from different fish species were found to adsorb at prism, secondary prism and pyramidal orientations, but not on the basal plane. This explains why the ice grows as needles parallel to the c-axis of the unit cell.)

Applications of antifreeze proteins, some based on their being potent inhibitors of recrystallization[6], have been sought for maintaining texture in frozen foods, improving storage of blood, tissues and organs, cryosurgery, and protecting crops from freezing. But just what is it that gives these molecules their activity? One side of them is relatively hydrophobic and the other relatively hydrophilic, and there has been disagreement over which side binds to ice[7]. Much of the evidence has come from freezing hysteresis measurements on synthesized versions of the natural proteins with amino-acid substitutions introduced into them.

The observations of Sidebottom *et al.*[1] raise the issue of the relationship between the two main effects of antifreeze proteins: freezing hysteresis (protection from freezing) and recrystallization inhibition (in organisms, presumably protection from freezing damage once freezing does occur). The general understanding has been that, like freezing hysteresis, recrystallization inhibition is a direct consequence of immobilization of solid–liquid interfaces in partially frozen samples. Sidebottom and colleagues' results suggest, however, that the two effects are uncoupled; if they are, then that understanding is probably flawed.

The molecular structures of antifreeze peptides described by Graether *et al.*[2] and Liou *et al.*[3] are the first from insects to be completely characterized. Both show beautiful structural matches between ice and the hydrophilic groups on one side of the molecule. Most of the fish antifreeze proteins shown to fit onto ice were linear molecules. Here, however, the arrays are two-dimensional in both cases, so there can now be little doubt that it is the hydrophilic sides that stick to the ice, leaving the relatively hydrophobic sides in contact with liquid water.

The structures[2,3] are similar in being β-helices, showing a new way in which peptide-chain folding can bring about the structural match that seems to be necessary for antifreeze activity. No doubt more ways will be found in the future. The very active spruce-budworm antifreeze of Graether *et al.*[2] is novel in yet another respect, in that the ice grows as basal plates from solution rather than as needles; this may be the first case of an antifreeze protein binding to the basal face of ice.

What of more general issues? The usual approach to antifreeze adsorption to ice has been to analyse the bonding between the antifreeze molecules and ice. In a different viewpoint, which I favour, the rather large antifreeze molecules can be viewed instead as very small particles. Their equilibrium positions at the ice–water interface can then be considered

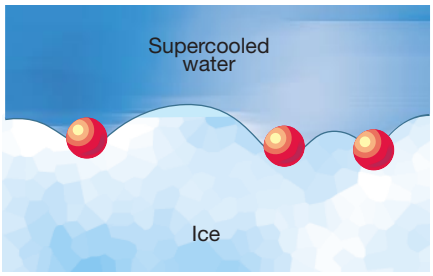

Figure 1 **Two-dimensional model of ice-growth inhibition by antifreeze molecules (red). One side of each molecule sticks to the ice, which grows between the molecules. But the curvature lowers the local freezing point through the Kelvin effect.**

in terms of interfacial energies, and insights from theoretical studies of the interactions of particles with growing crystals can be applied[8,9]. The important parameter is the difference in energy between the antifreeze–ice and antifreeze–water interfaces, and the principle is minimization of the total interfacial energy. Accordingly, even if an antifreeze molecule has no preference between contacting ice or water (its interfacial energies with them are the same), it may adsorb strongly because the area of the ice–water interface is reduced when it resides there.

Another point is that antifreeze proteins must match the ice structure well enough to prevent water molecules diffusing into the interface and pushing the protein ahead as the crystal grows. This 'pushing ahead' is the crystallization pressure familiar from many frost-heaving studies[10], in which the particle can maintain its equilibrium position at the surface as the crystal grows. Water molecules diffuse behind the particles, pushing them ahead and forming ice lenses in freezing soil. Considerable pressures are generated in this way, even over a supercooling temperature range of 1 °C or less.

With an array of antifreeze molecules anchored permanently to the ice and unable to be pushed ahead, ice growth only occurs at a supercooling sufficient to engulf the molecules, which then depends on their spacing on the ice surface. The depressed 'freezing point' presumably comes from the Kelvin effect, the local decrease in freezing point at the curved ice interface that bulges forward between the adsorbed antifreeze molecules (Fig. 1). The three-dimensional geometry of this is complicated, however, and has not been worked out.

The question that seems hardest to answer is that, if the adsorption is effectively permanent (as is necessary to stop the ice growth), why does the observed freezing point depend on the solution concentration? Especially at low solution concentrations, one would think it ought to depend more on the time available for antifreeze molecules to adsorb, but experimentally it does not seem to.

