Crystalline Phase Separation of Racemic and Nonracemic Zwitterionic α-Amino Acid Amphiphiles in a Phospholipid Environment at the Air/Water Interface: A Grazing-Incidence X-Ray Diffraction Study

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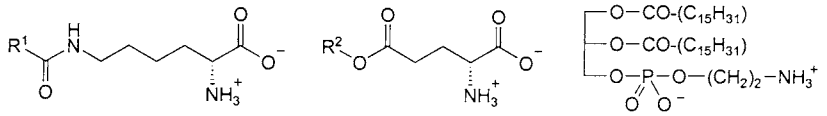
Dedicated to Professor *Duilio Arigoni* on the occasion of his 75th birthday

A grazing-incidence X-ray-diffraction (GIXD) study of the self-assembly, on water, of nonracemic γ-stearyl glutamic acid (pure or as a mixture with racemic or (*S*)-1,2-dipalmitoyl-glycero-3-phosphoethanolamine (DPPE)) demonstrated a phase separation of the α-amino acid amphiphile into racemic and enantiomorphous two-dimensional crystallites within the phospholipid domains. The packing arrangements of the two α-amino acid crystalline phases were identical to those found in the absence of DPPE and have been determined, at almost atomic resolution, by X-ray structure-factor calculations. By contrast, racemic and nonracemic N^{ε}-stearoyllysine spontaneously segregated into two-dimensional enantiomorphous domains within the DPPE environment that induced a change in the tilt direction of the hydrocarbon chains of the α-amino acid molecules. Phase separation of nonracemic amphiphiles, originating from preferred lateral homochiral or heterochiral intermolecular interactions, is in agreement with the formation of enantiomerically pure or enriched homochiral oligopeptides in overrepresented amounts in the polycondensation of activated nonracemic amphiphilic α-amino acids on plain water or within phospholipid monolayers.

Introduction. – As a part of our studies on the formation of homochiral oligopeptides from nonracemic activated α-amino acid monomers, we have proposed a general mechanism for the amplification of chirality that encompasses a phase separation of the nonracemic monomers into two-dimensional (2D) crystallites, either on H_2O or within a membrane-like environment of a phospholipid monolayer, followed by a lattice-controlled polymerization. In the preceding article of this journal issue [1], we demonstrated that polycondensation of nonracemic *S*-ethyl thioesters of both lysine and glutamic acid amphiphiles gives rise to homochiral oligopeptides, when embedded within a phospholipid monolayer, as determined by MALDI-TOF MS of deuterium-enantiolabeled monomers. Grazing-incidence X-ray-diffraction (GIXD) studies of the films composed of an equimolar mixture of racemic phospholipids with racemic thioesters clearly showed a phase separation between 2D crystalline domains of phospholipid and those of the thioesters, very similar to those observed for the pure components. However, it was not possible for these systems to demonstrate, by direct GIXD measurement, the occurrence of a phase separation of the nonracemic thioesters into a mixture of racemic and enantiomorphous domains. This was due to either similar X-ray diffraction patterns or an induced line-epitaxial crystallization of the enantiomorphous crystalline phase alongside the racemic crystallites, as discussed elsewhere [2].

Here, we provide direct experimental evidence that such a phase separation occurs for an appropriate system. Nonracemic γ-stearyl glutamic acid (=2-amino-5-oxo-5-stearyloxypentanoic acid; C_{18}-Glu), mixed with (*S*)- or *rac*-1,2-dipalmitoylglycero-3-phosphoethanolamine (DPPE), self-assembled into a mixture of three distinct types of 2D crystallites, as determined by GIXD at the air/water interface. Racemic C_{18}-Glu formed racemic 2D crystallites, whereas racemic N^{ε}-stearoyllysine (C_{18}-Lys) self-assembled into a conglomerate of enantiomorphous 2D domains, comprising molecules of single handedness, even in the presence of *rac*-DPPE.



