

Laser-Induced Alignment of Self-Assembled Films of an Oligopeptide β Sheet on the Water Surface**

Atalia Birman, Kristian Kjaer, Yehiam Prior, Iftach Nevo,* and Leslie Leiserowitz*

Two-dimensional (2D) crystals at the air–water interface may be obtained by spreading a solution of amphiphilic molecules in a volatile solvent onto the water surface. On solvent evaporation the amphiphiles may self-assemble into monolayer crystals,^[1] invariably oriented randomly about the water surface normal, yielding “2D powders”. Our goal is to develop a new approach to induce at various interfaces, such as air–liquid and liquid–solid, molecules that self-assemble into aligned 2D crystals.

Pioneering studies in light-induced crystallization were conducted by Garetz, Myerson et al.^[2] They irradiated a supersaturated solution of urea with nanosecond, linearly polarized IR pulses from a Nd:YAG laser. The first needle-shaped crystal observed was aligned approximately parallel to the beam’s electric field; tens of seconds later the complete sample crystallized into a complex mass.

Recently we reported^[3] the laser-induced alignment of two-dimensional (2D) crystals of α helices formed from poly- γ -benzyl-L-glutamate (PBLG) and alamethicin; PBLG was polydisperse with 130 peptide units on average, whereas alamethicin comprises 20 peptide units. Here we focus on peptidic amphiphiles tailored to form on the water surface

films of aligned β sheets (Figure 1 a) which, unlike α helices, comprise extended H-bonded networks.

Peptides composed of alternating hydrophobic and hydrophilic residues will tend to adopt on the water surface a conformation with the former groups above the water surface and the latter below,^[4] namely a β strand (Figure 1 a). Such strands can interlink by N–H $\cdots$ O bonds along a 4.8 Å repeat to form 2D crystalline β sheets (Figure 1 a). The molecule synthesized for our studies (see the Supporting Information), Pro-Lys-Phe-Glu-Phe-Ser-Phe-Lys-Phe-Glu-Pro (**1**), is similar to Pro-Glu-(Phe-Glu)₄-Pro, which forms a β -sheet monolayer on the water surface,^[5] but with a fundamental difference: Instead of all hydrophilic residues being Glu, they alternate with Lys, except for Ser introduced at the chain center. The idea is that the molecules will form, in the solution prior to spreading on the water surface, cyclic β -strand dimers **1a,b** (Scheme 1) through Glu–Lys acid–base interactions. The advantage of bilayer **1a,b** vis-a-vis monolayer **1a** for enhancing induced alignment, is twofold: the number of N–H $\cdots$ O bonds along the 4.8 Å β sheet repeat, as well as the hydrogen bonds that separately interlink the Ser and charged Lys and Glu residues by means of interleaving H₂O molecules (forming OH_{Ser} $\cdots$ OH_W $\cdots$ OH_{Ser} $\cdots$ OH_W and NH₃⁺_{Lys} $\cdots$ OH_W $\cdots$ O₂[−]_{Glu} $\cdots$ HO_W $\cdots$ NH₃⁺_{Lys} bonds, respectively) along the 4.8 Å repeat, will be doubled.

The nonilluminated film of **1** floating on water was characterized by surface pressure/molecular area (π –A) isotherms, IR reflection absorption spectroscopy (IRRAS), and grazing incidence X-ray diffraction (GIXD). A π –A isotherm recorded with a solution of **1** in trifluoroethanol (TFE)/chloroform (1:9) displayed a limiting molecular area of 135 Å² (see Figure 1S in the Supporting Information). The IRRAS measurements (see Figure 2S in the Supporting Information) provided evidence in favor of the antiparallel β -sheet motif.^[6] Definitive information on the crystalline film of **1** was obtained by GIXD (see the Supporting Information). A fresh solution of oligopeptide **1** (0.29 mg mL^{−1}) in TFE/chloroform (1:9) was spread on deionized water at ambient temperature, then cooled to 5 °C. The GIXD measurements on the resulting film yielded two Bragg peaks (Figure 2 a,c) with d spacings of 42.7 Å and 4.8 Å. The former corresponds to the crystalline repeat along the direction of the molecular chain (b axis in Figure 1 c), and the latter to the distance between β strands, which are interlinked by N–H $\cdots$ O bonds (a axis in Figure 1 c), forming a β sheet with a molecular area 42.7 $\times$ 4.8 = 205 Å². According to IRRAS,^[6] the β sheet adopts the antiparallel motif signifying that the adjacent β strands are related by twofold symmetry (Figure 1 b); this is consistent with the Bragg rod shapes (Figure 2 b,d) that peak at $q_z = 0$ Å^{−1} and with the packing arrangement.^[7] According to an

