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30. We thank K. Terakura and T. Ikeda for discussion on the protonic diffusion mechanism in high-pressure ice.

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Chiral Amplification of Oligopeptides in Two-Dimensional Crystalline Self-Assemblies on Water

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Differences in the two-dimensional packing arrangements of racemic and enantiomeric crystalline self-assemblies on the water surface of amphiphilic activated analogs of lysine and glutamic acid have been used to prepare oligopeptides of homochiral sequence and oligopeptides of single handedness from chiral nonracemic mixtures. The crystalline structures on the water surface were determined by grazing incidence x-ray diffraction and the diastereomeric composition of the oligopeptides by matrix-assisted laser desorption time-of-flight mass spectrometry with enantio-labeling. These results suggest that reactivity of ordered clusters at interfaces might have played a role in the generation of early homochiral biopolymers.

Theories on the emergence of the homochiral biopolymers of life at prebiotic times suggest the involvement of enantioselective reactions starting from heterochiral mixtures of α–amino acid and nucleic acid precursors (*1–7*). Polymerization reactions of racemates in isotropic media would lead, however, to formation of polymers comprising a random sequence of left (*S*)- and right (*R*)-handed repeat units in a binomial distribution (*8*). Thus, the probability of obtaining oligomers with homochiral sequence will become negligible with increasing length (*9–11*). A possible way to obtain oligopeptides of homochiral sequence from racemic mixtures would be through the self-assembly of the precursor molecules into ordered architectures followed by lattice-controlled reactions.

Two-dimensional (2D) self-assemblies formed at the water surface provide an ideal medium for the performance of stereospecific and enantioselective chemical reactions because (i) they promote effective molecular ordering, (ii) they permit substantial molecular flexibility, and (iii) they allow access to the ordered, flexible molecules by other reagents from the aqueous subphase (*12–14*). Moreover, with the recent advent of grazing incidence x-ray diffraction (GIXD) using synchrotron radiation, it became possible to determine the structure of the crystalline components of these assemblies at the molecular level, providing an important tool for the design of new architectures and control of their reactivity (*15*).

Racemic mixtures of amphiphilic molecules at interfaces can self-assemble into 2D crystallites of three types: (i) racemic compounds in which both enantiomers are packed together, (ii) enantiomorphous conglomerates involving segregation of the enantiomers (*16*), and (iii) enantiomerically disordered solid solutions. Here, we focus on activated α–amino acid derivatives that self-assemble into 2D crystallites as racemic compounds that, upon polymerization, yield oligopeptides with an enhanced homochiral (isotactic) sequence, as shown, in principle, in Scheme 1A. This concept is illustrated with the polycondensation of racemic N^{ε}-stearoyl-lysine-thioethylester (C_{18}-TE-Lys).

