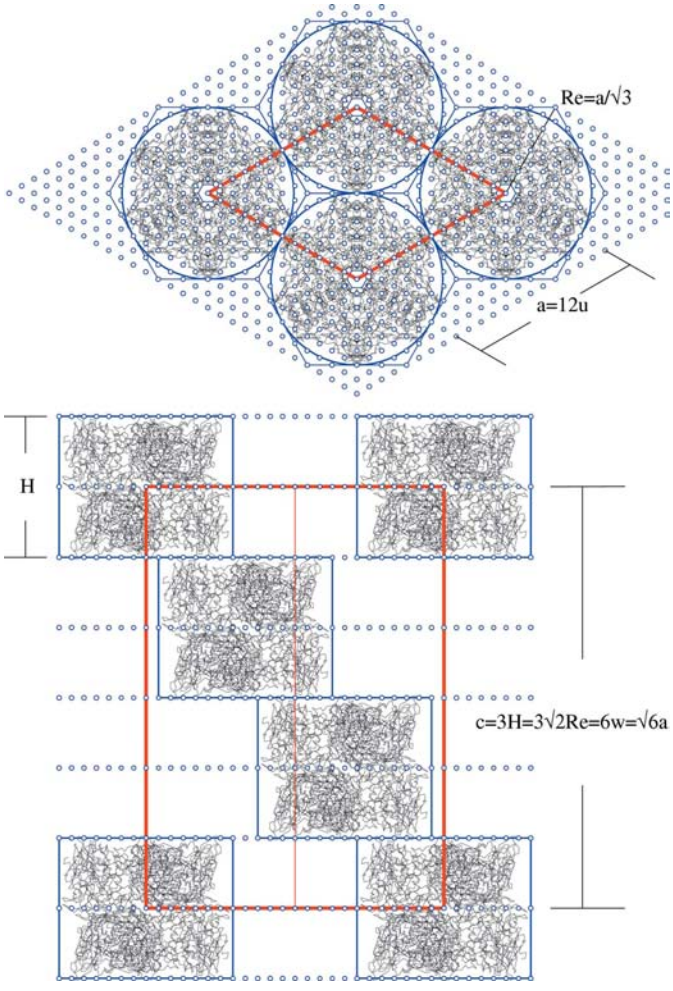

Figure 3
The crystal of metcyanin II is a cubic close packing of hexagonal prismatic enclosing forms (or alternatively of inscribed cylinders) with space-group symmetry $H32$. The packing lattice $\Lambda_P(u, w)$, turned by 60° with respect to the form lattice, is related to the hexagonal crystal lattice $\Lambda(a, c)$ by $a = 12u = 3^{1/2}R_e$ and $c = 6w = 3H = 3(2)^{1/2}R_e$, so that the axial ratio c/a is $6^{1/2}$ and Λ is in fact f.c.c.

The *crystal space group* is $H32$ and the centres of the packing forms are at the Wyckoff position 3(*a*) 000, $\frac{1}{3}\frac{2}{3}\frac{2}{3}$, $\frac{2}{3}\frac{1}{3}\frac{1}{3}$, which for the axial ratio $c/a = 6^{1/2}$ are at f.c.c. lattice points. For $a_c = 2^{1/2}a_h$ one has

$$\Lambda(a_h, c_h) = 6^{1/2}H(a_h, 6^{1/2}a_h) = \Lambda_{fcc}(a_c). \quad (2)$$

3.3. Vanillyl-alcohol oxidase

In the third example the molecular axes of the biomolecule are turned by an angle $\varphi = 7.12°$ around the common tetragonal *c* axis, with respect to the crystal axes *a* and *b*.

The *biomolecule* is an octamer with point-group symmetry $K_M = 4/mmm$. The molecular enclosing form has been described in a previous publication (Janner, 2005). The central channel along the tetragonal axis has a square basis with radius $R_0 = r_0 = 12.18$ Å and height $H = 6r_0$. The external enclosing square, turned by 45° with respect to the square with edge $L = 12r_0$ as indicated in Fig. 13 of Janner (2005), has radius $R_e = 8r_0$. The form lattice $\Lambda_F(a_0)$ is cubic with lattice parameter $a_0 = r_0$. The rotation of the biomolecule being rational [$\tan(\varphi) = 1/8$], the form lattice and the packing lattice are mutually commensurate. But instead of choosing a packing form oriented as the molecule, it is more convenient to adopt a crystal-oriented tetragonal form, even if at the price of some small protruding chain segments and with an overall fitting less good than for the molecular form indicated above.

The basis of the crystal-oriented *packing form* has an external square basis with edge $L_e = 10a_0$, which is truncated at $2a_0$ from the square vertices. The internal square, turned by 45°, has an edge $L_0 = 2^{1/2}a_0$. In the axial tetragonal direction the minimal height is $H_1 = 4a_0$ and the maximal one is $H_2 = 6a_0 = H$, so that $a_0 \simeq r_0$ [see Fig. 4, expressed, however, in terms of the packing lattice $\Lambda_P(u, w)$ instead of the form lattice $\Lambda_F(a_0, c_0)$].

The *crystal packing* is tetragonal centred. The crystal lattice is $\Lambda(a, c) = 4I$ with $a = 130.24$ and $c = 135.51$ Å, whereas the packing lattice $\Lambda_P(u, w)$, also tetragonal centred, has lattice parameters $u = a/10$, $w = c/10$. The packing forms are close packed (as already mentioned) leading to the relations

$$L_e = a, \quad H_1 + H_2 = c, \quad (3)$$

so that $u = a_0 = 13.024$ Å $\simeq r_0 = 12.18$ Å and $w = 13.351$ Å. Both the packing lattice and the crystal lattice are nearly cubic. Their axial ratio $c/a = w/u = 1.025$ is comparable with the ratio $a_0/r_0 = 1.07$. The tetragonal deviation from the cubic lattice can be seen as a consequence of the molecular φ rotation.

The *crystal space group* is $I4$ and the centres of the packing forms are at the Wyckoff position 2(*a*) 000, $\frac{1}{2}\frac{1}{2}\frac{1}{2}$.

3.4. Octameric SAP-like pentraxin from *Limulus polyphemus*

The octameric pentraxin is a *biomolecule* with the point-group symmetry 822, which is crystallographic in four dimensions but not in three, because only a four-dimensional

lattice is left invariant by 822 and no three-dimensional one. Nevertheless, indexing the vertices of an enclosing form having the symmetry of the biomolecule is still possible by four (rational) indices, which are the coordinates with respect to a $\mathbb{Z}$-module M_F of rank 4 and dimension 3. In the present case M_F may be improperly called an *octagonal lattice*, even if M_F is in fact the projection of a four-dimensional lattice. This is known from the theory of quasi-crystals and is discussed in detail for biomacromolecules in Janner (2005). The metric of M_F is fixed (as in other axial-symmetric cases) by two parameters a and c, with a the radius of the regular polygon (the octagon) and c the height (along the rotational axis) of the octagonal form.

