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Crystallization of amino acids on substrates with superficial chiral reliefs

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Graphoepitaxial crystallization of amino acids in anisotropically shaped droplets is studied on silicon substrates with striped and chiral microreliefs to simulate the formation of ordered planar biological structures and films.

Organic–inorganic hybrid materials demonstrate unusual chemical and physical properties, which are principally important for the development of bioimplants, biosensors and drug carriers with a prolonged effect.[1] Attention is focused on the nanostructuring or precise binding of biological molecules to technologically relevant solid interfaces for the development of new devices with smart inorganic surfaces and interfaces designed for clinical testing, chiral marker and antibody recognition receptors, structure–function elucidation, new interface probes, *etc*.[1] In a fundamental sense, studies of the interface interactions of biocrystalline planar structures with inorganic substrates are of great current interest.[2,3]

Among different crystallization factors inducing formation of these structures, surface patterning and artificial reliefs[4–6] would play a unique role; however, unfortunately, such interaction effects are still rarely and insufficiently studied. Being a particular case of a fundamentally important phenomenon of crystal growth in confined geometries, crystallization on patterned substrates (graphoepitaxy[4]) may result in a high degree of crystallite orientation of various materials on arbitrary shaped and untextured substrates. This work discusses the results of phenomenological investigations of the crystallization behaviour of model biological objects on substrates with different types of artificial surface microreliefs.

High biological importance, chirality and amphiphility combined with a low molecular weight and relative simplicity of molecular structures make amino acids the most suitable model objects representing typical features of natural bioactive sub-

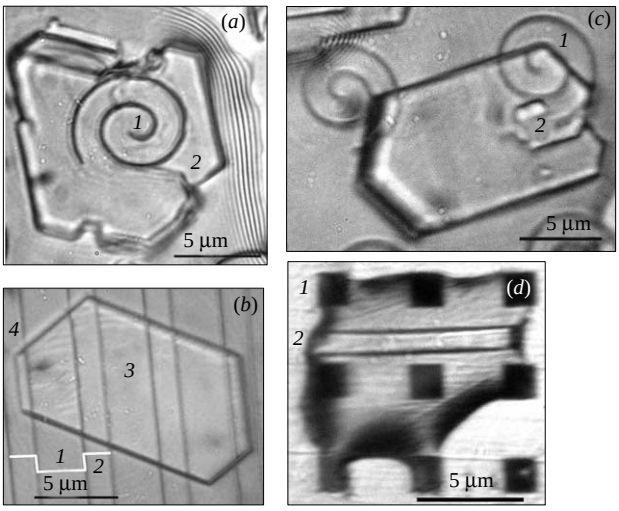

Figure 1 Crystal morphology of amino acids formed on asymmetrical and symmetrical reliefs: (*a*), (*b*), (*c*) monoclinic L-Val formed on left (*a*) or right (*c*) spirals and the striped relief (*b*) with grooves (*1*) and walls (*2*), the line profile is schematically shown in white; (*d*) square pitches on a (100) grain of silicon (*1*) arrange a needle-like crystal of D-Glu in a droplet of an aqueous solution (*2*). Spiral elements above a flat surface of silicon are marked with *1* (*a*), (*c*). The marker *2* shows the crystals of L-valine with mirror images of screw growth defects (*a*), (*c*), crystallization takes place in a droplet of solution. The principle of graphoepitaxy[10] is demonstrated in figures (*b*), (*d*): the rim of L-valine crystal (*b*): (*3*) is well-oriented along the edge of elements of the striped relief (*b*): (*4*); a needle of D-Glu is oriented in accordance with a square pitches arrangement (*d*): (*2*); reliefs of these kinds result in the growth of flat well-shaped crystals. To the contrary, developing (macro)spirals [(*a*), (*c*): (*2*)] originate from spiral elements of the asymmetric relief.

stances.[7–11] Amino acids are important food additives and have many applications in pharmaceutical industries. They are also the building units of other complex biomolecules such as proteins. Therefore, a study of the crystallization properties of amino acids[7,9–11] can provide valuable information on the interfacial interactions of larger biomolecules and on the biomimetic crystallization of biologically important inorganic substances.[12] Here, the room-temperature crystallization of chemically pure and XRD-pure D,L-enantiomers and racemates of glutamic acid, valine, alanine, phenylalanine, leucine, lysine, serine, cystine and proline on silicon wafers with different orientations, hydrophilic properties and reliefs was studied. Striped or chiral (spiral) microreliefs were prepared photolithographically with typical sizes of the relief elements of about 5 μm and wall heights of about 1 μm (Figure 1). The chiral relief was used since the structure of chiral lattices in two and three dimensions may provide insights into chiral discrimination, and chiral phases play an important part in the physics and applications of liquid–crystal and amphiphilic films. Crystallization was studied *in situ* in the view field of an optical microscope in polarised light by the visualization of evaporating micrometre-scale droplets of aqueous amino acid solutions deposited on the substrates from ultrasonic aerosol (mist). In order to vary the viscosity of solutions and the relaxation rate of supersaturation, multifunctional alcohols were used; the effects of the initial concentration of amino acids and surfactants and pH were also studied. Post-crystallization analysis was performed using scanning electron microscopy and X-ray diffraction.

