Stereochemistry at the Interface between Crystals and Biology

Extended Abstract of the 1998 *Prelog* Lecture

Foto: R. Häfliger

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So much has been said about *Vlado Prelog*, that I cannot add much. I feel that there is only place for personal reminiscence.

I pride myself in having been a friend of *Vlado*'s, although I know I must have been among the latest. I treasure the book of his autobiography, which bears written on the frontespice: 'To *Lia*, from *Vlado Prelog*'.

I met *Vlado* more than 20 years ago, in the mid seventies, when I was a young Ph.D. student at the *Weizmann* Institute. He was confronted by all our group, and submitted to a cross-fire of reports on the different projects each one of us was involved in. After a couple of hours, it was clear that he was tired, and wanted to stop without hurting anybody's feelings. He then stood up and said: 'I'm afraid I got to an age when, for each new piece of information I store in my memory, something else gets buried. I've heard from you many wonderful stories that I very much want to keep with me. I have, however, to keep a few other important bits of knowledge. I ask you thus to excuse me if, just in order not to forget those, I'll stop you here and thank you.'

It is his extraordinary grace, unassuming and never presumptuous, that I became acquainted with then, and met again over the years. I loved to talk to *Vlado*, on the few occasions I had an opportunity to meet with him since. I always came out of these conversations feeling somehow enriched.
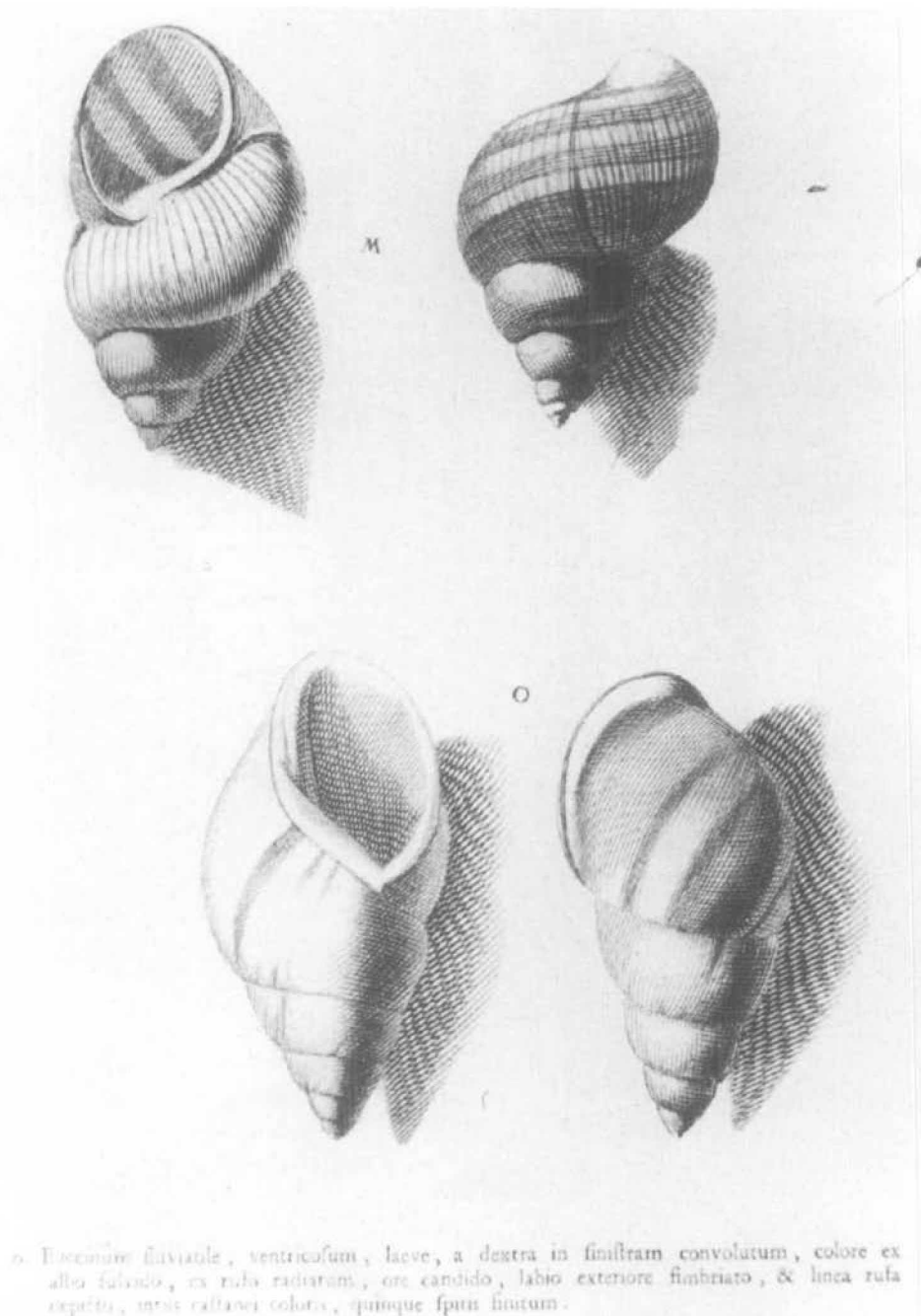
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Fig. 1. *A dextrorotatory* (M) *and a levorotatory* (O) *snail shell.* Copies of etchings taken from [1]. The legend refers to (O).