(*R*)-C_{18}-Lys ($R^1 = C_{17}H_{35}$) (*R*)-C_{18}-Glu ($R^2 = C_{18}H_{37}$) DPPE

Experimental. – Enantiomerically pure and racemic γ-stearyl glutamic acid (C_{18}-Glu) and N^{ε}-stearoyllysine (C_{18}-Lys) were synthesized as reported [2a]. The phospholipids 1,2-dipalmitoyl-*rac*-glycero-3-phosphoethanolamine (*rac*-DPPE) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine ((*S*)-DPPE; *Sigma*) were used. GIXD Measurements were performed with the liquid-surface diffractometer mounted at the *BW1* synchrotron beamline at HASYLAB, DESY, Hamburg. Details about the experimental technique and the instrument were reported elsewhere [3]. The recorded GIXD patterns are represented as 2D contour maps of the scattered intensity (I) as a function of the horizontal (q_{xy}) and vertical (q_z) components of the scattering vector. The unit-cell dimensions of the 2D lattice were derived from the q_{xy} positions of the *Bragg* peaks. The full-width at half-maximum of the *Bragg* peaks (corrected for instrument resolution), FWHM(q_{xy}), gave an estimate of the crystalline coherence lengths ($CL_{hk} \approx 0.9(2\pi/\mathrm{FWHM}(q_{xy}))$ associated with each h,k reflection. *Bragg*-rod-intensity profiles represent the intensity distribution along q_z, $I(q_z)$, derived by integrating across the q_{xy} range for each *Bragg* peak. The intensity at a particular value of q_z in a *Bragg* rod gives the experimental value of the square of the molecular-structure factor $|F_{h,k}(q_z)|^2$. The 2D packing arrangement was determined by performing X-ray-structure-factor calculations with atomic-coordinate molecular models constructed with the CERIUS^2 molecular package [4], and rigid-body structure refinement by means of the SHELX-97 program [5] adapted for 2D structures.

Results and Discussion. – Two-dimensional crystallites from racemic C_{18}-Lys and from enantiomerically pure, racemic, and nonracemic C_{18}-Glu were self-assembled on a H_2O surface at 20° by spreading their $CHCl_3$ solutions or those of their mixtures with (*S*)- or *rac*-DPPE (DPPE/α-amino acid 1:2 (molar ratio)) for a nominal molecular area (defined as the trough area divided by the number of spread molecules) of 35 Å^2, without any increase in surface pressure. The GIXD patterns were recorded from such films after cooling the H_2O subphase to 4°.

N^{ε}-Stearoyllysine (C_{18}-Lys). – The GIXD pattern of the mixture of *rac*-DPPE and *rac*-C_{18}-Lys is shown in *Fig. 1,a*. By comparison with the GIXD patterns of the pure components (*Fig. 1,b* and *1,c*), the *Bragg* peaks corresponding to the *rac*-DPPE phase and those of the C_{18}-Lys phase could be assigned. As previously reported, analysis of the three *Bragg* peaks for pure racemic C_{18}-Lys gave rise to a 2D oblique unit cell (a = 4.92 Å, b = 4.98 Å, γ = 108.5°) containing a single molecule, implying that the molecules are packed only by translation symmetry [6]. The hydrocarbon chains were tilted by 34° from the normal to the H_2O surface, with an azimuth angle of 10° from the