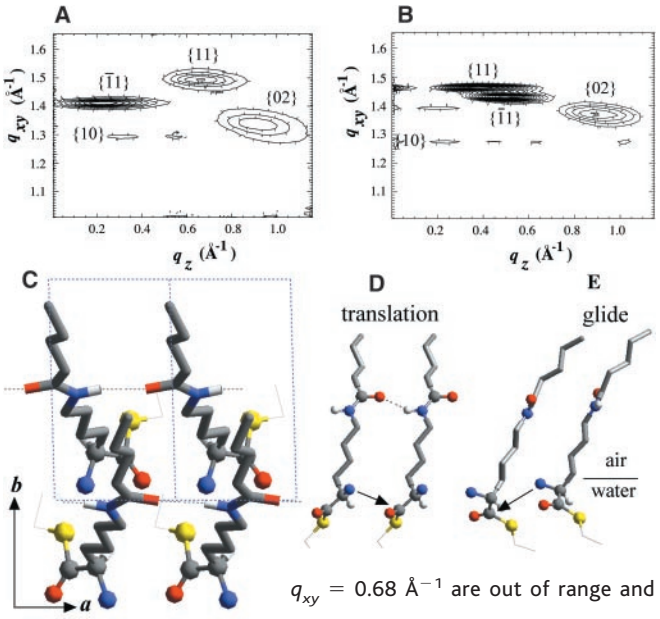

Fig. 1. GIXD patterns $I(q_{xy}, q_z)$ of the self-assembled 2D crystallites of C_{18}-TE-Lys on water at 4°C, where q_{xy} *and* q_z are horizontal and vertical components of the x-ray scattering vector. (**A**) Enantiomerically pure. (**B**) Racemate. (**C**) The packing arrangement of the racemic compound viewed perpendicular to the water surface. (**D** and **E**) Pairs of molecules related by translation and by glide symmetry, respectively, viewed parallel to the water surface. For clarity, part of the hydrocarbon chains is not shown. In (A) and (B) the {01} Bragg peaks at $q_{xy} = 0.68$ Å^{-1} are out of range and are not shown for clarity.

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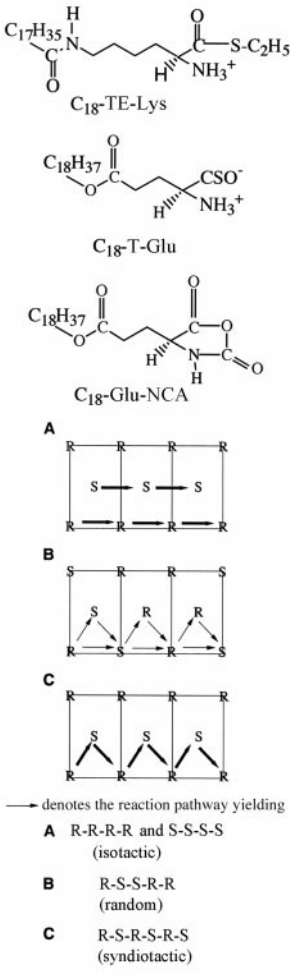
For comparison, we present the related racemic γ-stearyl thioglutamic acid (C_{18}-T-Glu), which self-assembles such that the two enantiomers are almost randomly distributed within the crystallites, thus forming a solid solution. Polymerization of this system yielded oligopeptides of almost random distribution of the *R* and *S* repeat units (Scheme 1B). The packing arrangements of the 2D crystallites on the water surface were determined by GIXD, and the composition of the oligopeptides was analyzed by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) with enantio-labeled samples.

A different type of process involves an amplification of chirality, starting from a low enantiomeric imbalance of the monomers. Oligopeptides exhibiting a single handedness can be prepared by self-assembly of chiral nonracemic mixtures of the monomers into separate racemic and enantiomorphous crystallites. This amplification process can be achieved efficiently provided that polymerization, within the racemic 2D crystallites, occurs between molecules of opposite handedness (Scheme 1C) yielding heterochiral (syndiotactic) oligopeptides, as illustrated for γ-stearyl-glutamic acid-*N*-carboxyanhydride (C_{18}-Glu-NCA). The enantiomer in excess will yield the homochiral oligopeptides.

Chloroform solutions of enantiomeric or racemic C_{18}-TE-Lys (*17*) in the form of their trifluoroacetate salts were spread on the water surface for 70% monolayer coverage. One of the enantiomers of racemic C_{18}-TE-Lys incorporated perdeuterated hydrocarbon chains (labeled S_d or R_d), leaving the other unlabeled (R_h or S_h). The GIXD patterns measured from the enantiomeric (*R*) or (*S*) and racemic (*R*,*S*) phases of C_{18}-TE-Lys on water (Fig. 1, A and B) are substantially different. The derived unit cell of the enantiomeric phase is centered pseudo-rectangular (a = 4.89 Å, b = 9.43 Å, γ = 93.9°) containing two molecules whose chains are tilted by 35° from the surface normal in a direction 15° off the b axis. The GIXD data from the racemic mixture yield a similar unit cell (a = 4.94 Å, b = 9.04 Å, γ = 91.4°) containing two molecules whose chains are tilted by 33° from the surface normal, but in a direction almost parallel to the b axis. This result, coupled with the observation that the dimensions of the unit cell projected along the chain axis (a_p = 4.9 Å, b_p = 7.5 Å, $\gamma_p \approx 90°$), is fingerprint evidence of



Scheme 1.