It was found that microrelief elements affect amino acid crystallization in a complex manner. It is evident that a symmetrical striped relief only aligns a valine crystal in Figure 1 along the walls of grooves, as would be expected from known experiments on graphoepitaxy for most of the test systems including inorganic salts, metals, semiconductors, superconductors and proteins.[4,5] Instead of the only effect of crystal orientation, an asymmetrical spiral relief unexpectedly causes a drastic change in the crystal habitus making it twisted. Thus, an unusual shape is found to be very reproducible as soon as a spiral relief is used (Table 1).

Table 1 Crystallization of L-Val on silicon wafers with left spiral elements of the superficial relief.[a]

Types of analysed crystals[b]	Twisting directions of growth spirals	
	Left, the number of cases (%)	Right, the number of cases (%)
Nucleated on the centres [as in Figure 1(*a*)] of relief spirals	59 (36)[c]	41 (64)
Interacting (as in Figure 3) with relief spirals	56	44
Free crystals (as in Figure 4)	52	48

[a]See Figure 1(*a*),(*c*) for the examples of left and right relief spirals, twisting directions of growth spirals are decided imaging a screw driver going from upper side part to the lower centre part. [b]About 100–200 crystals are counted. [c]The values in parentheses correspond to the opposite direction of twisting of relief spirals (silicon wafers with right spirals).

The pattern influence on nucleation rates and embryo-crystal migration is based on many factors like topographic wall effects, crystallization pressure, surface tension, solute redistribution and capillarity.[4,5] An understanding of possible mechanisms for the observed growth features can be achieved by considering the system-specific changes induced by the artificial relief in the chain of major solidification phenomena – the elementary events of nucleation, growth of individual crystals and interactions in an ensemble of growing crystals artificially constrained in their growth.

The influence of the relief on nucleation is important because crystals should grow close to relief elements in order to be influenced by these elements (Figures 1, 2). Indeed, it was found that the elements of reliefs act as heterogeneous nucleation sites (Figure 2), at least in the cases of valine, phenylalanine, lysine and glutamic acid. In the case of valine, crystals form on the spirals at the beginning of solvent evaporation, while in other cases the crystals start to grow on spirals later, at the stage of location of residual droplets of a solution at individual spirals.

The growth of individual crystals of amino acids is controlled by the spacing of the confining elements of the artificial relief, which permanently make the diffusion field anisotropic, change the local rate of consumption of supersaturation and physically limit the average size of crystals (Figure 3, 4) or inhibit the propagation of certain crystal facets (Figure 3). This is helpful for tuning the size-dependent mobility of crystals and also for the suppression of undesired nucleation, *e.g.*, due to the mutual influence of growing crystals in the close proximity. An optimal

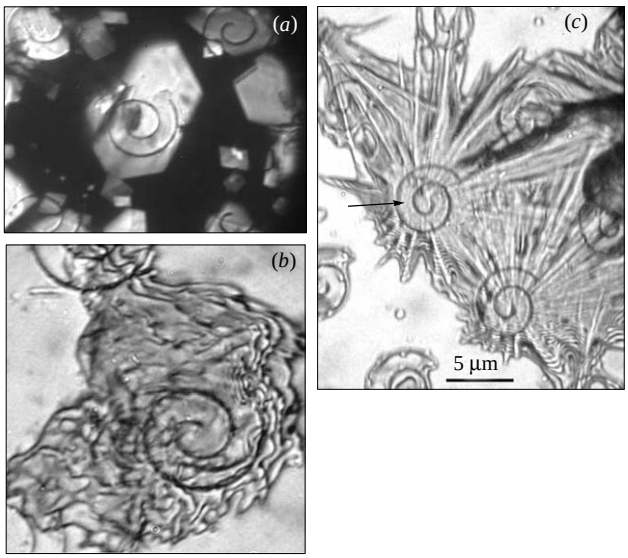

Figure 2 Nucleation of amino acids on the elements of a spiral relief: (*a*) D-Glu (polarised light, a view throughout the layer of solution), (*b*) DL-Lys (spirally distorted ensemble of crystallites) and (*c*) DL-Phe (a nucleation centre is shown by the arrow).