When I was chosen for the great honour of being the '98 *Prelog* lecturer, I was happy and touched in a very special manner, because stereochemistry is really close to my heart, and because *Vlado* was very close to my heart. A few weeks later, I was told that *Vlado* died. It was so sad that what I was looking forward to as a celebration for *Vlado* and with *Vlado*, was now going to be a memorial. But in a strange way this was still a celebration for *Vlado* and with *Vlado*, because we all loved him, and because all of us have been touched and influenced by him in our minds and in our research.

I will discuss biomineralization, the study of the mineralized hard parts made by organisms, as well as antibodies and cell adhesion. So where does stereochemistry come in?

A picture of a dextrorotatory and a levorotatory shell (*Fig. 1*), directly connects biomineralization to stereochemistry. This picture was taken from a book of etchings dated 1742, that belonged to my great-grandfather. The book collects, in a series of original etchings, the accurate image and description of thousands of samples from the shell collection of *Nicolao Gualtieri* from Firenze [1]. Looking through the book, one notices that the very large majority, in fact almost all the snail shells, are dextrorotatory (*Fig. 1*, top). This is also emphasized by the description of the shell in the lower part of the figure (*Fig. 1*, bottom), where we read '... a dextra in sinistram convolutum', turning from right to left. For none of the other shells is the direction of rotation specified, meaning that, already at that time, it was known that a levorotatory snail shell is very rare. We still do not know why. This is another reason I chose this picture, as a symbol of what is a unifying theme in my research: the breaking of symmetry in organisms; a fact with which we are constantly confronted in nature.

We are, unfortunately, still very far from understanding all the steps that result in snail shells being mostly dextrorotatory. The helicity of shells is a matter of development, determined at the cellular level. The transitions from molecular control to cellular control to development are indeed areas where information is still scant. This is what my research is attempting to contribute to, even in a very small measure. *Vlado Prelog* and others advanced enormously our understanding of stereochemical control at the molecular level. A new generation of researchers, myself included, are now trying to use the base that has been established to move to a larger length scale. We wish to understand how stereochemistry influences biology at the length scales of nanometers and microns; up to 10'000 times larger than the Ångström scale of molecules.

Mollusk shells are composite materials of mineral and biological fibers. They are among the 'smartest' materials we can think of, if what is defined as 'smart' refers to the strategy of building and to the control that is exercised on the construction at all length scales, from Ångströms to millimeters [2]. Together with my colleague *Steve Weiner*, my partner for the last fifteen years in the research on biomineralization, we have been (and still are) studying the secrets of shell construction and control at the molecular level. The important lesson that emerged early on from these studies is that proteins control many aspects of crystal formation in biomineralization, and they do so by virtue of their structure being matched to the structure of the crystal on one particular plane [3].
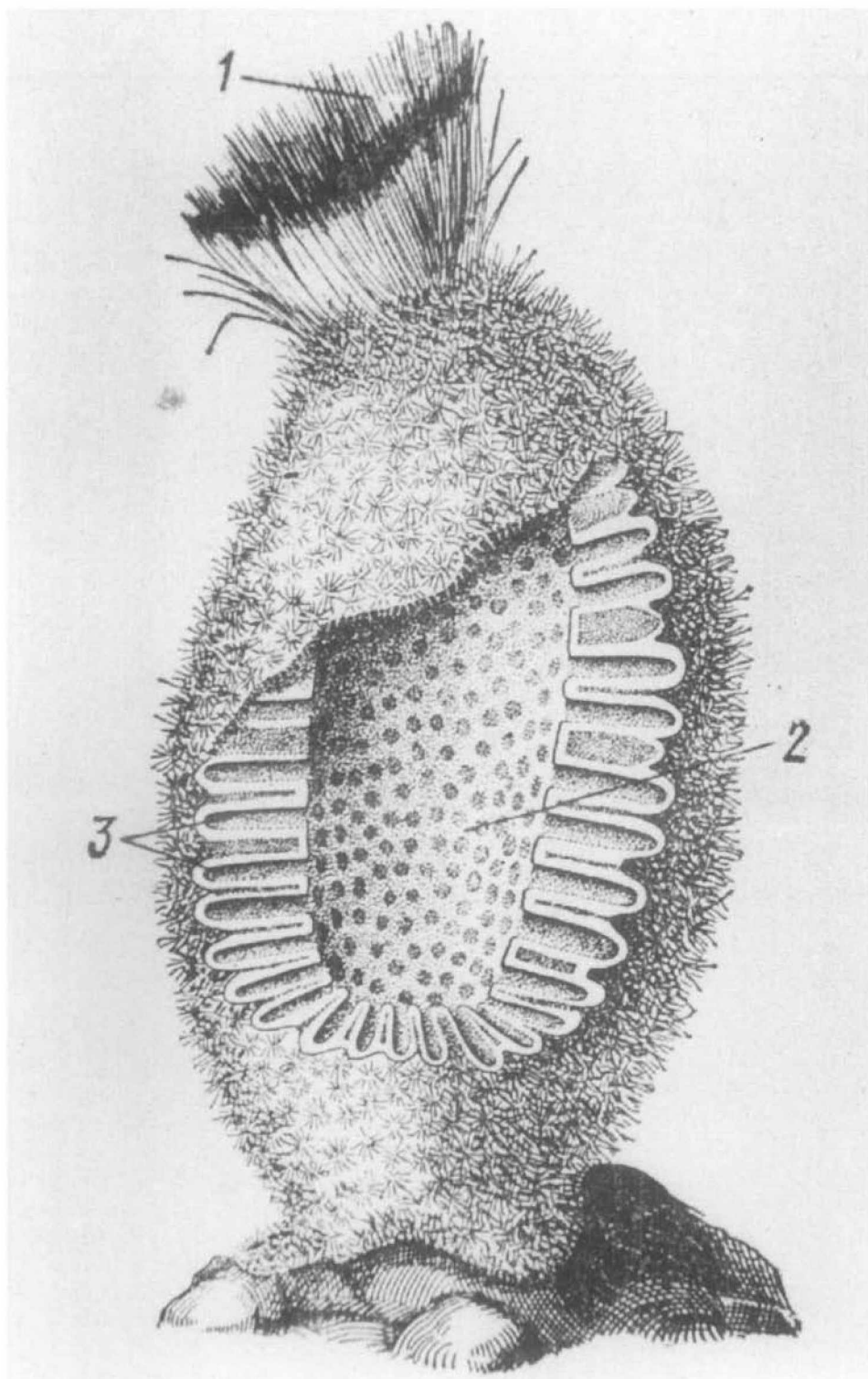