[*] A. Birman, Prof. L. Leiserowitz
Department of Materials and Interfaces, Weizmann Institute of Science
76100, Rehovot (Israel)
Fax: (+972) 89-344-138
E-mail: leslie.leiserowitz@weizmann.ac.il

Dr. I. Nevo
Department of Chemistry, Aarhus University
8000 Aarhus C (Denmark)
E-mail: iftachn@chem.au.dk

Prof. Y. Prior
Department of Chemical Physics
Weizmann Institute of Science, Rehovot (Israel)

Dr. K. Kjaer
Max-Planck-Institut für Kolloid- und Grenzflächenforschung
Am Mühlenberg (Germany)
and
Niels Bohr Institute, University of Copenhagen (Denmark)

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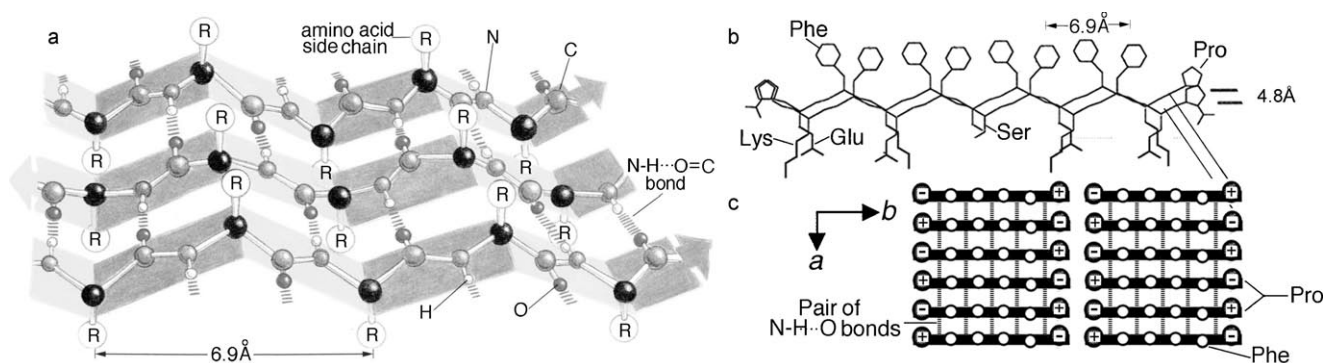
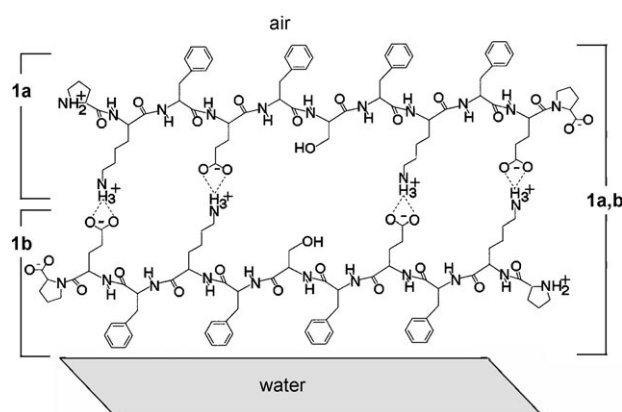


Figure 1. Schematic diagrams: a) A β sheet made up of an antiparallel set of β strands interlinked by $\text{N-H}\cdots\text{O}=\text{C}$ bonds; b) two β strands of peptide **1** separated by the $\text{N-H}\cdots\text{O}$ bonding distance of 4.8 Å and arranged antiparallel; each CONHCH unit is depicted by a slanted line; c) antiparallel β sheets of **1** viewed normal to their plane. Figure 1 a was reproduced from *Molecular Biology of the Cell*, 3rd ed. by permission of Garland Science.



Scheme 1.

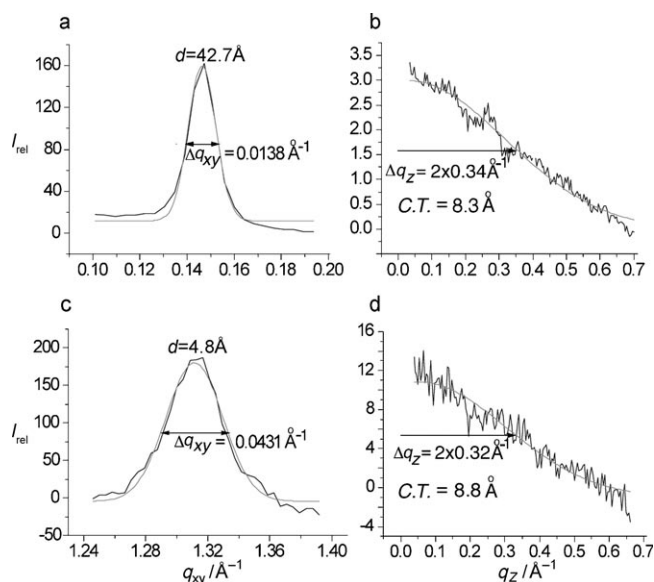


Figure 2. GIXD pattern of film **1** floating on water. a,c) Two Bragg peaks $I(q_{xy})$; b,d) corresponding Bragg rods $I(q_z)$. Also displayed are the Bragg peak d spacings and the FWHM of the Bragg peaks and rods, which yield the crystal coherence lengths and thickness (C.T.), respectively. Fitted curves are also shown.