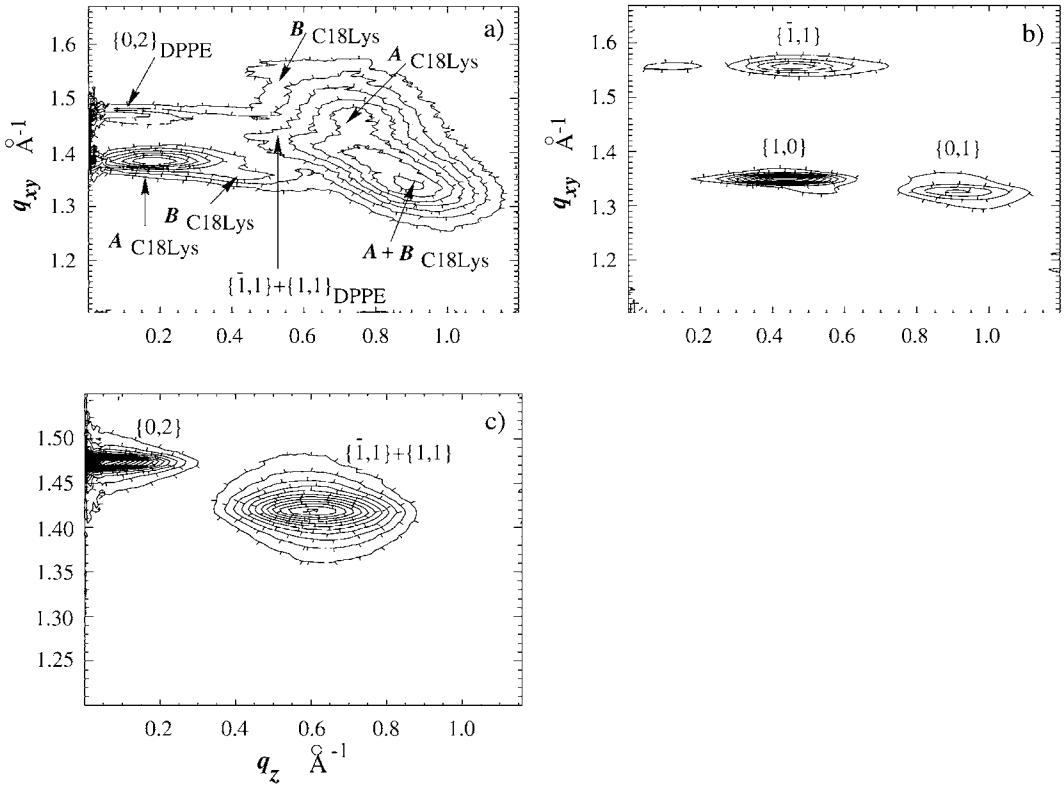


Fig. 1. *GIXD Patterns of the 2D crystallites of self-assembled amino acids and/or phospholipids obtained by spreading $CHCl_3$ solutions of* a) a *1:2 molar mixture of* rac-*DPPE and* rac-C_{18}-*Lys* (*A* and *B* phases shown), b) *pure* rac-C_{18}-*Lys*, c) *pure* rac-*DPPE*. The GIXD patterns are represented as 2D contour maps of scattered intensity *I* as a function of the horizontal (q_{xy}) and vertical (q_z) components of the scattering vector, measured on H_2O at 4° for a nominal molecular area of 35 Å^2. The *Bragg* peaks are labeled by their {*h*,*k*} *Miller* indices.

a-axis to the projection on the H_2O surface of the chain. The 2D packing arrangement was determined by X-ray-structure-factor calculations (*Fig. 2*). For this system, not even a pseudo-crystallographic glide symmetry was possible, since the oblique cell could be transformed into a larger unit cell ($a = 4.92$ Å, $b = 9.64$ Å, $\gamma = 100.2°$), which is far from being rectangular, the molecular tilt direction not following a unit-cell axis. Chiral disorder can occur only *via* an interchange of the C–H and C–NH_3^+ groups at the asymmetric C-atom, since the hydrocarbon chain must remain in its original position. But such an interchange would involve the loss of one H-bond and, therefore, a decrease in lattice energy of *ca.* 6 kcal/mol. Based on these considerations, racemic C_{18}-Lys undergoes a spontaneous segregation into enantiomorphous domains containing single-handed molecules.