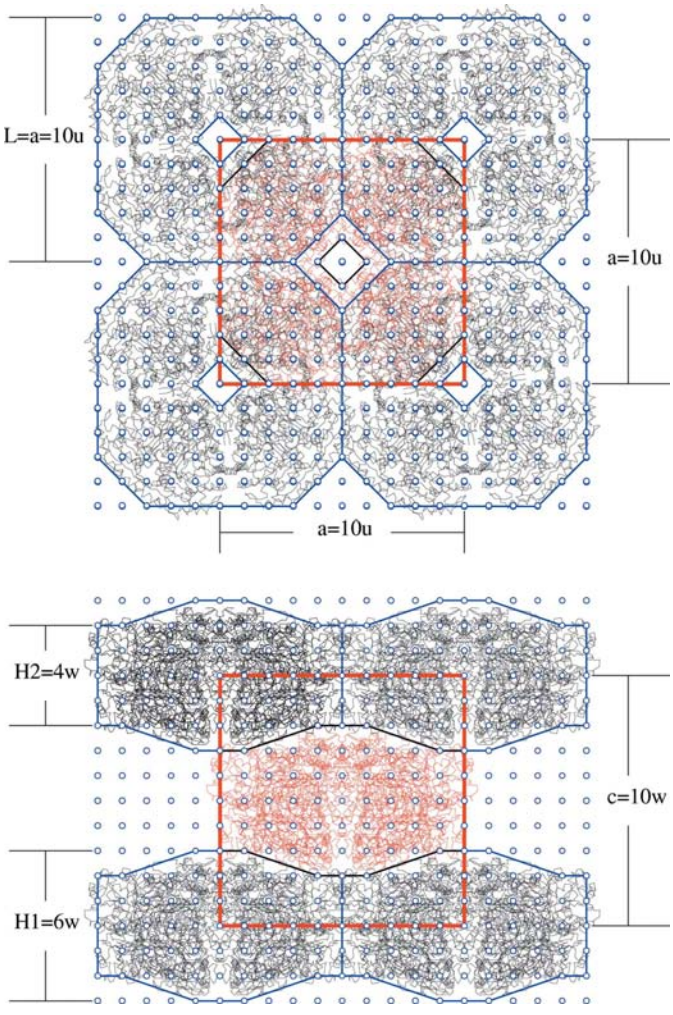

Figure 4
Crystal close packing of the vanillyl-alcohol oxidase packing forms with vertices at the points of the tetragonal packing lattice $\Lambda_P(u, w)$, having parameters related to the tetragonal centred crystal lattice $\Lambda(a, c)$ by $a = 10u$ and $c = 10w$, which only slightly deviates from a cubic lattice ($u = a_o$ = 13.0 Å, w = 13.5 Å). The tetragonal central channel of the packing form has radius $R_0 = u$ and height $H = H_1 = 6w$, whereas a smaller height (minimal) occurs in the enclosing form $H_2 = 4w$, ensuring close packing: $c = H_1 + H_2$. The protruding chain segments are due to a mismatch between the packing form and the underlying molecular form, turned by an angle of 7.12° around the tetragonal axis. The basis square with edge $L_e = a = 10u$ is truncated by $2u$.

The molecular form of the pentraxin has an octagonal central hole with radius $R_0 = r_0$ = 29 Å connected to the external envelope by a star octagon, with Schäfli symbol {8/3}. The external octagon has radius $R_e = \lambda_{\{8/3\}} R_0$, where $\lambda_{\{8/3\}} = 1 + 2^{1/2}$. The height H of the octagonal form is equal to the radius R_e, so that $M_F(a, c)$ is isometric octagonal, as shown in Fig. 5: $c/a = H/R_e = 1$ and is fixed by one single parameter: $R_e = H$ = 70 Å.

The *packing form* is a small tetragonal deformation of the molecular form and is built from two squares in a relative 45° orientation, and with a height H_0 slightly shorter than H (Fig. 6).

The *packing lattice* $\Lambda_P(u, w)$ is tetragonal 422P, whereas the crystal lattice $\Lambda(a, c)$ is tetragonal centred 422I. The lattice parameters are: a = 173.33, c = 98.81 Å and $u = a/5$, $w = c/6$. Disregarding the contraction of the packing unit height H_0 with respect to H, the mutual relations between form and packing are: $R_e = 2u = 69.33$ (74), $H = 4w = 65.27$ (70) and because $H = R_e$ the axial ratio of the crystal lattice is given by

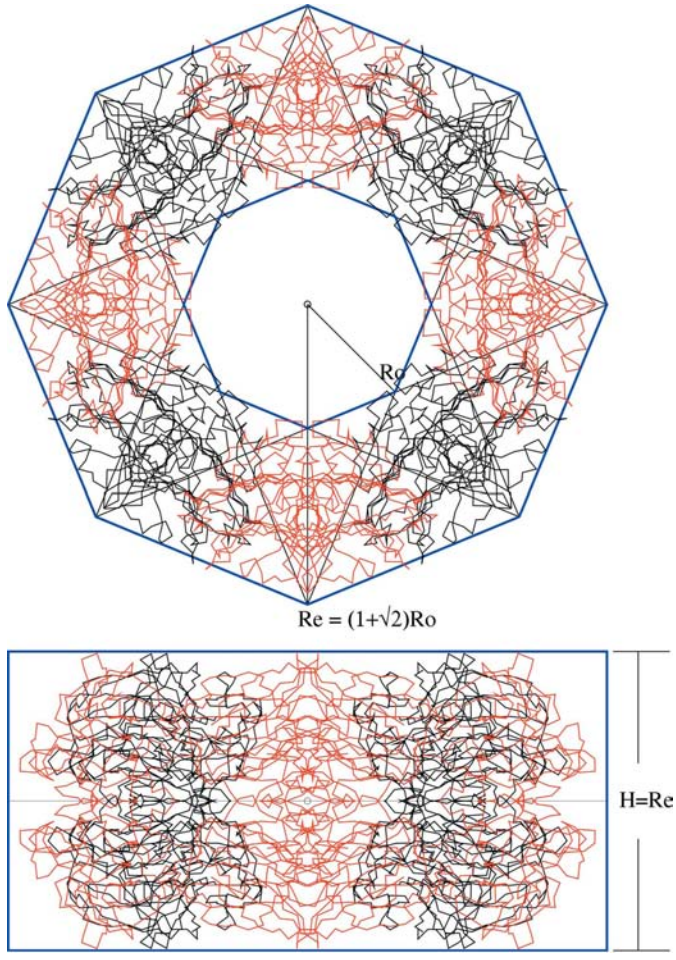

Figure 5
The octagonal molecular form of the octameric SAP-like pentraxin from *Limulus polyphemus* has a central hole (with radius R_0) related by a star octagon {8/3} to the external envelope (with radius R_e), so that $R_e = \lambda_{\{8/3\}} R_0$, with $\lambda_{\{8/3\}} = 1 + 2^{1/2}$. The (projected) octagonal form lattice $M_F(R_e, H)$ is isometric, because $H = R_e$ (axial ratio 1).