All in all, the three papers[1–3] discussed here do indeed provide thought-provoking information on the structure and behaviour of antifreeze proteins. But clearly there remains a lack of consensus on some of the fundamental issues to do with antifreeze action. ■

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Mathematics

Best packing in proteins and DNA

Andrzej Stasiak and John H. Maddocks

A pragmatic way to store a rope is to coil it loosely, and drop it in an appropriately shaped box. But if you are extremely parsimonious with space, as nature often is, this solution is suboptimal — there is a lot of unused space in the centre of the coil. So what is the longest piece of rope you can pack into a particular box? Such questions of optimal packing are addressed by Maritan *et al.*[1] on page 287 of this issue. Some of the optimal shapes they find are the familiar, naturally occurring, helical structures of proteins and DNA.

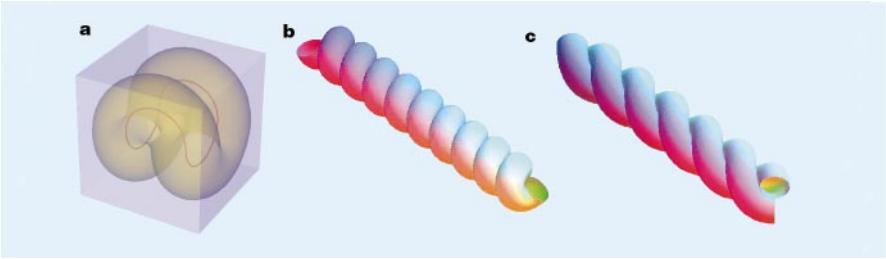
Optimal packing is a classic area of discrete geometry. Perhaps the best known example is Kepler's problem of the densest packing of identical spheres[2], which has important applications in such fundamental physical phenomena as crystallization and melting of condensed matter. But most physical objects cannot be modelled as simple spheres. In particular, many polymers, including DNA molecules and portions of proteins, might more reasonably be modelled as deformable tubes. For example, if we consider how DNA molecules could be packed within a small virus[3], we arrive at the question of optimal packing of tubes. Unlike

100 YEARS AGO

It is a remarkable sign of the times when the head of a firm principally distinguished for the introduction into this country of American methods of dealing with drugs, i.e. by putting them up in new and convenient shapes and doses, goes out of his way to fit up extensive research laboratories. This is what Mr. Wellcome has done... A well-built modern house has been secured at No. 6 King Street, Snow Hill, and has been converted into a series of three commodious and well-fitted laboratories, a library and office, and a store-room and workshop. Each laboratory is self-contained and each is connected with the other and with the directors' office by means of telephones… Mr. Wellcome intends to carry on his laboratories in no narrow spirit; this means, I presume, that he has other views then the conversion of his business into a chemical manufacturing concern. Though much work is done towards the perfection of the firm's preparations, time has been found for several researches which have been published, and other work of this kind is in hand… All interested in the advance of chemistry, whether pure or applied, will wish Mr. Wellcome success, and also that he may find imitators among the numbers of firms who are meditating an advance in the direction of a more scientific method of conducting their manufactures.
From *Nature* 19 July 1900.

50 YEARS AGO

Crystalline inclusion bodies in tobacco plants infected by tobacco mosaic virus have been known since 1903, and circumstantial evidence has made it appear likely that these crystals are composed largely of the virus protein. The present work makes it appear even more likely than before that the crystals are pure virus protein, and shows the crystals to be of considerable interest from several quite different but related points of view. On account of the exceptionally large dimension of the protein particle, it has been possible for the first time to make, in part at least, a structure analysis of the crystal using visible light in a manner analogous to that of X-ray diffraction. As a result, it has been possible to settle the controversial question of the length of the rod-shaped virus particle in the living plant. Also, the interpretation of the appearance of the crystals, as seen with the microscope, leads to a theory of the formation of images of three-dimensional objects. **M. H. F. Wilkins *et al.***
From *Nature* 22 July 1950.



P. PIERANSKI/A. DOBAY

Figure 1 **Tightly packed tubes.** a, **Packing of a tube into a box whose sides are four times the radius of the tube.** b, **A single helical tube whose centre line has the critical pitch/radius ratio of 2.512.** c, **Double-helical tubes in which the ratio between pitch and tube radius has the critical value 2π. Helices with parameters as in** b **closely resemble those observed in α-helical segments of proteins and are also obtained in numerical simulations of optimal packing of individual tubes by Maritan *et al.*[1], whereas the parameters of the double-helical tubes in** c **closely match those for DNA.**

spheres whose geometry is fixed, the optimal shape of the centre line of a deformable tube must be found. Consequently it is not a simple matter to find the optimal packing of even a single tube.

