Yves Bouligand’s analysis of the organizations of biological materials in relation to those of liquid crystals enabled the development of the idea that physical forces exerting their actions under strong spatial constraints determine the structures and morphologies of these materials. The different levels of organization in collagen have preoccupied him for a long time. We present here our recent works in this domain that we were still discussing with him a few months before his death at the age of 76 on 21 January 2011. After recalling the hierarchical set of structures built by collagen molecules, we analyse them, exploiting the properties of the curved space of the hypersphere and of the algorithm of phyllotaxis. Those two geometrical concepts can be proposed as structural archetypes founding the polymorphism of this complex material of biological origin.

Keywords: Yves Bouligand; biological materials; liquid crystals

1. YVES BOULIGAND, AT THE INTERFACE BETWEEN INERT AND LIVING MATTERS

At the end of the 1960s when the French community of liquid crystals was being formed, a somewhat unexpected figure in those times of effervescence met with it. Dressed in a conservative, dark-grey suit and manifesting an attentive courtesy in answer to the amazement of the audience, Yves Bouligand presented a work on crab shells with a discreet touch of passion. By means of a geometrical analysis of cuts made in those shells, he showed that crustaceans had secreted cholesteric liquid crystals a long time ago before us, and that they create growing forms using their defects and mineralize them in order to build their exoskeleton. We found all this a bit strange and timeless. On the one hand, much excited by the fundamental aspects of liquid crystals and their promises of applications, we were not far from thinking that crab shells could stay hidden in the drawers of our Museum of Natural History. On the other hand, we had the feeling that the work by Georges Friedel, characterizing the main liquid crystalline structures by the study of their defects, was the summit of the use of geometry in this domain and was the only basis we needed to put to work our fresh knowledge in statistical physics.

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On the whole, we were therefore rather disconcerted by Yves Bouligand’s programme of research, but some of us were fascinated and searched to understand it better. Several informal meetings took place in each other’s laboratories. For instance in the building occupied by Yves Bouligand—the hall of the great spark gaps and Faraday’s cages of the laboratory of Ivry, as drawn in the adventures of ‘Black and Mortimer’ by E. P. Jacobs, or the beetle gallery of the Museum, as filled with wonder as a cabinet of curiosities of the eighteenth century—cannot but create an aura of strangeness around him. An aura that we suspected, he was pleased to strengthen by searching his references, not only in Friedel, Nageotte or d’Arcy Thompson, but also in Buffon or again further in Bacon and even Aristotle. Each of our meetings with Yves Bouligand, always accompanied by his colleagues Françoise Gaill, Marie-Madeleine Giraud-Guille, Lién Lepécheux and Françoise Livolant, was the source of new findings. Thus, we discovered the remarkable structures and morphologies of many biological materials, not only crab shells, but also membranes of light-emitting animals, annelid cuticles, DNA aggregates and connective tissues such as skin, tendon and bones. The observations of this team highlighted the relations that exist between those structures and those of liquid crystals, and its analysis emphasized the role of their topological defects in the morphological development [1,2]. Beyond the originality of the materials studied by Bouligand, we were at last able to appraise the coherence and the topicality of the programme.

Yves Bouligand was therefore working at the interface between the inert and living worlds. This position enabled him to show how physical forces, exerting their action under strong spatial constraints, model the structures of the living world and determine their
morphogenesis, so that cells can use those processes to orient these structures and forms towards a function within the frame of evolution. He contributed to show that a limited number of fundamental structures is indeed at the origin of a large variety of natural forms, owing to subtle interplays between topological defects. This pushed him to also intervene against the recent questionings of Darwinism [3].

The encounter with Yves Bouligand was at the origin of an unforeseen opening towards the problem of the morphogenesis of living organisms. This was an old problem but it seems very new for the condensed matter physicists we were, first formed in solid-state physics and then orienting ourselves towards the studies of liquid crystals and their applications.

We present here some of our works on the different organization levels of collagen that we had the pleasure to discuss with him during these last years. We are indebted to him for having drawn our attention to this complex problem, suggesting that it should be approached by examining first the spatial constraints imposed at the different steps of the self assembly in order to identify the geometrical foundation of this hierarchical polymorphism. The necessary geometrical tools we use having been described in a few recent publications [4–6], we directly examine their implementation.

2. COLLAGEN FIBRILS

Collagen fibrils, cable-like assemblies of long biological molecules, are the major constituents of connective tissues such as bone, cartilage, myofibrils, ligament, skin and cornea. Typical fibrils of type I collagen are shown in figure 1.