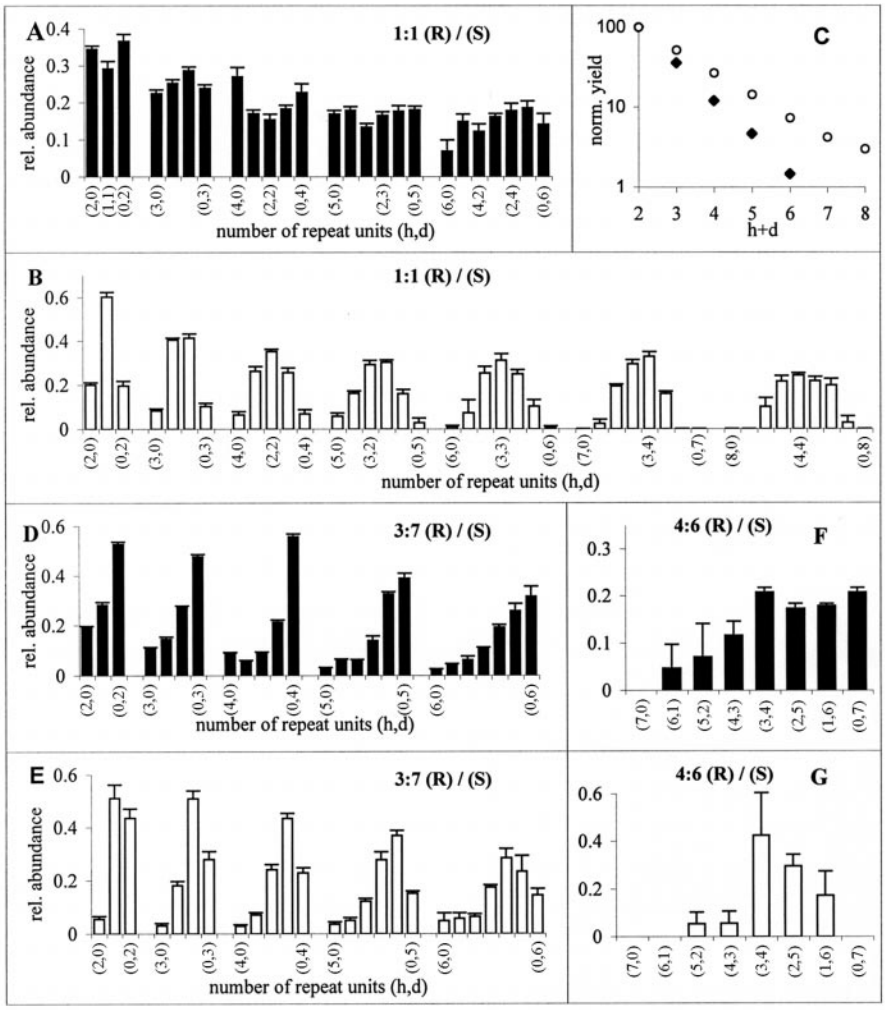


Fig. 2. MALDI-TOF MS analysis of the oligopeptides. The histograms show the relative distribution of each oligopeptide obtained from racemic and chiral nonracemic mixtures of monomers for C_{18}-TE-Lys (solid bars) and C_{18}-T-Glu (open bars). The vertical axis represents the relative abundance of each type of oligopeptide (*h*,*d*), where *h* is the number of *R* (unlabeled) repeat units and *d* the number of *S* (deuterated) repeat units; e.g., (4,0) is the tetrapeptide containing four *R* repeat units and no *S* repeat units. For oligopeptides with the same number of repeat units, ion intensity (*I*) and amount are reliably proportional. The relative abundance was calculated according to the equation exemplified below for the (4,0) tetrapeptide: relative abundance (4,0) = I(4,0)/I[(4,0) + (3,1) + (2,2) + (1,3) + (0,4)]. Space limitations prevent the inclusion of all the (*h*,*d*) symbols; e.g., for the tetrapeptides, symbols (3,1) and (1,3) are not shown. (**A** and **B**) Racemic monomers. (**C**) The relative intensity of the various oligopeptides normalized to that of the dipeptide: (◆) -C_{18}-TE-Lys, (○) -C_{18}-T-Glu. (**D** and **E**) 3:7 (*R*:*S*) mixtures. (**F** and **G**) The heptapeptides of 4:6 (*R*:*S*) mixtures of C_{18}-TE-Lys and C_{18}-T-Glu, respectively. No substantial isotope effect was observed on interchanging the isotope labeling of the two enantiomers.