Fig. 2. *Schematic representation of the anatomy of the calcareous sponge* Sycon. Taken from: Dogel VA, 'Invertebrate Zoology', 6th edition, Moscow, 1975.

In contrast to mollusk shells, many skeletal elements of different organisms are single crystals of calcite with beautiful, convoluted shapes. Biological macromolecules are intimately involved in their construction, also operating through recognition and complementarity to specific crystal planes. These macromolecules are selectively adsorbed on some crystal surfaces during growth, and are then incorporated by overgrowth inside the crystal [4]. They have two distinct functions; to modulate the shape of the crystal, because growth is selectively retarded where a protein is adsorbed, and to reinforce the crystal against fracture, by interfering with the propagation of cracks along cleavage planes inside the single crystal.

One example that we have investigated in some detail is the calcareous sponge spicules, of which *Sycon* is a representative (*Fig. 2*). The spicules fulfill a structural function by providing the necessary rigidity to the soft tissues [5]. They have different sizes and shapes. Each is a single crystal of calcite (*Fig. 3*), and each is oriented differently relative to the crystallographic axes of calcite. How are shapes and orientation controlled? There are proteins inside these crystals, albeit in small amounts (0.02 weight-%). Proteins are very large relative to the unit cell of calcite. Thus, where they are located, they interrupt the regular growth of the crystal creating an imperfection. The average distribution of these imperfections in the different crystallographic directions can be mapped, and, consequently, the average distance between them can be deduced. The measured distribution of the imperfections inside the *Sycon* curved monaxon spicule was found to faithfully reproduce the macroscopic morphology of the spicule (*Fig. 4b*) [6]. We, thus, believe that the proteins are targeted to the surface of the growing crystal in a controlled manner, such that they modulate