$c/a = 6w/5u = 3/5$ (0.57), so that this lattice is (nearly) integral.

The *crystal space group* is *I*422. The centres of the packing units are at the Wyckoff position 2(*a*) 000, $\frac{1}{2}\frac{1}{2}\frac{1}{2}$.

3.5. Human Dmc1 protein

The *biomolecule* Dmc1 is an octamer with non-crystallographic symmetry 822 as in the previous example. The integral scaling transformation that leaves the octagonal molecular module M_F invariant relates the radius of the central hole to that of the external boundary R_e by six successive star polygons {8/2}, where each star polygon is obtained by connecting alternative octagonal vertices. So

$$R_0 = r_0 = \lambda^6_{\{8/2\}} R_e, \quad \lambda_{\{8/2\}} = 0.765\ldots \tag{4}$$

and the height of the enclosing form is $H = R_e/2^{1/2}$. Therefore the $\mathbb{Z}$-module $M_F(R_e, 2^{1/2}R_e)$ is integral. In the crystal the packing unit is a pair of stacked octamers, twisted by $\pm 7.5^\circ$ around the tetragonal *c* axis and forming a double octagonal ring with external radius R_d = 67.1 Å (Fig. 7). The height of the layer is $H_d = 2^{1/2}R_d \cos(\pi/8) = 2^{1/2}R$.

The *crystal packing* has a layer structure. Each layer builds an octagonal tiling of the double-stacked octamers. The *packing lattice* is tetragonal $\Lambda_P(u, w) = 422P$ and the crystal lattice tetragonal centred $\Lambda(a, c) = 422I$, as in the previous example. The lattice parameters are: a = 124.09, c = 218.83 Å and $u = a/4$, $w = c/10$. The relations with the double octahedral ring are

$$a = 2R^d_e \cos\left(\frac{\pi}{8}\right) = 4u \text{ and } H_d = 4w = \frac{a}{2^{1/2}}, \tag{5}$$

implying the axial ratios $w/u = 1/2^{1/2}$ and $c/a = 5(2)^{1/2}/4 = 1.767$ (1.613) (the experimentally observed value is given in brackets) (Fig. 8). The crystal lattice is, therefore, integral, as also are (approximately) the packing lattice $\Lambda_P(u, w)$ and the octagonal form lattice $M_F(R_e, H)$.

The *crystal space group* is *I*422 and the centres of the packing units are at the lattice points 000, $\frac{1}{2}\frac{1}{2}\frac{1}{2}$, as in the previous example.

3.6. Heptameric SAP-like pentraxin from *Limulus polyphemus*

The heptameric pentraxin *biomolecule* has point-group symmetry $K_M = 722$, which is non-crystallographic in three dimensions, like the octameric pentraxin considered above. In the present case, 722 has no axial-symmetric crystallographic subgroups allowing consideration of an indexable enclosing packing form, as was the case for the octameric pentraxin. Therefore, for the heptameric pentraxin molecule two enclosing forms are considered: an heptagonal form and a

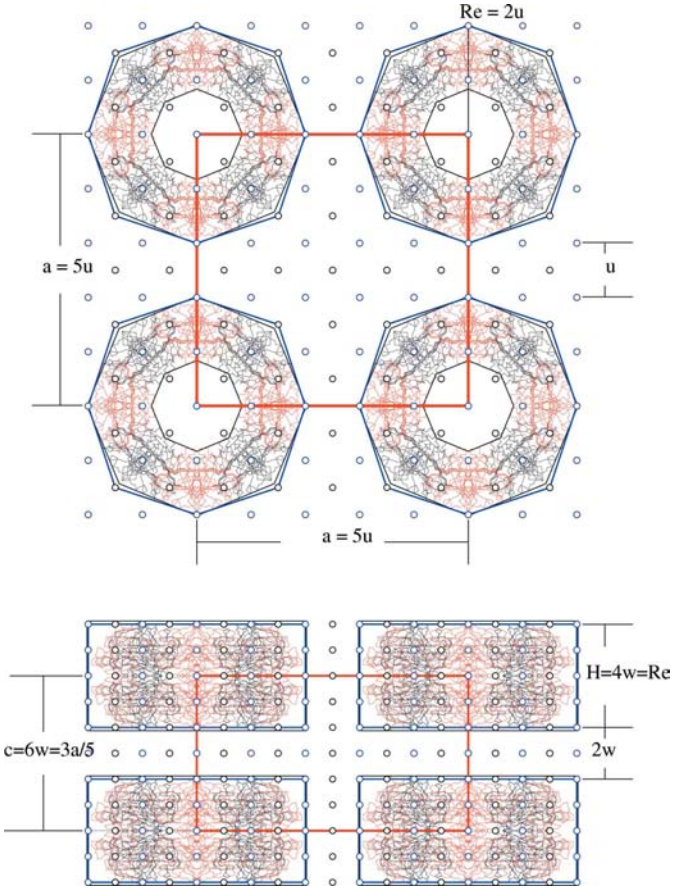

Figure 6
The space group of the octameric SAP-like pentraxin crystal is *I*422, with lattice parameters *a* and *c*, while the molecular symmetry is 822, as shown in Fig. 5. The packing lattice $\Lambda_P(u, w)$ has parameters $u = a/5$ and $w = c/6$, and the corresponding indexed packing form has radius 2*u* and height 4*w*. Disregarding the small deviations between the molecular and the packing forms shown (which are incommensurate), one has $R_e = 2u$ and $H = 4w$, implying by $H = R_e$ an axial ratio $c/a = 3/5$ instead of the observed 0.57 value.