herring-bone chain packing achieved by pseudo-glide symmetry relating two molecules of opposite handedness. The molecular packing arrangement, determined to near atomic resolution by x-ray structure factor–constrained least-squares analysis (*18*, *19*), is shown in Fig. 1C.

Polycondensation of racemic C_{18}-TE-Lys was achieved by injection of I_2/KI (*20*), or $AgNO_3$ (*21*) solutions into the aqueous subphase at 20°C or 1°C. After 2 to 3 hours of reaction, the samples were collected from the surface and analyzed by MALDI-TOF MS (*22*). As a result of the enantioselective labeling of only the *S* enantiomer with deuterium (see above), we obtain a mass difference of 35 mass units per monomer (*23*, *24*). Consequently, the number of *R* and of *S* monomer units could be determined for each diastereoisomeric oligopeptide. The total ion abundance of the different oligopeptides, normalized to that of the dipeptide product (Fig. 2C), exhibits an exponential decrease with increasing length of the oligopeptides. The distribution of the different diastereoisomers for each oligopeptide length (Fig. 2A) reveals a clear trend toward enhanced formation of homochiral R_h and S_d peptides vis-à-vis their heterochiral counterparts. For comparison, Fig. 2B shows the distribution of oligopeptides obtained from the polycondensation of racemic C_{18}-T-Glu (*25*). This racemate self-assembled into an enantiomerically disordered solid solution (Scheme 1B) that, upon polycondensation, yielded essentially a random distribution of oligopeptides (Fig. 2B), providing a reference system more realistic than the theoretical calculation for a random system (*8*).

A comparison between Fig. 2, A and B, shows a distinct bias for the C_{18}-TE-Lys system to form homochiral diastereoisomers, labeled (*h*,0) and (0,*d*), at the extremities of each distribution for di- to hexaoligopeptides.

The enhanced concentration of the homochiral C_{18}-TE-Lys oligopeptides (Fig. 2A) is in agreement with the packing arrangement of the racemic monomer (Fig. 1C). The amino group of one molecule is appropriately oriented and in close proximity to a carbonyl group of a nearest-neighbor homochiral molecule (~5.0 Å) to react and form a peptide bond (Fig. 1D). In contrast, the amine and carbonyl groups of two heterochiral molecules related by glide symmetry are less appropriately aligned for a nucleophilic attack (Fig. 1E).

Next, we describe amplification of homochirality starting from chiral nonracemic mixtures in systems where a phase separation occurs: The racemic fraction forms a racemic compound and the enantiomer in excess assembles into enantiomorphous 2D crystallites (*26*). This concept is illustrated first for 7:3 and 6:4 (*S*:*R*) mixtures of C_{18}-TE-Lys. A distinct enhancement of the homochiral fraction of the S_d oligopeptides [labeled (0,*d*) in Fig. 2, D and F] relative to that of the heterochiral molecules (*h*,*d*) was obtained especially for penta- to heptapeptides (*27*) as compared, once again, with the products obtained from the corresponding mixtures of C_{18}-T-Glu with random polycondensation (Fig. 2, E and G).