Analysis of the GIXD pattern measured in the presence of the *rac*-DPPE (*Fig. 1,a*) showed that the latter induced a change in the diffraction pattern of the C_{18}-Lys domains. The observed *Bragg* peaks were rationalized in terms of a mixture of two phases, a major phase *A* of dimension $a = 5.01$ Å, $b = 5.21$ Å, $\gamma = 114.9°$ (after

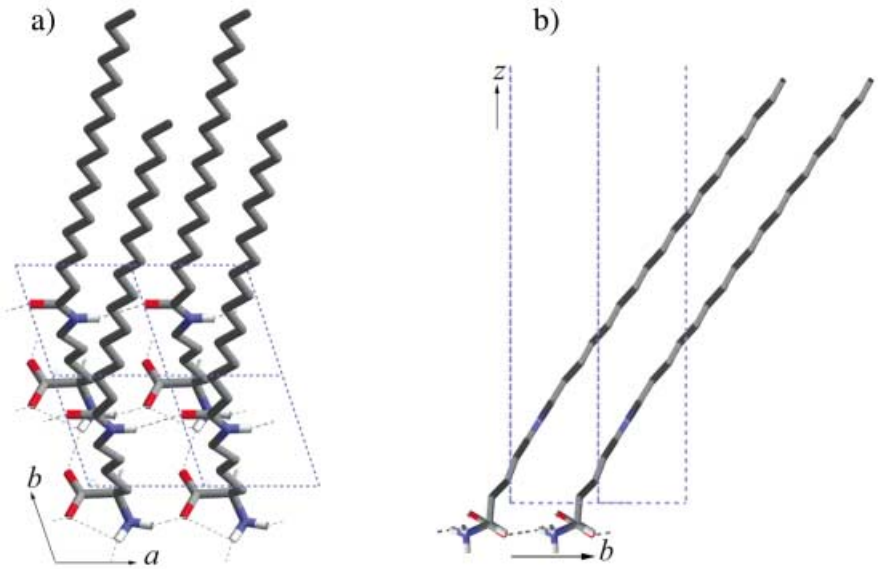


Fig. 2. *Two-dimensional-packing arrangement* – a) *view perpendicular and* b) *view parallel to the H_2O surface – of (*R*)-C_{18}-Lys crystallites obtained by spontaneous segregation of racemic C_{18}-Lys into a mixture of enantiomorphous domains.* O-atoms are marked in red, N-atoms in blue.

transformation: $a = 4.91$ Å, $b = 9.63$ Å, $\gamma = 98.8°$), and a minor phase *B*, similar to that of pure C_{18}-Lys. The oblique unit cell of phase *A* also contains one molecule. Thus, the packing arrangement is *via* translation symmetry with the hydrocarbon chains tilted in a slightly different direction (azimuth angle of 12.9° in *A vs.* 10° in *B*). Based on the overall similarity of the two phases, we concluded that, even in the *rac*-DPPE environment, racemic C_{18}-Lys spontaneously segregated into a conglomerate of enantiomorphous 2D crystallites. However, the crystalline coherence lengths (*CL*s) along the {0,1}, {1,0} and {1, – 1} directions, calculated from the full-width at half-maximum (FWHM(q_{xy})) of the *Bragg* peaks, were 300, 110. and 110 Å, respectively, much smaller than those calculated for the pure C_{18}-Lys crystallites (500, 320, and 470 Å, resp.). Note that the racemic phase of *rac*-DPPE caused only a slight change in the structure of the C_{18}-Lys 2D crystallites and did not prevent the segregation of the racemate into an enantiomorphous conglomerate.

γ-Stearyl-Glutamic Acid (C_{18}-Glu). a) *Enantiomerically Pure Form.* The GIXD patterns (*Fig. 3*) of the (*R*)- and racemic 2D crystallites indicated different packing arrangements. Analysis of the X-ray-diffraction pattern of (*R*)-C_{18}-Glu (*Fig. 3,a*) yielded an oblique unit cell of dimensions $a = 4.86$ Å, $b = 5.28$ Å, and $\gamma = 110.9°$, with *CL*s of 330, 840, and 250 Å along the {0,1}, {1,0} and {1, – 1} directions, respectively. The derived unit-cell belongs to the plane group *p*1 and contains a single molecule (packing by translation only). According to the q_z position of the intensity maximum in the *Bragg* rods (*Fig. 3,a*), the molecules are oriented with their hydrocarbon chain tilted by 37° from the normal to the H_2O surface, with an azimuth angle of 18° from the *b*-axis. X-Ray-structure-factor calculations were performed using an atomic-coordinate