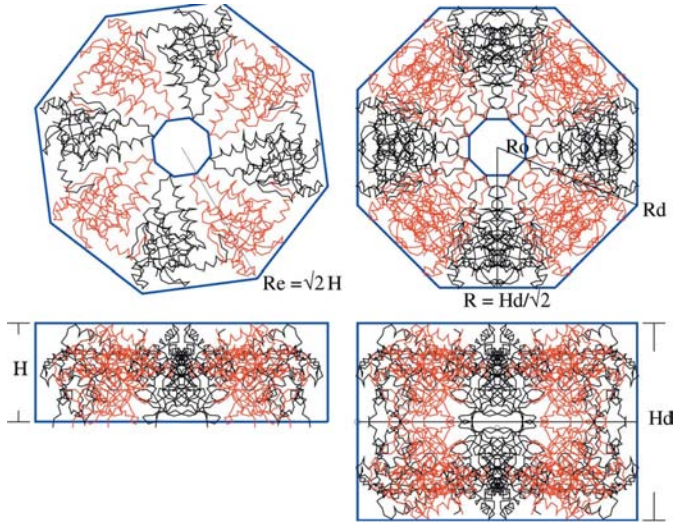

Figure 7
Comparison between the molecular enclosing form of the single human Dmc1 octamer (left-hand side) and the double-stacked ring of two such octamers (right-hand side). The central hole of the octamer has radius R_0 related by the octagrammal scaling factor $\lambda^6_{\{8/2\}}$ (where $\lambda_{\{8/2\}} = 0.765\ldots$) to the radius R_e = 65.5 Å of the external boundary. The height is $H = R_e/2^{1/2}$. In the double-stacked ring, the corresponding values are R_{ed} = 67.1 Å and $H_d = 2^{1/2}R_{ed}\cos(\pi/8)$.

cylindrical form. The heptagonal form has the expected properties: an external envelope with as basis a regular heptagon with radius $R_e = 68$ Å and height $H = (10/9)R_e$, and a central hole with radius R_0 scaled with respect to the external envelope by a factor $\lambda^3_{\{7/2\}}$, where $\lambda_{\{7/2\}} = 0.6920\ldots$, so that the corresponding heptagons are mutually related by three successive star heptagons {7/2}.

The enclosing cylindrical ring is inscribed in the heptagonal form. It has an outside radius $R_c = R_e \cos(\pi/7)$ and an inside radius given by $R_{0c} = R_c/3$. The last relation expresses the fact that $\lambda^3_{\{7/2\}} = 0.331$ is a good approximation of $\frac{1}{3}$ (see Fig. 9).

A cylindrical form is non-indexable. Nevertheless, one finds in this case also a natural *packing lattice* Λ_P having the same symmetry as the monoclinic crystal lattice $\Lambda(a, b, c, \beta) = 121P$ and lattice points at the (external) cylindrical envelope (Fig. 10). In the present case $a = 98.32$, $b = 167.56$, $c = 140.87$ Å and $\beta = 92.50°$. For $\Lambda_P(u, v, w, \beta)$ one finds $u = a/8$, $v = b/8$, $w = c/16$ and $\beta = 92.50°$.

The relations with the cylindrical enclosing ring are

$$H = \frac{10}{9}R_e = 6u = \frac{3}{4}a = 73.74\ (73.74)\ \text{Å}$$
$$R_c = \cos\left(\frac{\pi}{7}\right)R_e = 7w = \frac{7}{16}c = 61.26\ (61.63)\ \text{Å}$$
$$R_{0c} = \frac{1}{3}R_c = v = \frac{b}{8} = 20.94\ (20.54)\ \text{Å},$$

where the numerical values are those obtained from the enclosing form and those given in brackets are derived from the crystal lattice parameters.

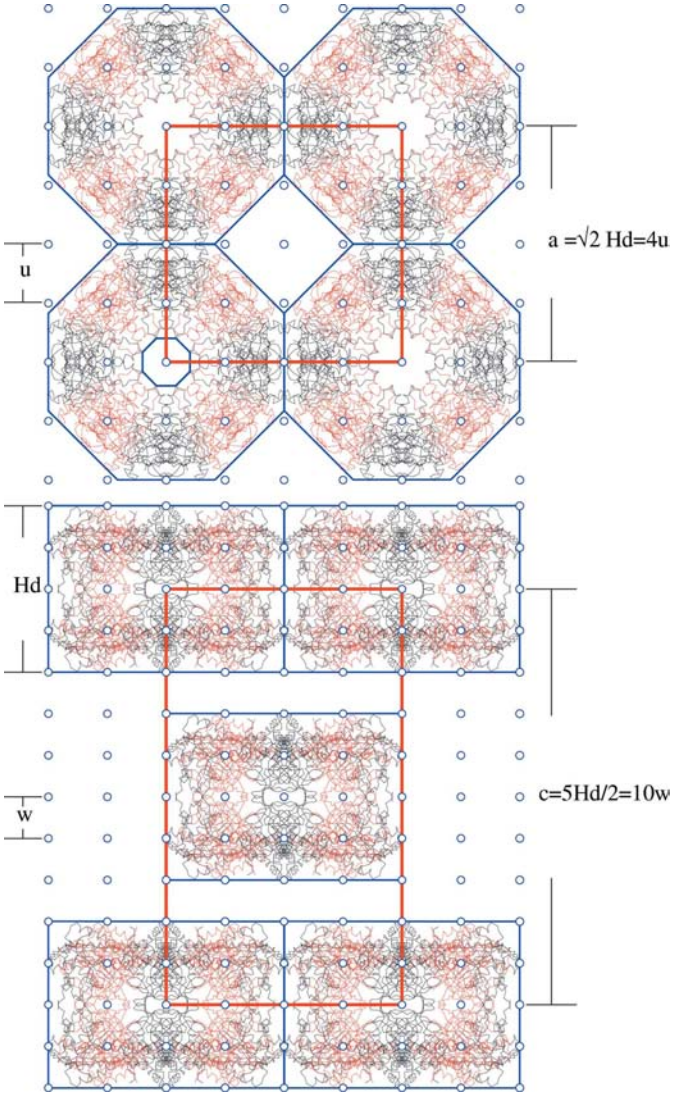

Figure 8
Crystal packing of the double-stacked octameric Dmc1 protein and corresponding tetragonal packing lattice $\Lambda_P(u, w)$ connected with the lattice $\Lambda(a, c)$ of the crystal space group $I422$ by the relations $a = 4u = 2R_{ed}\cos(\pi/8)$, $c = 20w$ and $H_d = 4w = a/2^{1/2}$, so that the axial ratio is $c/a = 5(2)^{1/2}/4$ and Λ is integral (approximately). The centres of the packing units are at the Wyckoff positions 2(*a*) 000, $\frac{1}{2}\frac{1}{2}\frac{1}{2}$.