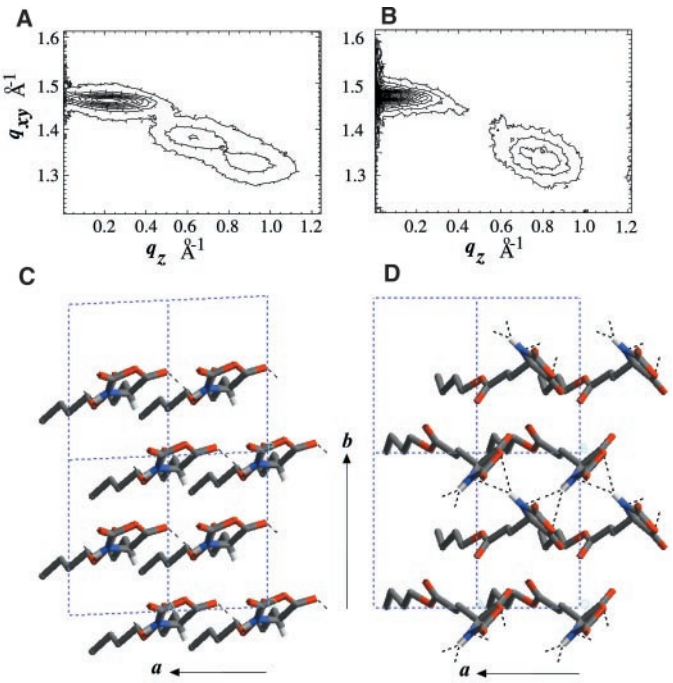


Fig. 3. GIXD patterns $I(q_{xy}, q_z)$ of the self-assembled crystallites of C_{18}-NCA-Glu on water at 4°C of (**A**) enantiomerically pure and (**B**) racemate. (**C** and **D**) The packing arrangements of the enantiomerically pure and racemic compound, respectively, viewed perpendicular to the water surface. For clarity, part of the hydrocarbon chains are not shown.

In the C_{18}-TE-Lys system, however, the formation of the oligopeptides with homochiral sequence of the minor component [labeled (*h*,0) in Fig. 2, D and F] cannot be prevented because of the intrinsic formation in the racemic phase of both homochiral R_h and S_d oligopeptides (Scheme 1A). This drawback can be circumvented (see above) in systems where the reaction in the racemic crystallites occurs primarily between heterochiral molecules (Scheme 1C) and the enantiomer in excess polymers of a single handedness. This concept is illustrated for C_{18}-Glu-NCA.

The GIXD pattern of enantiomeric C_{18}-Glu-NCA (Fig. 3A) on water yields a pseudo-rectangular unit cell (a = 5.52 Å, b = 8.62 Å, γ = 92.5°) with a molecular arrangement shown in Fig. 3C. The chains are tilted by 34.8° from the surface normal in a direction 15° off the *a* axis. Injection of the nickel acetate catalyst into the aqueous subphase induces a reaction that could be monitored by GIXD. A minor, yet important, change occurs in the unit cell that becomes rectangular (a = 5.54 Å, b = 8.58 Å, γ = 90°), in keeping with chains of the polymerized film tilted by 34° from the surface normal in a direction parallel to the *a* axis.

The GIXD patterns of racemic C_{18}-Glu-NCA on pure water and on aqueous solution containing a catalyst are similar to that shown in Fig. 3B, namely, no change in the diffraction pattern was observed during the reaction. According to the proposed packing arrangement of the racemic monomer (Fig. 3D), a lattice-controlled polymerization occurs between heterochiral molecules related by glide symmetry (Scheme 1C), which is strongly supported by the MALDI-TOF MS analysis (Fig. 4A). This histogram shows the formation of heterochiral oligopeptides in concentrations beyond the random distribution obtained from C_{18}-T-Glu (Fig. 2B). When starting from chiral nonracemic mixtures of 3:7 and 4:6 (*S*:*R*) compositions, the generated short oligopeptides are rich in heterochiral diastereoisomers, whereas the longer oligopeptides are rich in homochiral sequences of single handedness (Fig. 4, B and C). Oligopeptides 9 or 10 units long obtained from the 3:7 (*S*:*R*) mixtures consisted only of compositions 1*S*:8*R* and 0*S*:9*R* or 1*S*:9*R* and 0*S*:10*R*. Similarly, the 4:6 (*S*:*R*) mixtures yielded oligopeptides of only 1*S*:7*R* and 0*S*:8*R* or 1*S*:8*R* and 0*S*:9*R* compositions (Fig. 4, B and C) (*28*).