This modelling problem makes sense only if the tube, like any real rope or molecule, has a definite non-zero thickness. Figure 1a shows a way of packing (presumably optimally) a tube of length 4π and uniform thickness r (or radius r, but be aware that sometimes thickness means diameter) into a cubic box of side length $4r$. The centre line of the tube is a saddle-like trajectory that is built from four semi-circles. The point of this example is that, whenever the parameters of the tube and the box are less simply related, it is hard to even guess the optimal packing. This is the general topic addressed by Maritan *et al.*[1] in their numerical simulations.

The basic issue to be overcome in numerical simulations is that the idea of thickness is not quite as simple as it might first seem[4,5]. In the tube model a non-zero thickness has two possible effects: first, the centre line cannot be bent too sharply; and second, any two points that are far apart along the curve cannot be too close to each other in space. So, for a given centre line, the maximum possible thickness is governed by either local bending or by non-local points of closest approach, or, apparently exceptionally, by both conditions simultaneously. For the centre line of the tube shown in Fig. 1a, both of these conditions are simultaneously realized at every point along the red centre line.

Maritan *et al.* characterize the thickness of strings or tubes in terms of a quantity called global radius of curvature[6]. For any curve made up of discrete straight lines joining node points, the thickness is taken to be the minimal radius of all possible circles passing through any three nodes of the curve. If the smallest radius is achieved by three adjacent nodes, the thickness is controlled by local bending, whereas if the nodes on the minimal circle are not all adjacent, the thickness is governed by the non-local condition of closest approach.

Armed with this tool, Maritan *et al.* use a Monte Carlo algorithm to move the nodes of a centre line with a given length into an optimal shape, which maximizes thickness when subject to one of several compactness constraints. Perhaps the simplest compactness condition is that the centre line is completely contained in a given box. In spirit, their procedure is similar to that of earlier studies (see examples in ref. 7) but, with the exception of ref. 8, all previous work has looked at the optimal shapes of closed, knotted curves, much beloved of mathematicians. (A curve is closed if its two ends are joined or glued together to form a loop. A loop is knotted if it cannot be smoothly deformed to a simple circle without cutting.) Indeed, from a solely mathematical point of view, Maritan *et al.*'s contribution is the elegant, and in retrospect delightfully obvious, idea that the constraints of closure and knotting can usefully be replaced by one of several compactness conditions on the centre line. (In the absence of any compactness constraint at all, the optimal centre line is merely a straight line of infinite thickness.)

What about the physical implications of the simulations presented by Maritan *et al.*? Perhaps their most intriguing results arise when they impose local compactness conditions that are independent of external constraints such as a box. They state that, for "a broad class of local constraints", the optimal centre line is a particular helix in which the ratio of the pitch (or period) p to radius r is such that the bending and closest-approach constraints are realized everywhere simultaneously ($p/r = 2.512$). Maritan *et al.* then consider crystal structures of various α-helical polypeptides (one of the basic structural motifs of proteins), and show that the helices formed by the α-carbons in the polypeptide backbone have almost the same optimal shape as found in their simulations (Fig. 1b).

Are optimal packings of tubes related to other basic structural motifs in biology? For example, does the DNA double helix also involve optimally packed tubes? A related problem studied by Pieranski[8] involves finding the densest coiling of two identical

interwound tubes forming a double-helical structure (Fig. 1c). As noted by Pieranski, for this problem there is a critical pitch-to-radius ratio ($p/r = 2\pi = 6.28$) above which the line of contact between the tubes is straight, and below which it is helical. The crystallographic diameter of the classic DNA double helix is 23.7 ångstroms (Å), which would correspond to a radius for each of the two idealized helical tubes of 23.7/4 = 5.92 Å (here the sugar-phosphate backbones lie on the outside of the helical tubes). The generally accepted value for the pitch is 10.5 base pairs or 35.7 Å, so $p/r = 35.7/5.92 = 6.03$, which is within 4% of 2π. Is this an insight or just a coincidence? We suspect it is the former. ■

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Cell biology

A protein accumulator

Jennifer C. Pinder and Anthony J. Baines

We thought we knew what spectrin does. Is it not the elastic, membrane-bound protein that prevents red blood cells from rupturing as they circulate in the bloodstream? And does it not have the same supporting function in other cells? The second assumption has seldom been questioned over the past two decades, but has just been overturned by the power of experimental genetics, as described in three reports[1–3] in the *Journal of Cell Biology*. The results may bear on human diseases such as muscular dystrophy.

Red blood cells with deleterious mutations or deficiencies in spectrin have weakened outer membranes, are misshapen and lack resilience[4], so it is not surprising that spectrin has long been assumed to be necessary for membrane integrity. Over the years, other functions have been ascribed to spectrin as well. For example, it is thought to be involved in generating the polarized morphology of epithelial cells, and to have roles in the functioning of the Golgi complex — an organelle involved in protein secretion from cells — and the organization of synaptic vesicles (see, for example, refs 5–7).