analysis of the full width at half maximum (FWHM) of the Bragg peaks, the crystal coherence lengths along the 42.7 Å and 4.8 Å axes are 410 Å and 130 Å corresponding to 10 and 27 molecular repeats, respectively.^[8] The two Bragg rods (Figure 2b,d) yield a thickness for the crystalline film of approximately 8.5 Å,^[8] namely a monolayer of **1a**.

For the IR illumination experiment, we used a coherent, linearly polarized 1.064 μm beam from a Nd:YAG Q-switched pulsed laser (40 mJ per pulse, 50 pulses per second, 10 ns pulse duration, beam diameter FWHM 3 mm). Illumination was perpendicular to the water surface of a cell (see the Supporting Information) onto which we had deposited 160 μL of a solution of peptide **1** (15.05 $\mu\text{g mL}^{-1}$) in TFE/chloroform (1:9) to achieve 80% coverage of **1**. Exposure to the laser beam was for 5 min until the solvent had evaporated. Immediately thereafter the formed film was transferred by horizontal attachment^[9] to a Si(111) wafer that had been coated with *n*-octadecyltrichlorosilane (OTS) to give the Si surface hydrophobic character. The bare Si-OTS surface, when studied by atomic force microscopy (AFM, see the Supporting Information), was featureless with a roughness of 0.2 nm. The transferred film was imaged by AFM in two regions separated by 1 mm within the illuminated region.

The two AFM topography images (Figure 3a_i and b_i) display rods oriented along the same diagonal direction. The tendency for alignment is also reflected in their Fourier transform (FT) patterns (Figure 3a_f and b_f). The FT lobes are highly anisotropic in shape, perpendicular not only to the mean direction of the rods in the AFM images but also to the direction of polarization of the laser electric field. However, there was an uncertainty of roughly 15° in relative azimuth of the Si wafer with respect to the polarization direction of the laser beam. A different film prepared under similar conditions gave the AFM image and FT pattern in Figure 3c_i and c_f, respectively, which are almost identical to those in Figure 3b. These results provide conclusive evidence that the laser illumination induces alignment persisting over macroscopic distances. The average film thickness, derived from the profile (Figure 3d) of the AFM image in Figure 3c_i, is approximately 2.3 nm.

In one control experiment the film-illumination procedure was repeated, but with circularly polarized light; in a

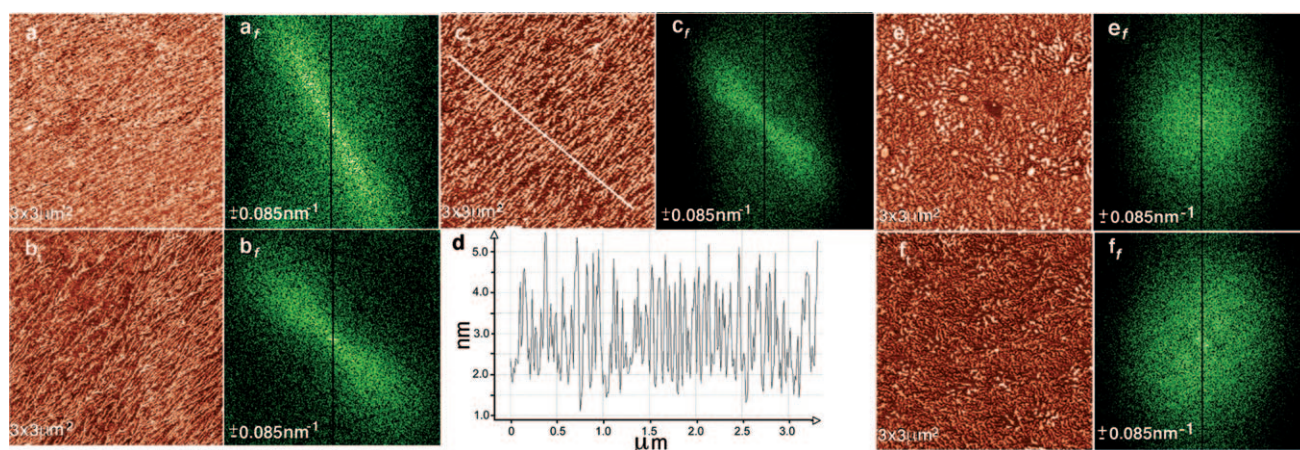


Figure 3. Effect of linearly (a–c) and circularly (e, f) polarized IR laser illumination of **1** floating on water. AFM images (a, b, c, e, f) and FT patterns (a_f, b_f, c_f, e_f, f_f) of film **1** after transfer to Si surface. Images (a_f) and (b_f) are from one sample recorded at positions roughly 1 mm apart. Image (c_f) is from another sample. The topography profile (d) was recorded along the white line on image (c). Images (e) and (f) are from one sample recorded at positions roughly 1 mm apart.