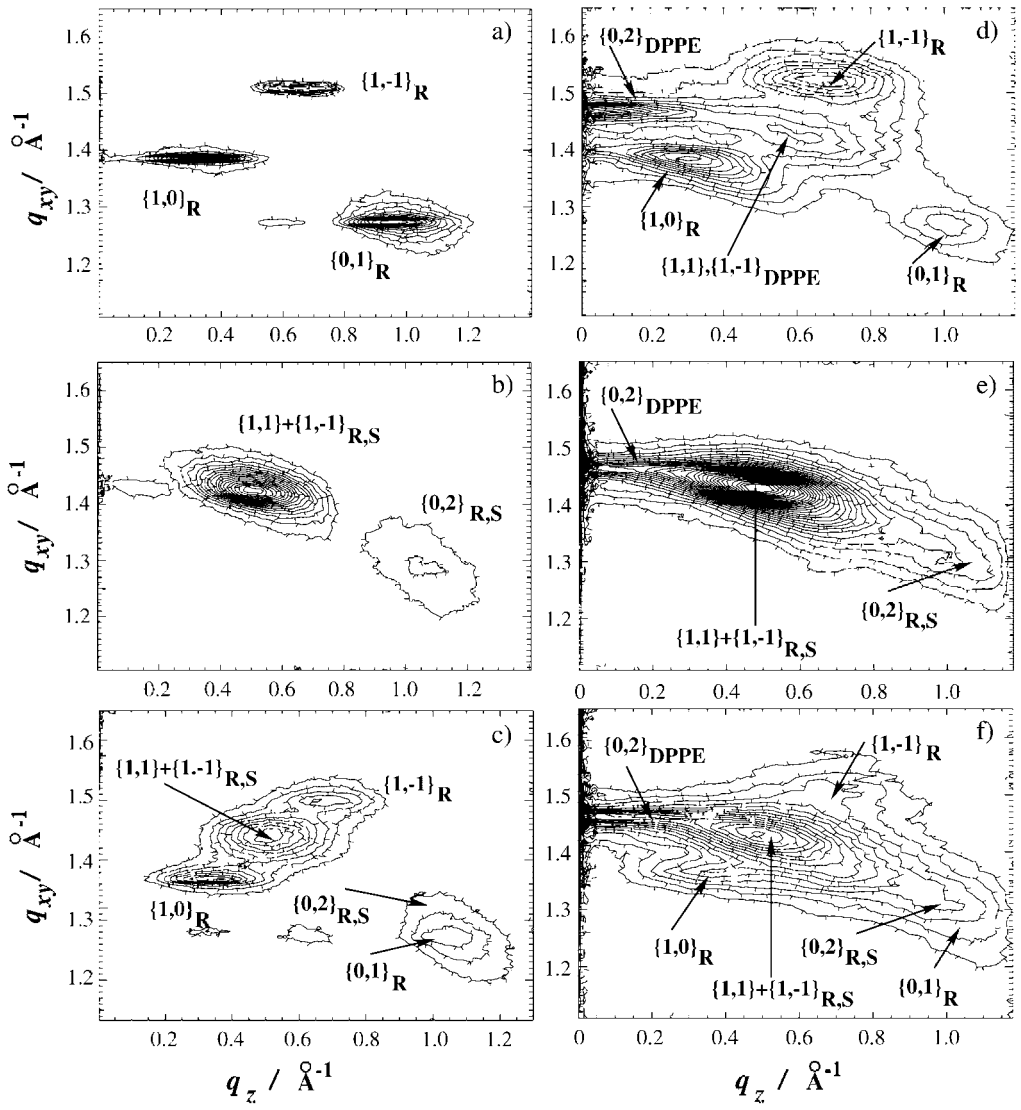


Fig. 3. *GIXD Patterns of the 2D crystallites of self-assembled amino acids and/or phospholipids obtained by spreading $CHCl_3$ solutions of* a) (R)-C_{18}-*Glu,* b) rac-C_{18}-*Glu*, c) nonracemic C_{18}-*Glu* ((*R*)/(*S*) 7:3), *and 1:2 molar mixtures of* d) (R)-C_{18}-*Glu,* e) rac-C_{18}-*Glu, and* f) *nonracemic* C_{18}-*Glu* ((*R*)/(*S*) 7:3) *with* (S)-*DPPE*. The GIXD patterns are represented as 2D contour maps of scattered intensity *I* as a function of the horizontal (q_{xy}) and vertical (q_z) components of the scattering vector, measured on H_2O at 4° for a nominal molecular area of 35 Å^2. The *Bragg* peaks are labeled by their {*h*,*k*} *Miller* indices, the subscripts '*R*' and '*R*,*S*' refer to the C_{18}-Glu phases. The patterns (not shown) of the corresponding mixtures with *rac*-DPPE are very similar. Only the {0,2} peak of the (*S*)-DPPE phase was observed in e) and f), the others overlapping with the strong $\{1,1\}+\{1,-1\}$ peak of the *rac*-C_{18}-Glu phase.

molecular model constructed on the basis of the glutamic acid 3D crystal. The structure was refined with SHELX97 adapted for 2D crystals to fit the measured *Bragg*-rod-intensity profiles (*Fig. 4*,*a*). The refined 2D packing arrangement is shown in *Figs. 4*,*b* and *4*,*c* viewed perpendicular and parallel to the H_2O surface, respectively.

b) *Racemic Form*. Although C_{18}-Glu has a zwitterionic α-amino acid head group identical to that of C_{18}-Lys, the ester or the amide group in the hydrocarbon chains induced a different packing arrangement for the two racemic amphiphiles. In contrast to C_{18}-Lys, the GIXD patterns of *rac*-C_{18}-Glu (*Fig. 3*,*b*) yielded a rectangular unit cell of dimension $a = 4.95$ Å, $b = 9.78$ Å, containing two molecules with their hydrocarbon chains tilted along the *b*-direction by an angle of 38° from the normal to the H_2O surface, in keeping with a 'herringbone' packing motif [3] in a racemic 2D crystal. The two molecules in the unit cell are related to each other *via* glide symmetry along the *b*-axis. The refined 2D packing arrangements (*Figs. 5*,*b* and *5*,*c*) were obtained from