Their properties and the rich polymorphism of their associations under different physico-chemical conditions allow the building of tissues adapted to the very diverse roles expected from them. Unfortunately, they can also underlie the development of crippling deformities associated with rheumatic diseases and congenital defects in the tissues. The structure of collagen fibrils and their associations have therefore been the subject of many studies but are not totally understood yet.

It is commonly accepted that the formation of type I collagen fibrils proceeds along two steps, as shown in figure 2: left-handed simple helices of collagen molecules build right-handed triple helices, which in turn build the fibrils (an intermediate step in which five triple helices would associate to build micro-fibrils was proposed earlier but is now questioned).

These fibrils can associate themselves following different schema according to the role expected of them, most often building long composite cables but not always, as shown in figure 3.

Thus, in figures 2 and 3, a remarkable sequence of hierarchical structures is displayed that exemplifies how complex the formation of the final architecture can be. Its detailed analysis would require a knowledge of molecular interactions, which is not accessible at the moment. However, in order to lay a basis for a further physico-chemical approach, we examined the successive steps of this sequence, just searching for organizations that would respect the compactness and symmetry constraints imposed by the interactions between chiral molecules at different structural levels.

3. FROM SIMPLE HELICES TO TRIPLE HELICES

The so-called simple helix is a long polypeptidic chain produced by specialized cells and represented in figure 4. Those chains associate three by three with the same orientation and keeping their chirality to build a right-handed triple helix. Crystallographic data obtained with crystals of synthetic polypeptides show that the glycines of each simple helix with rapid left pitch occupy the core of the triple helix and draw a helix with a slow right pitch. Hydrogen bonds between the nitrogens of the glycines and the oxygens of the prolines connect the three simple helices and stabilize the triple helix. The simple helix has a non-integer number of amino acids per turn of the triple helix, and the periodicity of the primary structure is not found in the secondary structure. If the symmetries of this organization are considered, it is possible to develop a geometrical approach [10] that justifies its broad lines referring to a particular geometrical object, the helix or column of Boerdjick–Coxeter represented in figure 5. The number of summits on a helix of type {2} is not an integer because the distance between the centres of the tetrahedra and the pitch of the helix are not commensurable. Such a column decorated as in figure 5c by Gly-Pro-Hyp amino acids along a {2}-helix and Gly along a {3}-helix can then be taken as a model for a simple helix.
A model for the triple helix must associate three of these simple helices; so we have to pack three Boerdjick–Coxeter columns of tetrahedra. Unfortunately, tetrahedra cannot be densely stacked in our Euclidean space \( \mathbb{R}^3 \); this is possible only in the curved space of the hypersphere \( S_3 \), where the summits of the tetrahedra are the 120 vertices of the polytope \( \{3,3,5\} \). It is possible to gather these vertices by sets of 10 on 12 great circles of \( S_3 \). Those great circles are members of the so-called Hopf fibration of \( S_3 \); they are ‘naturally’ twisted as represented in figure 6a.

The Hopf fibration admits a spherical basis on which one fibre is represented by one point, and the representative points of those 12 circles are the 12 summits of a spherical icosahedron. A simple helix is then represented on this basis by an equilateral triangle and three simple helices twisted in a triple helix by three such triangles each attached at the summit of a central triangle, as drawn in figure 6b.

It appears that this geometrical model of a triple helix corresponds well to what is known from structural studies of crystalized synthetic polypeptides: glycines

Figure 2. Left-handed collagen molecules (a) assemble in right-handed triple helices (b,c) represented by rods (d), which in turn are proposed to be longitudinally assembled with a regular shift (e) in order to create zones of gap ‘g’ and overlap ‘o’ giving account of the striations; the transverse bi-dimensional distribution of triple helices in (e) is still a matter of debate.

Figure 3. Precipitate of collagen fibrils extracted from calf skin [8] (a), the fibrils seen along their normal section at the centre are seen along more and more oblique sections, thus revealing the existence of a double twist, and structure of an annelid cuticle [9] (b), the fibrils are stacked in orthogonal layers crossed by microvilli issued from the epidermic cells and the molecular orientation within the fibrils are represented by nails of variable lengths.
are localized in the core of the triple helix; there are 3.75 amino acids per turn of a simple helix; and the hydrogen bonds linking the simple helices are quasi normal to the triple helix axis. But this triple helix is built in the curved space \( S_3 \) where it follows the trajectories of great circles; this is a toroidal object which should be projected as a straight object in \( R_3 \). This was not done; nevertheless, the local features of this structure in \( S_3 \) are not strongly affected by the projection and give account of the local organization in \( R_3 \) in a rather satisfactory way.