In conclusion, we have demonstrated that polymerization within 2D crystallites composed of racemic compounds appropriately packed can lead to the enhanced generation of oligopeptides with homochiral sequences through lattice-controlled reactions between molecules of the same handedness. Furthermore, an efficient amplification of homochiral oligopeptides starting with monomers of low enantiomeric imbalance was accomplished by segregation into racemic and enantiomorphous phases followed by reactivity. This process takes advantage of the differences in the packing arrangement and kinetics of polymerization within the racemic and enan-

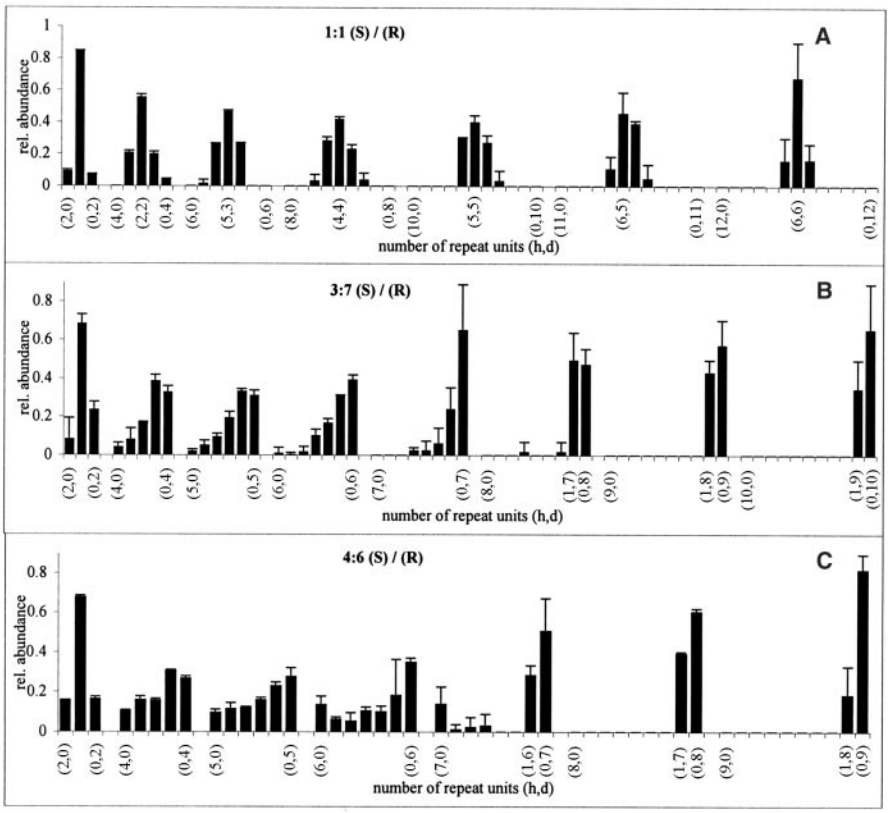


Fig. 4. MALDI-TOF MS analysis of the oligopeptides obtained from racemic and chiral nonracemic mixtures of C_{18}-NCA-Glu monomer. (**A**) Racemic. (**B**) The 3:7 *S:R* and (**C**) 4:6 *S:R* mixtures. For clarity, the distribution of only some of the oligopeptides are shown. Note that the percentage error in the relative abundance is larger for long oligopeptides that are formed in low chemical yield.

tiomorphous crystalline phases of the monomers, thus introducing nonlinear effects (*29*) and resulting in libraries of oligopeptides of different lengths and handedness.

Scenarios describing the appearance of chiral amino acids under prebiotic conditions such as irradiation of racemates with circularly polarized light (*30*), stochastic crystallization processes (*31*), or amino acids that had reached Earth by way of meteorites (*32*) are always of low enantiomeric imbalance. The mechanism of amplification of chirality described here might be relevant in converting such mixtures of amino acids into oligopeptides of single handedness.

Finally, it has been proposed that self-assemblies of amphiphilic molecules have played a ubiquitous role at early stages of evolution in the formation of primitive membranous "minimum protocells" (*33*, *34*). The present results suggest that ordered self-assembled architectures of appropriate amphiphiles in aqueous media or on the surfaces of minerals might have also been instrumental in the formation of the first optically active biopolymers.

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