its growth defining microscopic domains, and, hence, eventually determine the morphology of the spicule. There is, however, a problem. The main axis of the spicule lies along the general direction [012]. There are three symmetry-related planes {012} that are perfectly identical. Yet, the crystal develops only along one of these directions, and not along the others. If proteins recognize the crystal surface based on its structure, how do they distinguish between three identical surfaces of the same crystal? One conceivable solution is that the crystal is nucleated from one of the three planes {012}. If this direction is blocked by nucleation, the crystal would develop growing in the opposite direction, [01$\bar{2}$], never
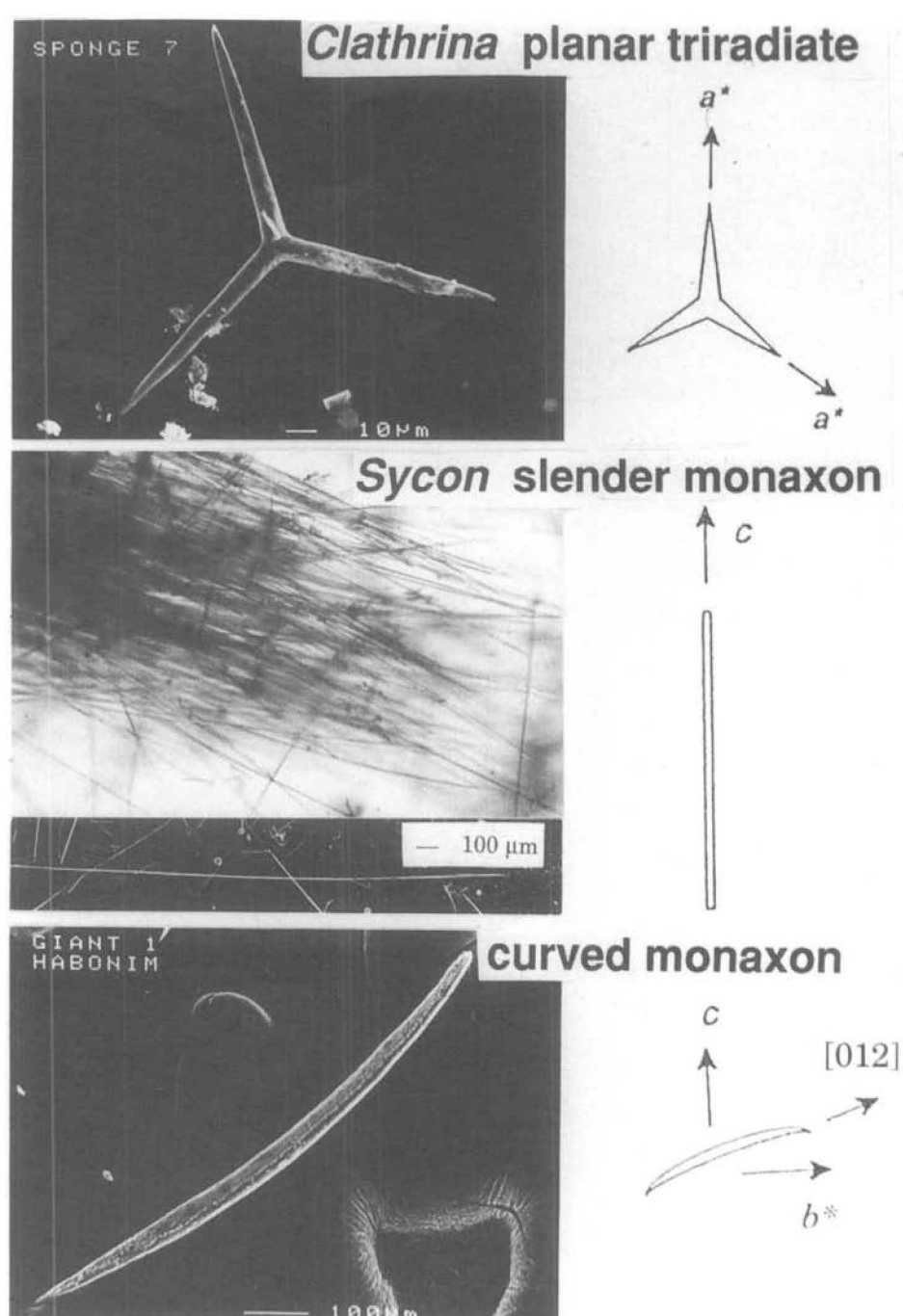

Fig. 3. *Scanning electron micrographs* (left) *and schematic representations of the crystallographic orientations* (right) *of three types of calcareous sponge spicules.* Each spicule is a single crystal of calcite.

exposing the plane to which the proteins can be adsorbed (*Fig. 4c*). The other two symmetry-related directions, in contrast, would be exposed during growth, would adsorb protein and be affected. This, in turn, implies that the protein can distinguish between the front and the back of the same plane, which is indeed chiral (*Fig. 4d*). The recognition of the chirality of this plane by a protein is certainly not trivial, and is, thus, a beautiful example of the strength of stereochemical recognition. We still do not have the protein in hand, and we may never know the trick involved.

What can conceivably be done to understand systematically how proteins recognize surfaces? Some answers can be obtained from the study of *antibodies* interacting with surfaces.

Antibodies are the tool that nature evolved in vertebrates to tackle all foreign invaders by virtue of molecular recognition. Crystals are also invaders of the organism, associated to well-known pathologies, such as atherosclerosis (cholesterol), gout (sodium urate), and kidney stones (calcium oxalate and others).