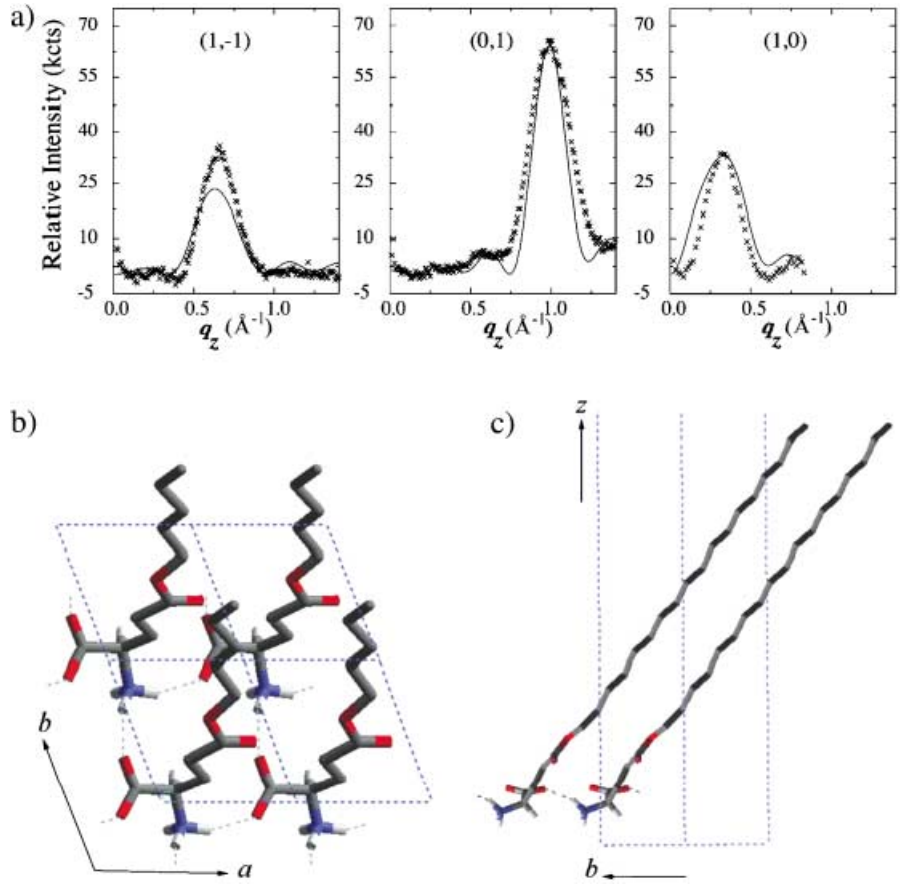


Fig. 4. a) *Measured* (×) vs. *calculated* (solid line) Bragg-*rod-intensity profiles corresponding to the {1, − 1}, {1,0}, and {0,1}* Bragg *peaks of* (R)-C_{18}-*Glu*; *also shown are the 2D packing arrangement of* (R)-C_{18}-*Glu crystallites viewed* b) *perpendicular and* c) *parallel to the* H_2O *surface, resp.* For clarity, only part of the chains are depicted in *b*). The O-atoms are marked in red, N-atoms in blue.