To probe spectrin's function, Hammarlund *et al.*[1] and Moorthy *et al.*[2] have taken advantage of the simplicity of the genome of the nematode worm *Caenorhabditis elegans*. This genome, like that of the fruitfly *Drosophila melanogaster*, has only three spectrin genes, encoding α, β and β_H forms of the protein (Fig. 1). Hammarlund *et al.*[1] looked at worms with a mutation called *unc-70*, which they discovered to lie in the β-spectrin gene. Moorthy *et al.*[2] used a now common technique for blocking protein expression: they injected double-stranded RNA into the worm's gonad to block the expression of one or more spectrin subunits individually and in combination. They then analysed the resulting embryos.

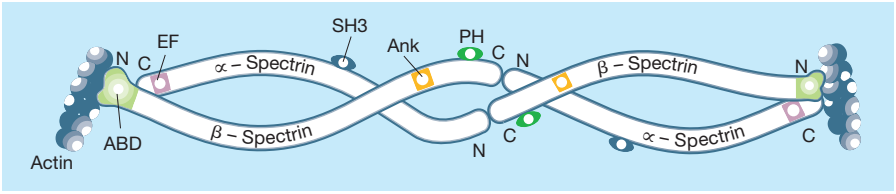

Figure 1 **The structure of spectrin[12,13]. Spectrin is a giant molecule comprising α and β subunits, of which there are different types. For example, *Drosophila* and *C. elegans* have α, β and β_H forms of spectrin. (β_H-Spectrin is a 'heavy' form of β-spectrin.) The α and β subunits associate to form an elongated $(\alpha\beta)_2$ tetramer. Lying near to the interior surface of the plasma membrane, spectrin forms a hexagonal lattice, the nodes of which are crosslinked by the cytoskeletal protein actin. This network is attached to the membrane in several ways, for example through the connecting protein ankyrin. β-Spectrins have binding sites for α-spectrin, actin and ankyrin (Ank). The pleckstrin-homology (PH) domain binds to certain membrane lipids. The Src-homology-3 (SH3) domain of α-spectrin probably accumulates signalling molecules close to the membrane. EF-hands are calcium-binding sites. ABD, actin-binding domain; C, carboxy terminus; N, amino terminus.**

Surprisingly, both groups find that β-spectrin is not essential for many of the processes suggested from previous investigations. Their worms do not lose general membrane integrity, and synaptic vesicles in nerve endings are clustered normally. The cellular secretory pathways appear unimpaired: the worms deposit cuticle and secrete collagen and components of the basement membrane as usual. The cells that should be polarized are polarized, suggesting that β-spectrin has no primary function in this process. Ankyrin is a connecting protein that is known to link spectrin to a variety of transmembrane proteins, including cell-adhesion molecules of the L1 family. Moorthy *et al.*[2] find that, in their worms, ankyrin apparently binds to L1 adhesion molecules normally.

However, the worms are paralysed and have a 'dumpy' appearance[1]. The main defects lie in the organization of muscle and nerve cells. The number of neurons is normal, but the patterns of axon outgrowth are altered[1,2], with few axons finding their targets. The muscle cells have disrupted sarcomeres (contractile units)[1,2], and the sarcoplasmic reticulum — an intracellular calcium store — is generally missing. It seems that the dumpy appearance is caused by a failure of the muscles to spring back after contracting. The muscle defects may arise during the course of contraction, as worms with both the *unc-70* mutation and the *unc-54* mutation, which results in a failure in muscle contraction, show less severe characteristics than the *unc-70* mutants[1].

What, then, has become of the anticipated functions of spectrin? Dubreuil *et al.*[3] have looked at fruitflies that lack β-spectrin, and provide some clues to the role of this protein in cell polarization. These flies live just long enough for 'copper' cells in the intestine to be analysed. In the mutant flies, the copper cells are polarized and ankyrin associates with the membrane of these cells as normal. So spectrin is not the main driver of cell polarization. It has been suggested[8] that what drives cell polarization is the contact of transmembrane cell-adhesion molecules with either their extracellular matrix ligands or their counterparts on other cells. So, in this case, a transmembrane L1-type adhesion molecule binds to its ligand and recruits ankyrin. But a transmembrane ion pump, the Na^+/K^+ ATPase, does not accumulate as normal in the plasma membrane of the mutant copper cells. Why is this?

Spectrin has been described as a 'protein-sorting machine'[9]. The new data[1–3] show that this model can be taken a step further: spectrin not only sorts, but also collects, proteins at the plasma membrane. Genetic evidence indicates that spectrin functions as a tetramer (Fig. 1). Each tetramer has two