There is no good reason to assume that the immune system should relate to crystals in a manner different from other invaders because of their intrinsic nature as crystals. A number of years ago, we introduced the idea that specific antibodies
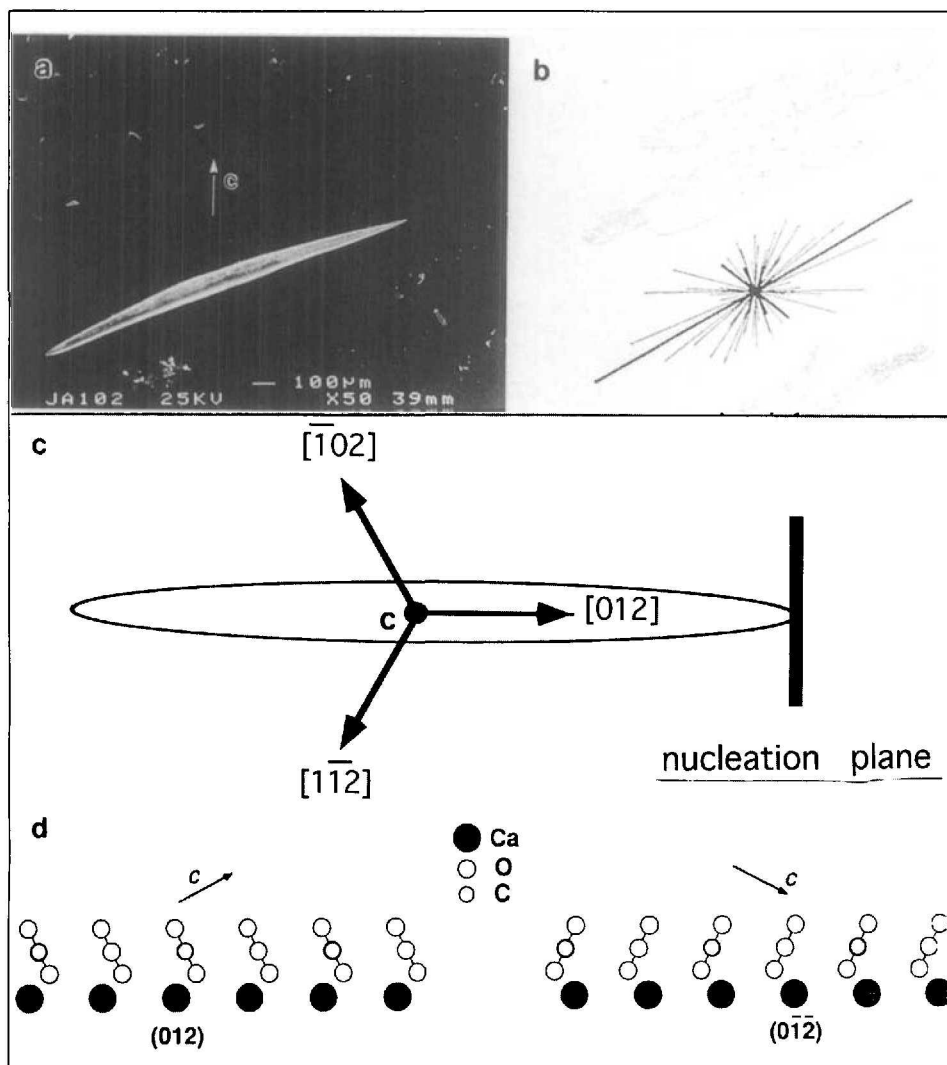
Fig. 4. *Curved monaxon spicules from the calcareous sponge* Sycon *sp.* a) Electron micrograph of one spicule; *b*) graphic representation of the microscopic distribution of imperfections inside the spicule: each vector is proportional to the length of perfect domains measured in the given directions; *c*) orientation of the three [012] directions relative to the spicule, and the suggested nucleation plane; *d*) structure of calcite on the (012) and (0$\bar{1}$2) chiral planes.

Fig 5. *Schematic representation of possible complementarity between antibodies and crystal surfaces*

may be produced against crystals [7]. We proposed that the structure of their binding sites may be complementary to the surface of the crystal exposed to solution; in essence a molecular mold (*Fig. 5*). Notice that an antibody binding-site, with an area of 600–900 Å^2, would typically cover an area on which 5–15 molecules are exposed at the surface of a molecular crystal, depending on the size of the molecules and on their orientation. Furthermore, each antibody would conceivably recognize just one surface type of the same crystal, while none would presumably recognize the isolated molecule.

Cholesterol crystals are associated with atherosclerosis and gall-stones. Both pathological conditions could, thus, generate an immune response to crystals of cholesterol. Antibodies which recognize cholesterol monohydrate crystals were generated and isolated after injection of the crystals in mice. One of these was shown to recognize specifically the {301} faces of the crystals [8a]. These faces are characterized by long hydrophobic stretches exposing the cholesterol backbones, separated by hydrophilic steps where water molecules and the 3β-hydroxy groups of the cholesterol molecules emerge. When the antibody binding-site was sequenced and its structure was modeled, we were delighted to find that it adopts the shape of a step, with five tyrosine hydroxy groups pointing out from one wall of the step, while hydrophobic residues pave the floor of the step. Docking of the binding-site model on the structure of the {301} face results in an extremely good match with cholesterol hydroxy groups and hydrophobic cholesterol backbones, respectively (*Fig. 6*) [8b].