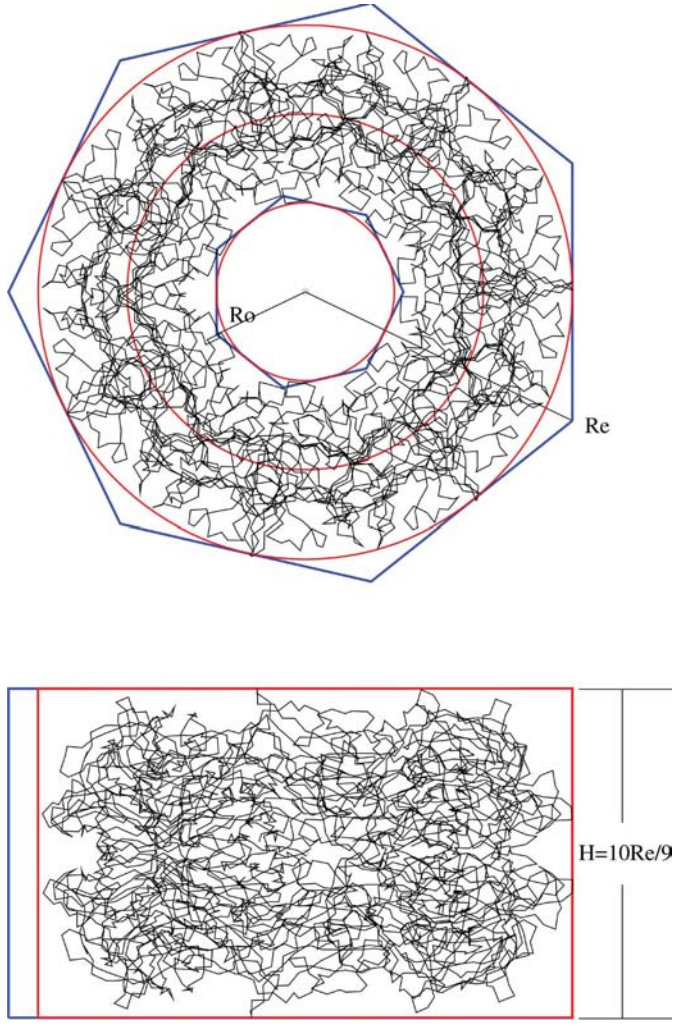

Figure 9
Heptagonal and cylindrical enclosing forms of the heptameric SAP-like pentraxin. The radius R_e of the external heptagon is related to the central hole radius R_0 by the heptagrammal scaling factor $\lambda^3_{\{7/2\}} = 0.331$ ($\lambda_{\{7/2\}} = 0.692\ldots$) and to the height by $H = 10R_e/9$. The enclosing cylinder ring has an outside radius $R_{ec} = R_e\cos(\pi/7)$ and an internal radius $R_0 = R_e/3$.

The *crystal space group* is $P12_11$ and the centres of the enclosing forms are at the Wyckoff position 2(*a*) xyz, $\overline{x}\ \frac{1}{2}+y\ \overline{z}$ with $x=y=z=\frac{1}{4}$.

3.7. Light-harvesting protein LH2

The nonameric light-harvesting protein is a *biomolecule* with point-group symmetry $K_M = 922$. Its enclosing form has already been discussed in Janner (2005). The nonagonal enclosing form has an external radius R_e = 36.7 Å and an internal radius of a regular nonagon obtained from R_e by the combination of a {9/2} and a {9/3} star polygon, so $R_0 = \lambda_{\{9/2\}}\lambda_{\{9/3\}}R_e$, where $\lambda_{\{9/2\}} = 0.815\ldots$ and $\lambda_{\{9/3\}} = 0.532\ldots$ [see Fig. 7 of Janner (2005)]. In the crystal, the packing unit is a double-stacked nonamer, compressed along the ninefold axis and with height $H = 3R_e$. For similar reasons as in the previous case, the enclosing form one has to choose is a cylindrical ring. The external cylinder has a radius $R_c = R_e$ and the radius of the internal cylinder $R_{0c} = 2R_e/5$ is slightly smaller than that of the cylinder inscribed in the central hole with radius $\cos(\pi/9)R_0$ (Fig. 11).

The *crystal lattice* is f.c.c., despite the fact that the space group is trigonal $G = H32$ (= $R32$). One indeed finds

$$\Lambda(a, c) = 32H(a, 6^{1/2}a) = 6^{1/2}H = 432F(a_0), \qquad (6)$$

with lattice parameters $a = 120.30$, $c = 296.20$ Å and an axial ratio $c/a = 2.462$ ($6^{1/2} = 2.449$). The cubic lattice parameter is $a_{f.c.c.} = 2^{1/2}a$.

The *packing lattice* is hexagonal $\Lambda_P(u, w)$ with lattice parameters $u = a/12$, $w = c/33$ and an axial ratio $w/u =$

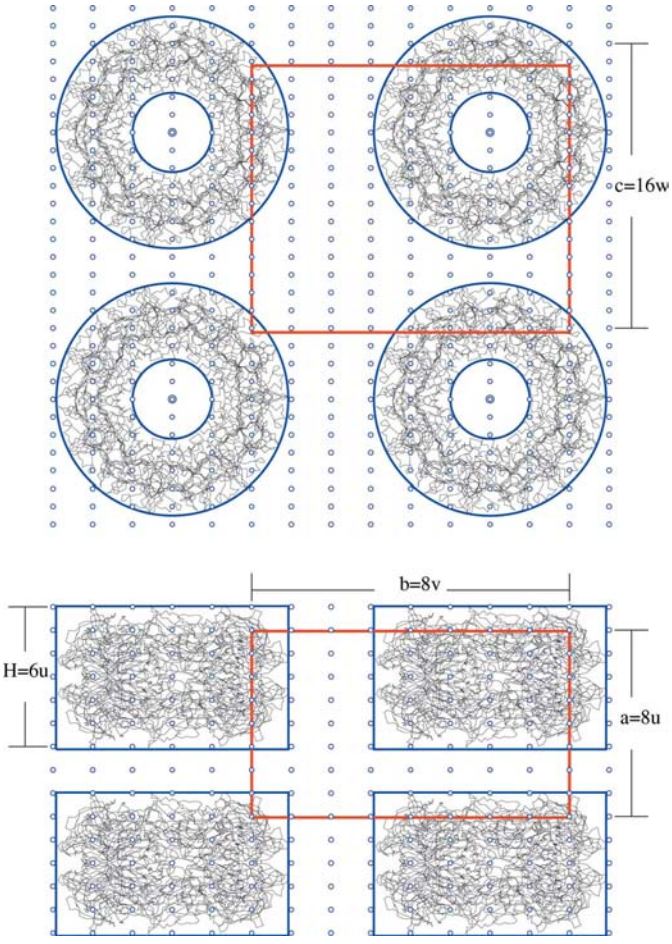

Figure 10
Monoclinic packing lattice $\Lambda_P(u, v, w, \beta)$ (unique axis *b*) with u, v, w parallel to the crystal axes a, b, c, respectively, and cylindrical packing form of the heptameric SAP-like pentraxin viewed in the (c, b) plane (top) and (a, b) plane (bottom). The corresponding crystal lattice $\Lambda(a, b, c, \beta)$ is the sublattice given by $a = 8u, b = 8v, c = 14w$. Both lattices have $\beta = 92.50°$. The relations with the enclosing cylinder are $H = 6u = 3a/4$, $R_{cc} = 7w = 7c/16$, $R_{0c} = v = R_{cc}/3 = b/8$. The centres of the packing units are at the Wyckoff position 2(*a*) of the space group $P12_11$: xyz, $\overline{x}\ \frac{1}{2}+y\ \overline{z}$ with $x=y=z=\frac{1}{4}$.