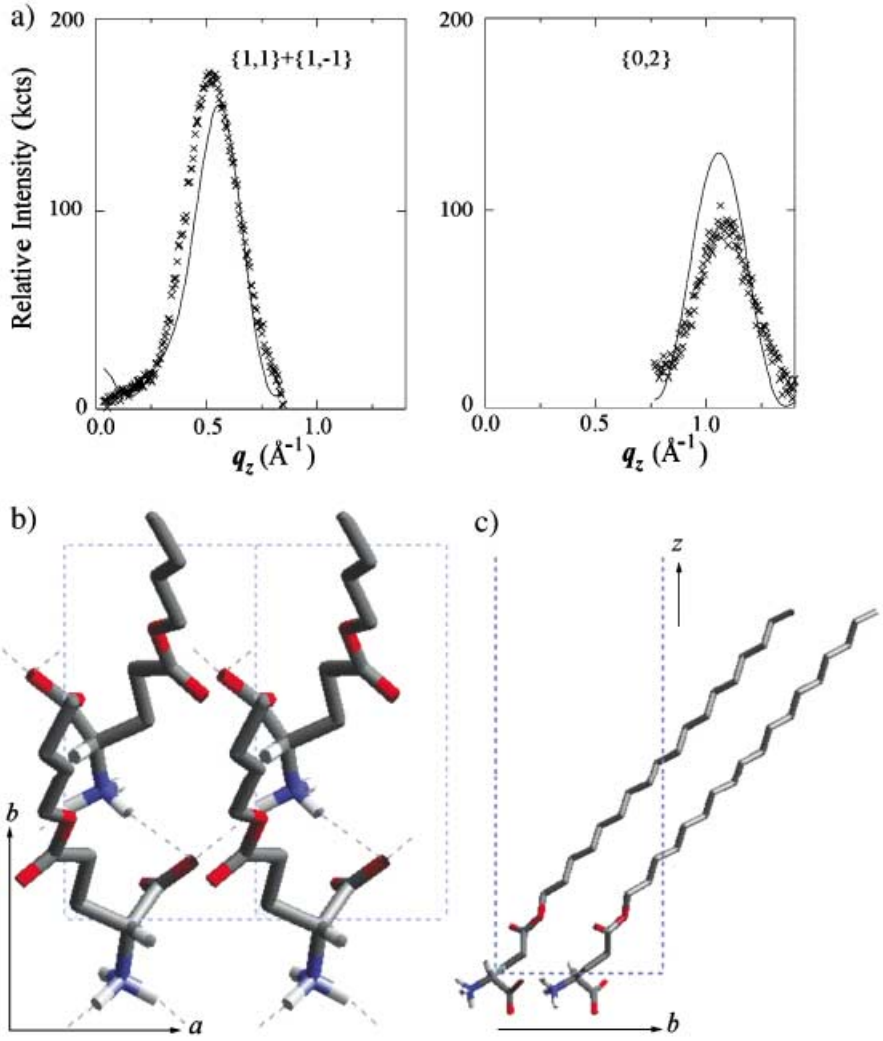


Fig. 5. a) *Measured* (×) vs. *calculated* (solid line) Bragg-*rod-intensity profiles corresponding to the {1,0} + {1, – 1} and {0,2}* Bragg *peaks of racemic C_{18}-Glu*; *also shown are the 2D packing arrangement of the* rac-C_{18}-*Glu crystallites viewed* b) *perpendicular and* c) *parallel to the H_2O surface*, *resp*. For clarity, only part of the chains are depicted in *b*). The O-atoms are marked in red, N-atoms in blue.

X-ray-structure-factor calculations, using an atomic-coordinate model, to fit the measured *Bragg*-rod intensity profiles (*Fig. 5*,*a*). The *CL*s along the {1,1} + {1, – 1} and {0,2} directions were 150 and 320 Å, respectively.

c) *Nonracemic Form*. The GIXD patterns of chiral, nonracemic C_{18}-Glu mixtures ((*R*)/(*S*) 7:3), self-assembled at a nominal molecular area of 35 Å^2, exhibited a clear phase separation into racemic and enantiomorphous ((*S*)) crystalline phases (*Fig. 3*,*c*).

It is evident that each phase retained its original structure observed, when either enantiomerically pure or racemic C_{18}-Glu amphiphiles were spread on the H_2O surface.

d) *Phospholipid Environment*. When nonracemic ((*R*)/(*S*) 7.3) C_{18}-Glu was mixed with either (*S*)- or *rac*-DPPE in a 2:1 molar ratio, the GIXD patterns, recorded under the same spreading conditions as above, showed the coexistence of three crystalline phases: DPPE, *rac*-C_{18}-Glu, and (*R*)-C_{18}-Glu (*Fig. 3*,*f*). For comparison, only two crystalline phases were observed when either (*R*)- or *rac*-C_{18}-Glu were mixed with DPPE (*Fig. 3*,*d* and *3*,*e*). Note that the chiral environment provided by (*S*)-DPPE