The next question was whether the antibody would be able to recognize cholesterol in a more relaxed, but still organized state, such as in a monolayer of molecules at the air-water interface. This type of organization is closer to that of cholesterol in membranes. In such monolayers, the molecules are aligned to each other, more or less perpendicular to the interface, with their hydroxy functions in water and the hydrophobic backbone pointing toward the air. It was seen that the antibody that recognizes the {301} face of cholesterol monohydrate crystals does also recognize cholesterol molecules in a monolayer at the air-water interface, while antibodies that are not specific to cholesterol crystals do not [9a]. Complementarity, however, might be manifested only at the level of interactions between hydrophobic and hydrophilic surfaces, or hydroxylated hydrogen-bonding surfaces, without a real
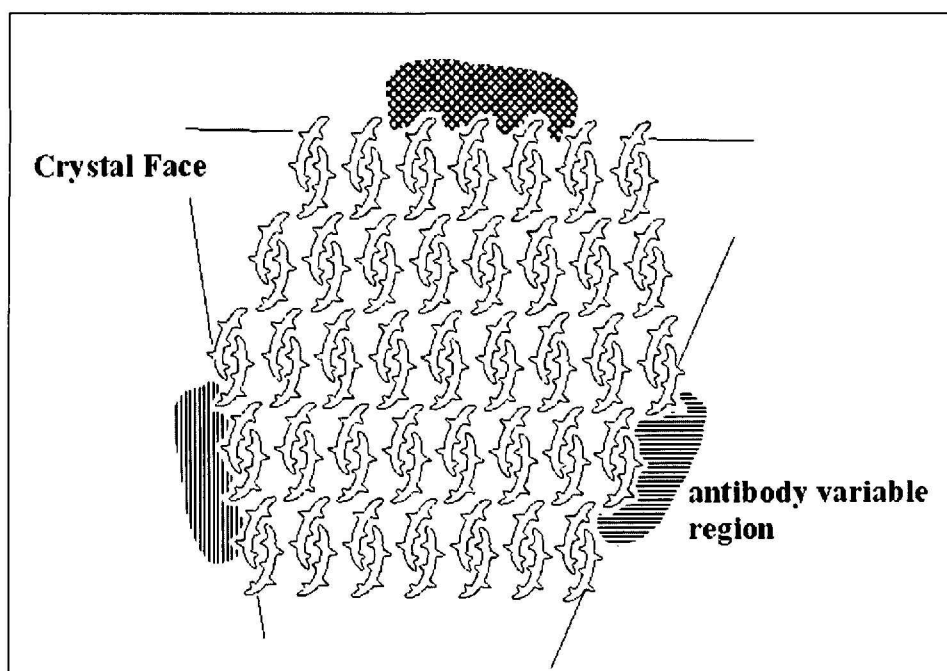
structural and stereochemical match. In order to clarify this issue, we formed monolayers of epicholesterol, which differ from cholesterol only in the orientation of the hydroxy moiety (3α rather than 3β). The schematic representation in *Fig. 7* shows that the specific antibody, with a binding-site structure akin to the modeled one, may not be able to complex with an epicholesterol monolayer, if the interaction is specific.

Indeed, epicholesterol does form monolayers at the air-water interface, with molecules oriented and structured in a manner very similar to that of cholesterol. These monolayers, however, are not recognized in any measure by the same antibody that recognizes cholesterol monolayers under exactly the same conditions [9b].

It is, thus, evident that stereochemistry operates at interfaces of organized surfaces as well as on separated molecules, and is an invaluable tool to discern the fine details of molecular and surface specificity in biological macromolecules.

We addressed an even more ambitious question, namely, whether *cells* have stereorecognition at the molecular level. Note that, if macromolecules such as antibodies operate at a length scale of one order of magnitude larger than molecules, the length scale of cells, with typical sizes of tens of microns, is four orders of magnitude greater than molecular size.

Most of the cells in multicellular organisms, apart from the blood cells, have to adhere to substrates in order to live, proliferate, receive and transmit signals. The adhesion process can best be described as a sequence of events, involving recognition, attachment, and spreading, culminating in the formation of the so-called focal adhesions. The question that we addressed here (together with my colleague *B. Geiger*) concerns only the very first step of this cascade of events, namely recognition. At which level does a cell recognize the surface to which it does, or does not, attach? Again, crystals, with their homogeneous and structurally defined surfaces, offer us an ideal tool to address such questions.