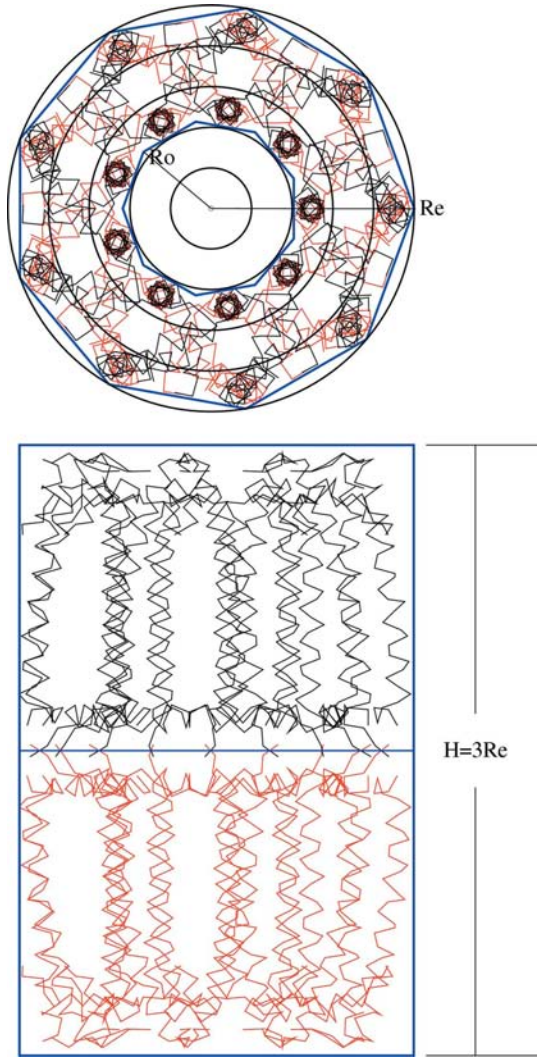

Figure 11
The double-stacked nonameric light-harvesting protein LH2 has a central hole with radius R_0 related by the nonagrammal scalings of the two star nonagons {9/2} and {9/3} to the external radius by $R_0 = \lambda_{\{9/2\}}\lambda_{\{9/3\}}R_e$, with $\lambda_{\{9/2\}} = 0.8152\ldots$ and $\lambda_{\{9/3\}} = 0.532\ldots$. The height $H = 3R_e$ is slightly compressed with respect to twice the height of each of the stacked nonamers. The enclosing ring has an external radius $R_c = R_e$ and an internal radius $R_{0c} = R_0\cos(\pi/9)$, satisfying the relation (for a somewhat smaller value) $R_{0c} = 2R_c/5$.

$4(6)^{1/2}/11$. The relations between the molecular form of the double-stacked ring and the lattices are

$$R_c = \frac{3^{1/2}}{6}a = 2(3)^{1/2}u, \quad H_c = \frac{4}{11}c = \frac{4(6)^{1/2}}{11}a, \quad \frac{H_c}{R_c} = 24\frac{2^{1/2}}{11}. \tag{7}$$

The ratio H_c/R_c of the cylindrical packing unit (Fig. 12) is a bit larger than the ratio H/R_e obtained from Fig. 11 for the double-stacked nonamers (3.085 instead of 3.0).

The *crystal space group* is $H32$. The centres of the packing forms (cylinders) are at the Wyckoff position 3(*a*) 000, $\frac{1}{3}\frac{2}{3}\frac{2}{3}$, $\frac{2}{3}\frac{1}{3}\frac{1}{3}$, so that the cylinders are cubic close packed.

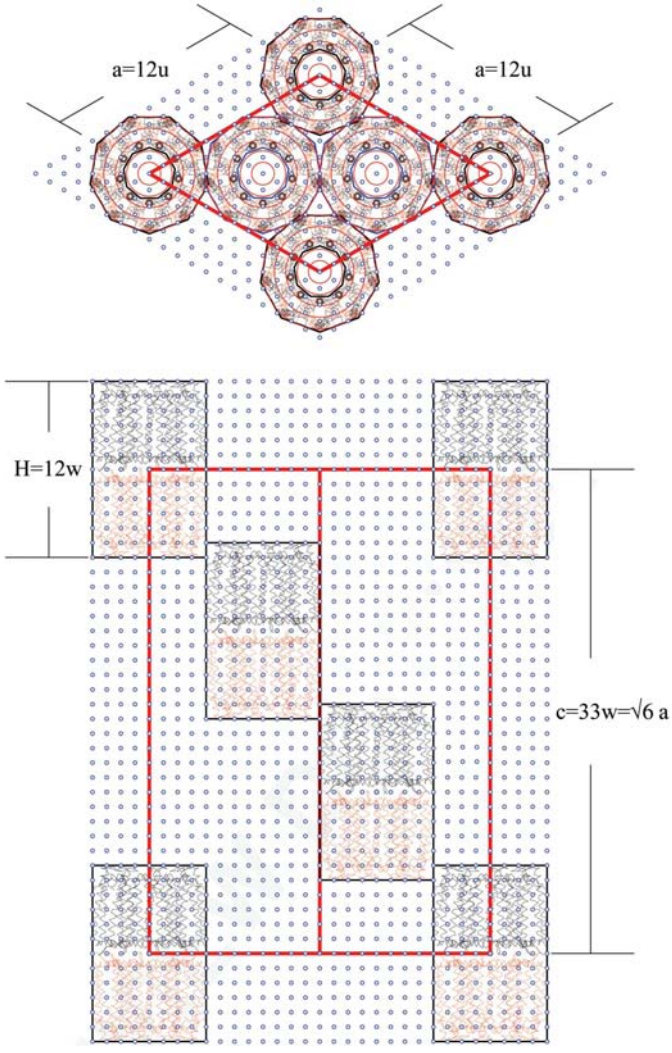

Figure 12
Packing of the double-stacked light-harvesting protein LH2 in the crystal with space group $H32$ and axial ratio $c/a = 6^{1/2}$, implying that the crystal lattice $\Lambda(a, c)$ is f.c.c. (cubic lattice parameter $a_{\text{f.c.c.}} = 2^{1/2}a$). The packing lattice $\Lambda_P(u, w)$ has parameters $u = a/12$, $w = c/33$ and axial ratio $w/u = 4(6)^{1/2}/11$. The packing form is a cylinder with radius $R_c = 3^{1/2}a/6 = 2(3)^{1/2}u$ and height $H_c = 4c/11 = 12w$, deviating a little from the corresponding values R_e and H of the molecular form (Fig. 11). The centres of the packing units are at the Wyckoff position 3(*a*) 000, $\frac{1}{3}\frac{2}{3}\frac{2}{3}$, $\frac{2}{3}\frac{1}{3}\frac{1}{3}$ of $H32$.