did not cause any change in the structure of the 2D crystallites of *rac*-C_{18}-Glu nor induce any separation of the racemate into an enantiomorphic conglomerate. Both *rac*- and (*R*)-C_{18}-Glu crystallites, self-assembled from nonracemic mixtures of C_{18}-Glu, gave rise to smaller *CL*s values than the corresponding pure phases, *e.g.*, 200, 280, and 250 Å along the {0,1}, {1,0} and {1, – 1} directions, respectively, of the enantiomorphous (*R*)-crystallites of the component in excess. Furthermore, the coherence lengths were even smaller in the DPPE environment, being 60, 85, and 170 Å, respectively.

Conclusions. – The occurrence of phase separation of nonracemic amphiphilic molecules of C_{18}-Glu into distinct racemic and enantiomorphous 2D crystalline domains, both on plain H_2O and within a monolayer of either enantiomeric or racemic phospholipid, was demonstrated by direct GIXD measurements on the H_2O surface. By contrast, slight changes in the tilt direction of the hydrocarbon chains of C_{18}-Lys induced by the phospholipid environment indicated an interaction between the 2D crystallites of the host and guest. Nevertheless, these interactions did not prevent the spontaneous segregation of the racemic C_{18}-Lys enantiomorphous 2D crystalline domains, even within the racemic phospholipid environment, as demonstrated by GIXD. Such phase separations in nonracemic mixtures of activated α-amino acids, undergoing lattice-controlled polymerizations, are important for enhanced formation of homochiral oligopeptides and might be of relevance to the emergence of homochirality in nature.

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