Calcium tartrate tetrahydrate crystals develop two types of faces with different structure of the exposed molecules, one dominated by hydroxy substituents and water, the other by carboxylate and calcium ions. Certain epithelial cells were shown to massively attach within minutes to the former, and not to attach even within hours to the latter. This effect could, however, be caused by differences in chemical potential at the two surfaces rather than molecular recognition.
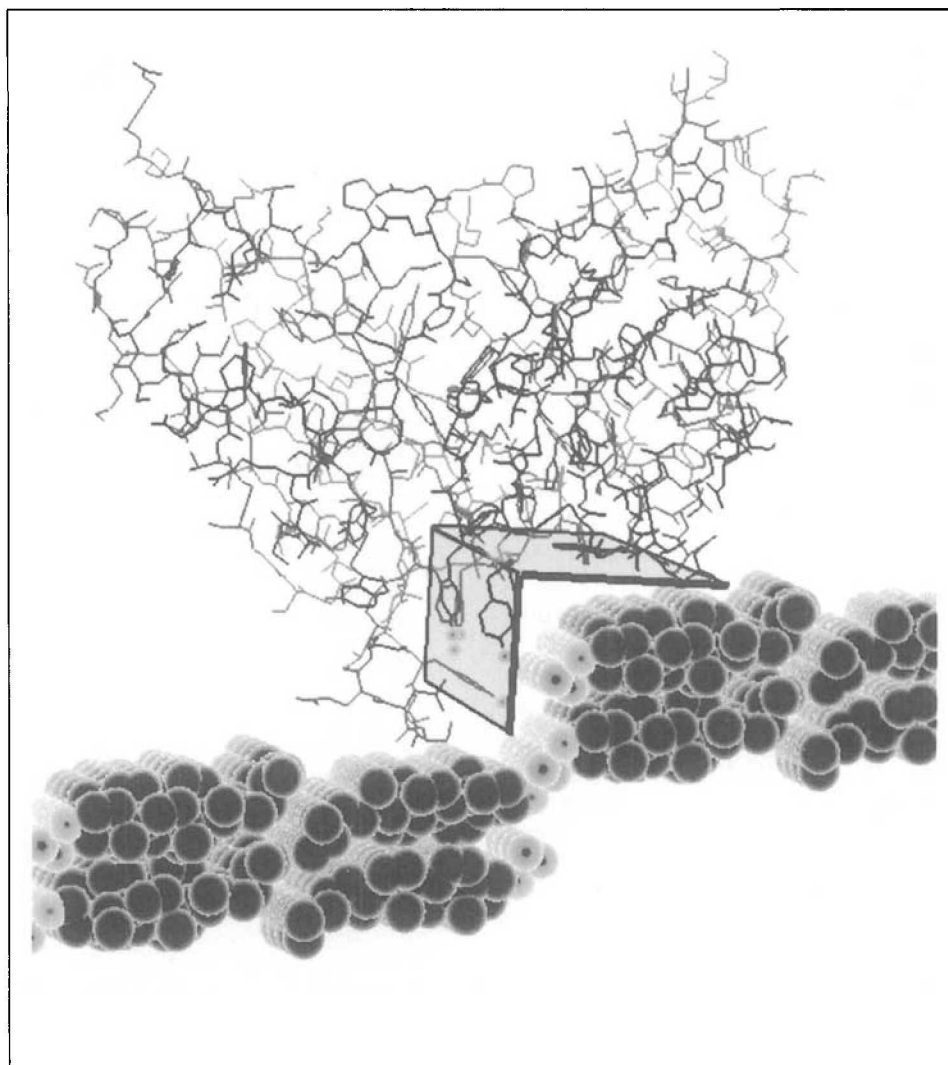

Fig. 6. *Docking of the modeled structure of antibody 36A1 on the surface of cholesterol monohydrate that it selectively recognizes*
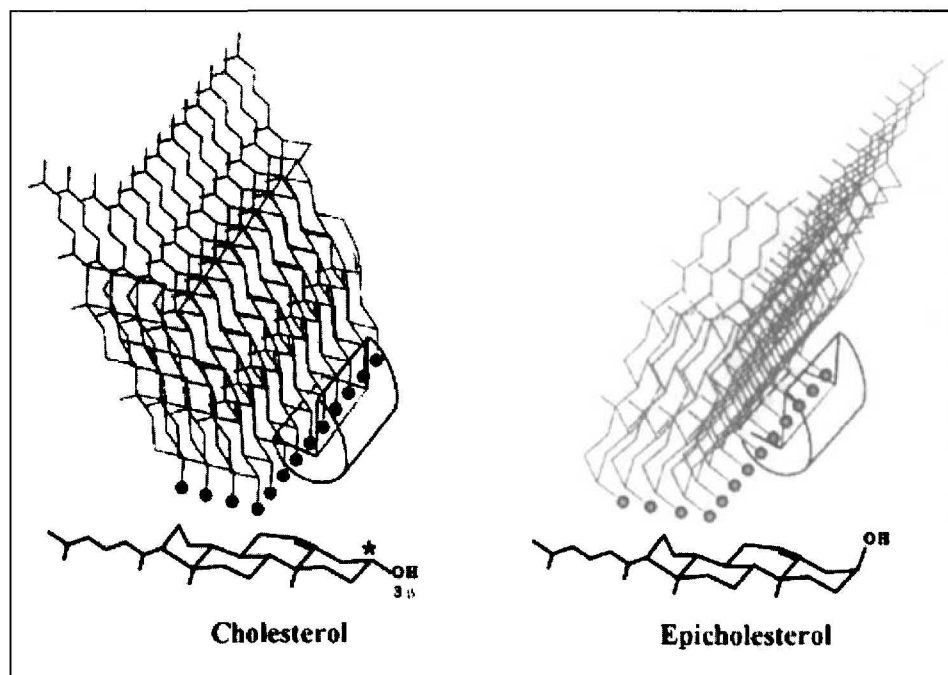

Cholesterol   **Epicholesterol**

Fig. 7. *Schematic representation of the good complementarity of antibody 36A1 with a cholesterol monolayer, and the bad complementarity with an epicholesterol monolayer*

Tartrate is, in fact, the original molecule with which *Pasteur* performed the experiment that led to the establishment of stereochemistry, the separation of a racemate into enantiomers. *Pasteur* separated the hemihedral crystals of sodium ammonium (*R,R*)-tartrate from its (*S,S*)-enantiomer, by virtue of their morphology, which reflects the asymmetry of the molecular structure [10]. The salt we used was calcium tartrate, and the crystals are not hemihedral. The cells, however, turn out to be even more efficient than *Pasteur*. They are able to recognize crystals formed of the (*R,R*)-enantiomer and adhere to them, while they do not adhere to the crystals composed of the (*S,S*)-enantiomer (*Fig. 8*). Equal-sized and equal-shaped crystals of the two enantiomers were mixed in equal amounts, and cells were seeded onto them. Crystals which had cells attached were separated. The separated crystals, once dissolved, turned out to be composed of (*R,R*)-tartrate with an enantiomeric purity of 94% [11]. Thus, even cells are able to recognize the surface to which they adhere at the molecular and stereochemical level.