3.8. Rhodopsin–retinal complex

The last example is only apparently simple: the enclosing form, the point-group symmetry and the crystal packing are trigonal or hexagonal and crystallographic in three dimensions. Their mutual relations reveal unexpected interesting aspects, which could hint at possible general properties.

The enclosing forms and form lattice of the trimeric rhodopsin with symmetry $K_M = 3$ have already been discussed in a previous paper (Janner, 2005). Here the rhodopsin–retinal complex is considered as a whole. This has consequences for the orientation and the characteristics of the hexagonal form lattice. While the form lattice of the rhodopsin has parameters R_0 and H, with R_0 the radius of the central hole and H the height of the trimer, the form lattice of the rhodopsin–retinal complex is given by $\Lambda_F(a_0, c_0)$ with $a_0 = R_0/3$ and $c_0 = H/30$ and axial ratio $c_0/a_0 = 3(3)^{1/2}/10$, with values $a_0 = 3.5$ and $c_0 = 1.82$ Å. The external envelope of the rhodopsin has radius $R_e = 9a_0$, which is related to the height by $H = 3^{1/2}R_e$. The corresponding parameters of the retinal enclosing form are

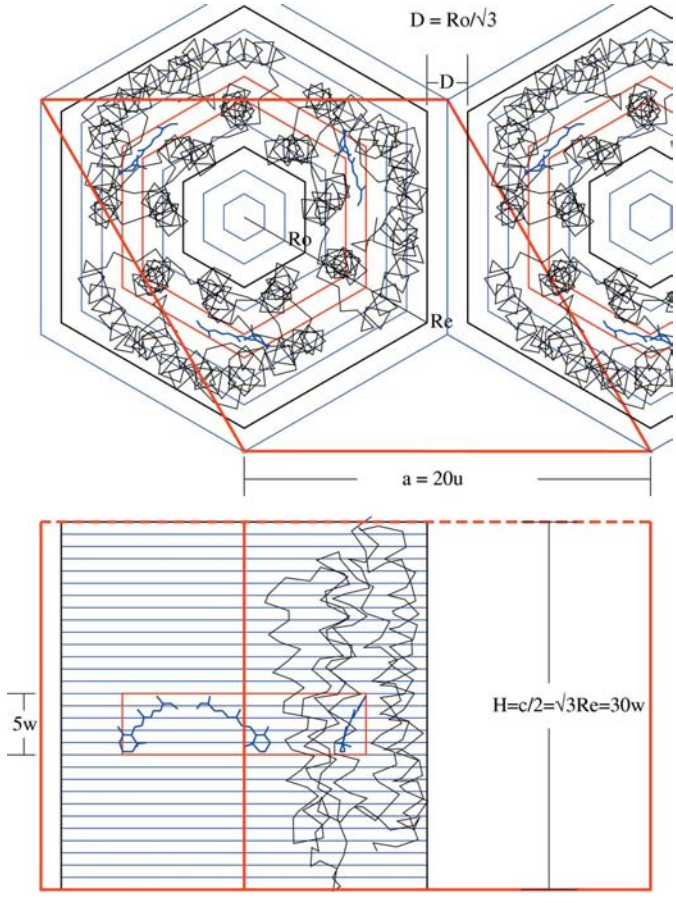

Figure 13
Packing unit of the rhodopsin–retinal complex in the crystal with space group $P6_3$. The packing lattice $\Lambda_P(u, w)$ is related to the crystal lattice $\Lambda(a, c)$ by $a = 20u$ and $c = 60w$. The packing form of the rhodopsin has height $H = c/2 = 30w$, internal radius $R_0 = 3a_0$, inter-packing distance $D = R_0/3^{1/2}$ and external radius $R_e = 9a_0 = H/3^{1/2}$, where $a_0 = u/\cos(\pi/6)$. The corresponding packing parameters for the retinal are $H_r = H/6 = 5w$, $R_{0r} = 5r_0$ and $R_{er} = 6r_0$.

$R_{0r} = 5a_0$, $R_{er} = 6a_0$ and $H_r = 5c_0$. The packing form of the complex is turned by 45° around the hexagonal axis with respect to the orientation of the molecular form indicated above, but the corresponding parameters are the same. The inter-packing distance is $D = 3^{1/2}a_0 = R_0/3^{1/2} =$ 6.06 Å, revealing the crystallographic compatibility between molecular form and crystal packing parameters (Fig. 13).

Indeed, the *packing lattice* $\Lambda_P(u, w)$ is connected to the hexagonal crystal lattice $\Lambda(a, c)$ by the relations $a = 20u =$ 61.06 Å, $c = 60w =$ 110.57 Å and to the form parameters by $u = 3^{1/2}a_0/2$ and $w = c_0 = 3(3)^{1/2}a_0/10$, leading to the axial ratios $w/u = 3/5$ and $c/a = 9/5$ (1.81), with the experimental values in brackets.

The *crystal space group* is $P6_3$ and the centres of the hexagonal prismatic packing units are at the Wyckoff position $2(b)$ $\frac{1}{3}\frac{2}{3}z$, $\frac{2}{3}\frac{1}{3}\frac{1}{2}+z$, for $z = \frac{1}{4}$.

The geometric relations between the molecular enclosing form, the space-group symmetry and packing (with packing units and their centres) are shown in Fig. 14, for the empty case (omitting the molecular chains), which recalls the space-group diagrams in Volume A of *International Tables for Crystallography*. Note that the origin of the packing lattice indexing the molecular forms is different for the two Wyckoff positions indicated above, and from that of the crystal lattice. The origins involved are related by shifts of $\frac{1}{3}(\mathbf{a}+\mathbf{b})$ along the diagonal of the two-dimensional hexagonal unit cell. This plot looks like an interference pattern of hexagonal waves satisfying appropriate boundary conditions.

A similar diagrammatic representation of the relation between forms and space-group unit cell for the case of the light-harvesting protein LH2 is shown in Fig. 15 (compare with Fig. 12).