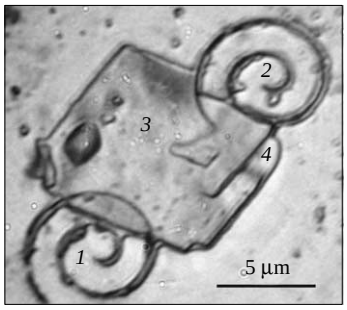

Figure 3 Interaction of curved walls (*1*, *2*) of a spiral artificial relief with a growing facet of a thin plate-like crystal (*3*) of D-glutamic acid. Blocking of the crystallization front movement and the formation of an upper crystallising layer (*4*) as a direct effect of such a disturbance are evident.

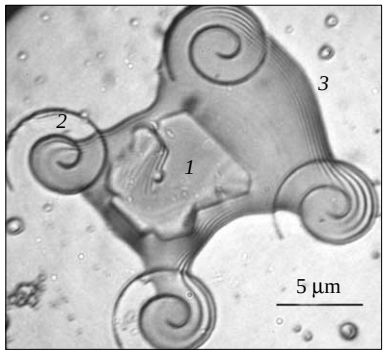

Figure 4 Formation of anisotropic droplets of an aqueous solution with crystallising L-valin (*1*) on chiral periodic elements of a superficial relief (*2*). Lines (*3*) are observed due to light interference in the liquid film (edge of the droplet) of different thickness.

spacing of the relief elements hinders the crystal size and further nucleation at a stage when the crystals are still mobile and do not interfere with each other. As soon as the artificial relief thus has provided a suitable ensemble of mobile growing crystals, further orientation of the crystals proceeds by their adjustment with respect to the relief. The compatibility of the relief architecture with the specific properties of the system becomes highly important at this stage yielding finally a good mutual orientation for most of the as-grown crystals, in the case of the simplest types of reliefs.

Interfacial tensions and free surfaces between crystals and substrate structures determine the ultimate position and orientation of the crystals. In this case, three-phase equilibria (crystal–substrate–liquid; crystal–liquid–gas/substrate–liquid–gas) play a major role (Figure 4), as is well known from experiments of classic graphoepitaxy[4] and its recent modification called as fluidic self-assembling,[6,13] in which inorganic or biological components at any scale (nano-, micro- or macro-) either separate or linked, spontaneously form ordered aggregates in microchannels due to capillary interactions. Similarly, a meniscus, the shape of a droplet and droplet edges become important factors because the surface tension tends to lower the area of free liquid between the crystal and the relief elements thus making a crystal to be rotated and aligned according to the relief element orientation.

In addition, the liquid phase redistribution, either local or long-range, becomes important in this particular system. A crucial influence of the relief consists in a change of the character of substrate wetting. The relief results in the formation of microdroplets with anisotropic shapes as determined by the asymmetry of relief elements (Figure 4). In turn, anisotropic wetting controls the superficial adsorption of surfactants, the local flows of solution, the gradient of surface tension and thus the concentration gradient in a droplet due to the Marangoni effect. The effects of rotational degrees of freedom, which are most important for adjustment of the crystal orientation, have not been treated by now for complex systems where surface tensions are dependent on concentration and thus lead to thermal/solutal Marangoni effects. These Marangoni effects will significantly alter the wetting behaviour – and small initial particle motion – and then will lead to local solute redistribution influencing both crystal growth and final crystal orientation.

The most essential effect introduced by the spiral relief as compared to the usual, striped, relief consists in changing crystal morphology. The crystal shape depends on relative growth rates of each of the crystal facets present. A typical growth mode of amino acids is the nuclei-above-nuclei mechanism.[10] As soon as a spiral relief presents, the growth mechanism may change (Figures 1, 3) and a facet grows by the Burton–Frank–Cabrera (BFC) spiral growth mechanism leading to a faster growth rate. Amino acid crystals often consist of alternating hydrophobic and hydrophilic layers parallel to the basal plane.[8] The hydrophobic layers contain nonpolar side chains, while the hydrophilic layers are composed of charged carboxylate and amino groups. The layer consists of two identical sheets related by a twofold screw axis. BFC growth demands for a screw dislocation to form due to shear and tensile strengths caused by a crystal interaction with a spiral element of the relief. A screw dislocation as a chiral object existing in a chiral environment (amino acid crystal lattice) will have a different energy and a different Burgers vector in different enantiomers thus predetermining parity violation and partial splitting the chiral forms of amino acids. Thus, a spiral relief (right- or left-type) replicates its asymmetry in the spiral growth morphology of the crystallites of amino acids (Figure 1) fascinating easier growth of a corresponding enantiomer. It seems that the chirality of relief elements is a strong factor affecting crystallization (Table 1) in both the cases of D- and L-enantiomers [Figure 1(*a*),(*c*)].

In conclusion, the artificial violation of the isotropy of a crystallising system on substrates with a surface relief can potentially allow us to control the formation of ordered biocrystalline planar structures for new hybrid materials. Symmetrical reliefs result in the co-orientation of crystallising amino acids as in classical graphoepitaxy, while the relief asymmetry results in deeper changes affecting crystallization morphology and the mode of crystal growth.

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