During the last years, we are witnessing a transition in chemistry research from the molecular to the supramolecular level, be it oriented towards materials, catalysis or biology, and stereochemistry is an important tool in this endeavour. Stereochemistry is, however, not only a tool, it is a concept and a manner of thinking about science. The contribution of scientists such as *Vlado Prelog* is indeed, over and above the practical applications, the establishment of a school of thinking in chemistry that will bear fruit for many years to come, in fields that may well have been inconceivable and unexpected to them and to us.

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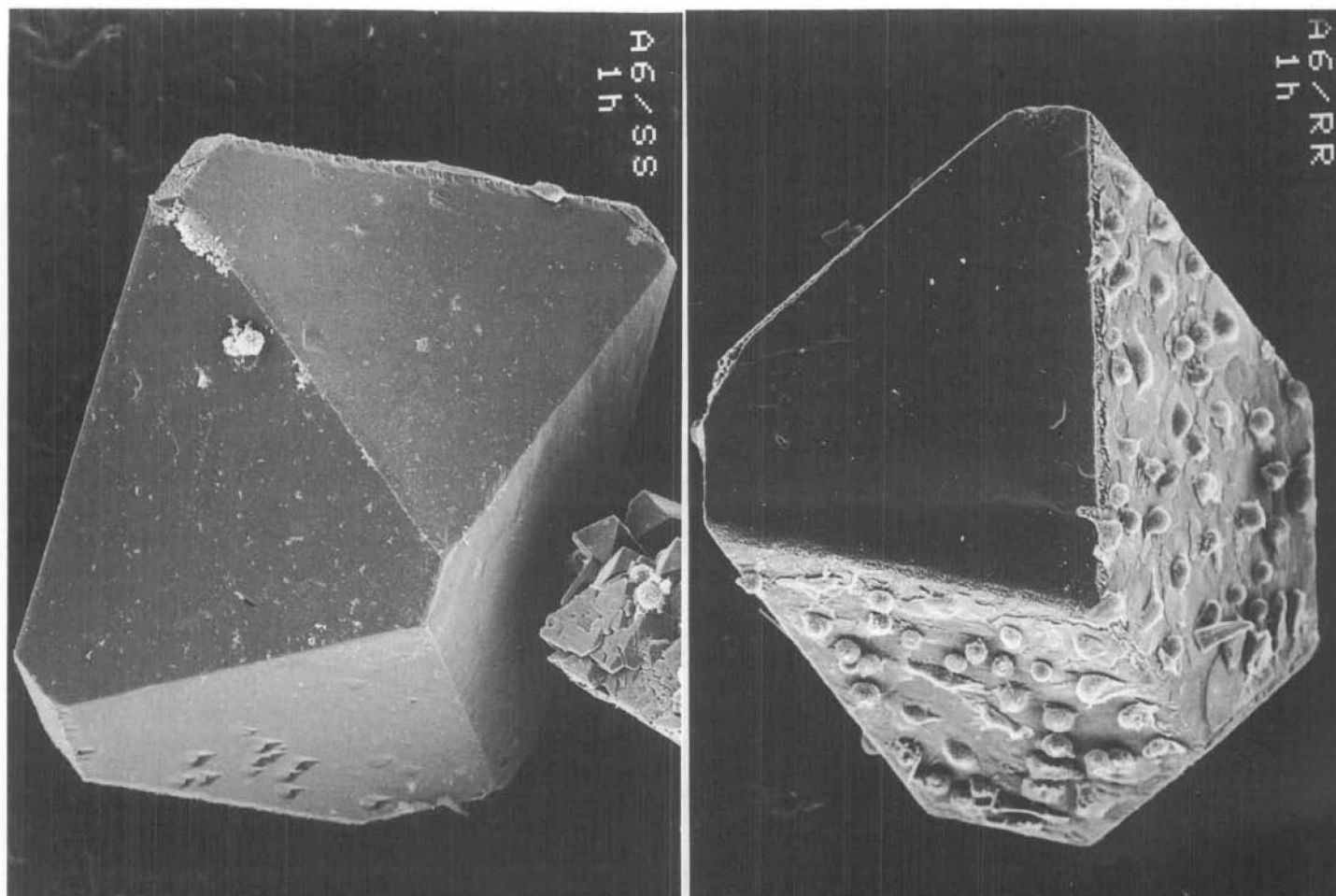[11] D. Hanein, B. Geiger L. Addadi, Science 1994, 263, 1413.

Fig. 8. *Scanning electron micrographs of epithelial cells strongly adhering to specific faces of a calcium (R, R)-tartrate crystal (right), and not adhering to a calcium (S,S)-tartrate crystal (left)*