Moreover, this model provides information about the stabilization of the structure when it shows that the two protons of each glycine must be localized in the core in a compact manner. This compactness would protect the hydrophobic CH\(_2\) of the glycines from getting into contact with water, taking advantage of the configurational freedom of the chain in their vicinity to adopt this geometry. This hydrophobic force would initiate the aggregation of three simple triple helices, which would then be locked by hydrogen bondings. Of course, this search for compactness cannot be the only term dominating the formation of a biological helix, but its intervention should have a certain value in as much as the collagen structure does not seem to have a great sensitivity to the chemical details of the other amino acids of the chain.

4. FROM TRIPLE HELICES TO FIBRILS

The internal structure of the fibrils and their stability have been the subjects of many works; they however do not give access to a definite perspective at the moment. This holds to the complexity of the X-ray-scattering spectra that suggest variable coexistences of order and disorder according to the preparations of the samples, but a clear relation was not established. We recently proposed to consider that triple helices in fibrils adopt a phyllotactic organization [11]. Phyllotaxis describes a self-organized growth process that might be well adapted here insofar as it leads to organizations with cylindrical symmetry in which each element has the most homogeneous and isotropic area per point, also characterized by an intrinsic coexistence of order and disorder.

A phyllotactic organization is obtained with an algorithm such that the position of point \( s \) is given by its polar coordinates \( r = \alpha \sqrt{s} \) and \( \theta(s) = 2\pi\lambda s \), that is \( r = (a/\sqrt{2\pi\lambda})\sqrt{\theta} \), which is the equation of a Fermat spiral here called the generative spiral. The best packing efficiency is obtained for \( \lambda = 1/\tau \), where \( \tau \) is the irrational golden ratio \((1 + \sqrt{5})/2\). The phyllotactic organization in a plane of a set of 2500 points with their Voronoi cells is shown in figure 7.

In this pattern, pentagons and heptagons are associated with dipoles distributed along narrow circular rings with constant widths that separate large rings of hexagons whose width increases progressively from the core to the periphery, according to the Fibonacci series. This organization is then characterized by a local disorder, the polygons being not regular, on which is superposed the long range order of the rings of dipoles, an order of topological nature. The role of these rings of dipole is essential to maintain the homogeneity and isotropy of the Voronoi cells; they can be seen as dislocations introducing new cells in a cylindrical configuration.

Such a pattern can be considered as representative of the organization of triple helices in the overlap regions of the fibrils but not in the gap regions where the shift of the alignment shown in figure 2e requires the suppression of one point over five in the phyllotactic

Figure 4. The amino acids, glycine, proline and hydroxyproline, and the chain of the left-handed simple helix; the glycine is important structurally as the small size of its CH\(_2\) radical does not restrict chain deformations.
pattern. If this suppression is done along the generative spiral of the algorithm, a new pattern is obtained which is shown in figure 8.

The superposition of the squares of the Fourier transforms of the patterns of figure 7 (overlap regions) and 8 (gap regions) gives a reasonable account of the equatorial traces of scattered intensities of X-rays, mainly as far as the intrinsic coexistence of order and disorder is concerned. This is however but a preliminary approach to a complex structural problem, and the correspondence...
observed between the experimental and calculated spectra should be investigated more deeply. We are examining two directions aiming at a more realistic representation of the assembly of triple helices: one is the introduction of an additional cause of metric disorder through fluctuations of the positions along the generative spiral, and the other is the presence of an eventual torsion in relation to the chirality of the triple helices. The first is obviously associated with thermal disorder. The second is suggested by the fan shape of the experimental scattered intensity curves outside the equatorial line and shown by the observation of twisted toroidal fibrils among normal straight fibrils during the precipitation of a solution of triple helices, as represented in figure 1b.