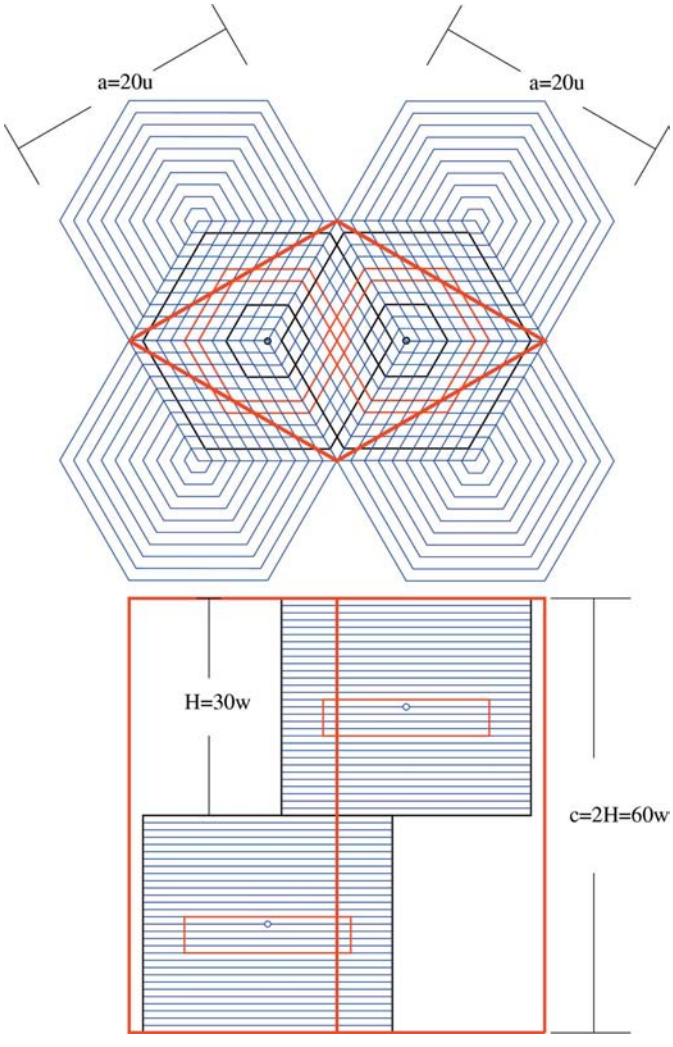

Figure 14
Geometric relations underlying the packing forms of the rhodopsin–retinal complex in the crystal with space-group symmetry $P6_3$. The centres of the packing forms (small circles) are at the Wyckoff position $2(b)$ $\frac{1}{3}\frac{2}{3}z$, $\frac{2}{3}\frac{1}{3}\frac{1}{2}+z$ with $z = \frac{1}{4}$.

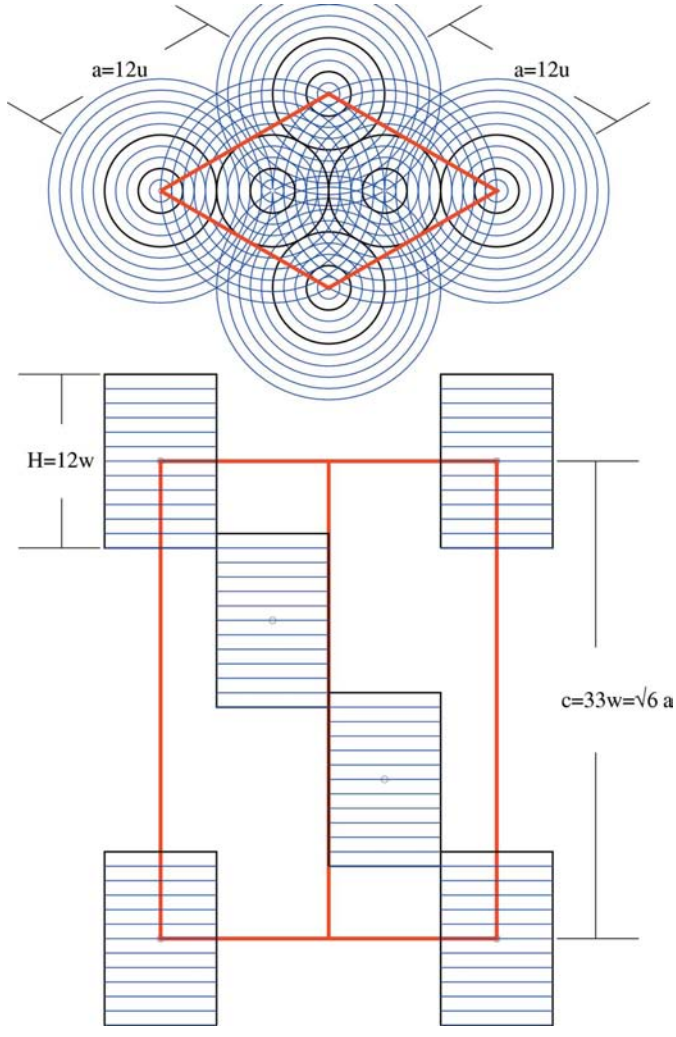

Figure 15
Empty geometric arrangement of cylindrical forms underlying, in a similar way as in Fig. 14, the packing of the forms enclosing the light-harvesting protein LH2 shown in Fig. 12. The pattern suggests standing cylindrical waves with appropriate boundary conditions, compatible with both the molecular and the crystal structures.

4. Final remarks

The validity of the concepts *packing form* and of the associated *packing lattice* (intended to represent a bridge between *enclosing form* and *form lattice* for single biomolecules, already introduced in previous publications, and *crystal lattice*) has been tested on a variety of concrete examples.

It has been shown how non-indexable packing forms, like spheres and cylinders, natural for biomolecules with non-crystallographic point-group symmetry, could nevertheless be associated with a *packing lattice*, by considering alternative forms circumscribing or inscribing the enclosing spheres or cylinders, respectively.

In many cases it is then possible to relate the integral property of form lattices to the observed parameter values of the crystal lattice, and to recognize its integral (or rational) character, even if not always structurally relevant, because of the possible deformation of the single biomolecules when considered in the crystal packed state.

The examples discussed support the evidence, pointed out in §1, of the non-accidental character of structural properties not explained by the current crystallographic laws, such as integral lattices and indexable molecular enclosing forms allowing one to relate external boundaries with possible internal holes and to find relations between the lattice parameters of a given crystal. In particular, intriguing structural properties have been found for trigonal proteins with hexagonal enclosing forms packed according to a cubic lattice.

Thanks are due to M. C. Feiters for having drawn attention to the peculiar properties of hemocyanin and of pentraxin in arthropods such as *Limulus*, and to A. van der Avoird for raising, after a talk given by the author, the general question of the relation between the crystals of biomacromolecules and their indexed enclosing forms.

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