Up to now, we have considered parallel triple helices, and they can be viewed as a fibration of the Euclidean space $R^3$ whose basis is drawn in a plane normal to the direction of alignment, the plane in which the phyllotactic patterns were built. If a double twist is present, the only template making this twist compatible with a homogeneous environment is the Hopf fibration in the curved space $S_3$ whose basis is a sphere in $R^3$ on which the phyllotactic patterns are to be built. Such patterns preserve the homogeneity of the area of the cells on the whole surface of the sphere but not the number of first neighbours that decreases from six to four when moving away from its polar regions, i.e. when the radius of the assembly increases. This might be related to the limited growth of the fibrils. Thus, the template of the assembly in the curved space would give an account not only of the nature of the organization but also of its size. This was indeed observed previously using a Seifert fibration in $S_3$ as a template for toroidal aggregates of DNA [12]. Moreover, this template in the curved space provides a clue to understand the formation of the toroidal fibril shown in figure 1b. This twisted fibril clearly puts in light the existence of a double twist in an assembly of triple helices. But a double twist cannot propagate without frustration in $R^3$; this is possible in $S_3$, only aligning the triple helices along Hopf fibres at a constant distance. The Hopf fibration therefore provides an ideal template which has to be projected in $R^3$. If this is done in the same manner as that in figure 6, the configuration obtained around the $C_6$ axis projected as a circle has the same toroidal topology than that observed. However, the stereographic projection, which is a conformal transformation, preserves the angles but not the distances. Thus, if it is chosen so that the distance between the projected fibres in the vicinity of this circle is equal to that of the triple helices, this distance cannot be preserved beyond and the double twist constraint can no longer be respected. This would bring in another reason for a limited lateral growth of the aggregate.

5. ASSOCIATION OF FIBRILS WITH DOUBLE TWIST

The precipitate of collagen of figure 3a clearly shows the existence of a double twist at this level of association. This is formally similar to what was discussed earlier, although on a different scale as the association concerns fibrils instead of triple helices. The Hopf fibration of the hypersphere also provides an ideal template, which has to be projected in our Euclidean space as shown in figure 6. The region of interest here is that limited
around the $C_\infty$ axis projected as a straight line. If the distance between projected fibres in this region is such that it is equal to that between fibrils, this distance cannot be conserved when moving away from it and this should limit the growth of the nucleus. This agrees well with the formation of the nuclei at the beginning of the association process, their dispersion in the field of observation and their finite size.

If those nuclei can be seen as finite solutions to the problem of the propagation of a double twist in the Euclidean space, the cuticular plywood structure presented in figure 3b can be seen as a step towards the building of an infinite solution. Here the propagation of the double twist is localized along the lines of contact between orthogonal layers of fibrils and this leads to the building of a nearly periodical structure of large size that is close to that proposed for a liquid crystalline blue phase and shown in figure 9.

Only cylinders are drawn on this figure, but it is an infinite crystal [13]. It can be described by joining the local replicas of the ideal structure built inside the hypersphere using disclinations to fill the angular deficit characterizing this curved space with respect to the Euclidean space. Those disclinations are periodically organized, and the structure is indeed a crystal of defects. In the cuticle, the double twist structure is discrete because of the presence of the microvilli going through it, whose cells provide the molecules needed for its formation.

6. CONCLUSION
From simple helices to triple helices then to fibrils and their associations, collagen displays an amazing series of diversified hierarchical assemblies, starting from a relatively simple molecule. This polymorphism is certainly put to use by biological cells to build tissues capable of ensuring the diverse functions expected from them in living organisms. The physico-chemical environment of the molecules and their associations obviously controls this diversification, but it is remarkable that at each step of their assembly, all the structures observed require for their description a unique geometrical object, the curved space of the hypersphere. Owing to its topology, only the Hopf fibration provides the ideal template with uniform double twist related to the chirality of the molecules. Geometrical transformations, adapted to the symmetries of this template, then make possible the descriptions of their assemblies in the Euclidean space. The distortions or defects introduced by those transformations bring in arguments to analyse the growth limitations or morphological evolutions at long distances. The collagen case therefore remarkably illustrates one of the preoccupation of Yves Bouligand, which was to show that a limited number of fundamental structures is indeed at the origin of a large variety of natural forms, owing to subtle interplays between topological defects.

Other properties of the hypersphere had already been used to describe two other systems of biological interest: Seifert’s fibrations in the case of the toroidal aggregates of DNA [12] and the family of parallel Clifford tori in the case of the cubic structures built by films of amphiphiles or membranes with symmetric interfacial curvatures [14]. The hypersphere can then be considered as a structural archetype founding organizations in which a local stacking order cannot propagate because of topological constraints associated with twist or curvature. But phyllotaxis, which we used to give account of the lateral organization of triple helices in fibrils, might claim for the same status. At the origin, its algorithm was used to analyse the self-organized growth process in plants showing spiral developments—for instance, daisies and sunflowers. Since then, it was also used to describe the formation of Bénard–Marangoni convection cells in a liquid layer contained in a cylindrical container submitted to a vertical temperature gradient [15,16], the distribution of ferrofluid droplets falling down in a silicone oil in the presence of a vertical inhomogeneous magnetic field with cylindrical symmetry [17] and the behaviour of bubbles at a circular air–water interface [18].

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