second experiment the film was illuminated with linearly polarized light only after the solvent had evaporated. Both experiments yielded no alignment, as is evident in Figure 3 e, f for the first case. The second experiment gave similar negative results (not shown).

The alignment experiments were repeated with a new batch of **1** with purity exceeding 90% (see the Supporting Information). The laser beam optics were modified to yield a linearly polarized beam more conveniently oriented relative to the Si wafer, but with an intensity about half of that used in the previous experiments. The side of the Si wafer onto which **1** was transferred was wet, implying that a significant part of the film had Glu, Lys, and Ser residues exposed to the water subphase, as a monolayer **1a**. The film alignment (Figure 4 a, and b_f) was poorer than in Figure 3, consistent with film distortion following transfer onto the Si wafer as a result of transport of the water drop. The lobes of the FT patterns (Figure 4 a_f, b_f), although diffuse, are nonetheless directed

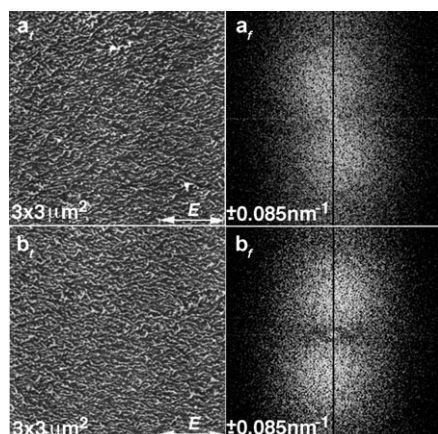


Figure 4. Effect of illumination of fresh solution of **1** on water with the linearly polarized IR laser beam; AFM images (a, b) and corresponding FT patterns (a_f, b_f). The double arrow shows direction of the laser beam's electric field *E*.

along the vertical axis, perpendicular to the laser electric field *E*.

The AFM images of laser-aligned crystals of β sheets of **1** (Figure 3 a–c) revealed a pattern of rods with average length and width of 250 nm and 30 nm, respectively. The β -sheet N–H $\cdots$ O=C bonds are surely parallel to the rod axis, for in amyloid β -sheet fibrils, the N–H $\cdots$ O=C bonds are parallel to the fibril axis,^[10] which we explain as follows: Given that the energy of interaction between β strands along the N–H $\cdots$ O bond axis of 4.8 Å is significantly greater than between β strands related by the 42.7 Å axis, the crystals grow faster along the former axis in accord with the Hartman–Perdok theory of crystal growth.^[11,12]

Having found that the laser-aligned rods of **1** tend to be parallel to the linearly polarized electric field, and having deduced that the N–H $\cdots$ O=C bonds are parallel to the crystal rod axis, we conclude that alignment was induced along the N–H $\cdots$ O=C bonds. The absence of alignment in case of circular polarization highlights the role of a linearly polarized field in creating an asymmetry, leading to preferred direction for self-assembly.

As to the thickness of film **1**, the non-laser-illuminated crystalline film on the water surface was a monolayer of **1a** according to GIXD. But, the limiting area per molecule of 135 Å² derived from the π -A isotherm (see the Supporting Information) measured on the Langmuir trough is roughly two-thirds of the molecule area of 205 Å² in the crystalline β sheet, indicating appreciable bilayer formation on the Langmuir trough. This result is in accord with the average 2.3 nm thickness of the aligned film determined by AFM, providing conclusive evidence in favor of the bilayer **1a,b** on the surface of the water cell. Bilayer formation after transfer of the monolayer onto the Si surface would require rupture of multiple hydrogen bonds.

The cyclic dimer of **1** is obviously polarizable, but the direction of induced alignment parallel to the linearly polarized electric field *E* proved to be along the crystal rod, namely the β -sheet N–H $\cdots$ O=C bonds. Given that the long

molecular axis of **1** is perpendicular to the crystal rod axis, we deduce that molecules of **1** were not polarized in the direction of their long axis by the electric field **E** at any stage of the crystal formation. Furthermore, since alignment was not induced after complete solidification of **1**, we conclude it was induced before the crystal rods of **1** became fully formed. We also argue that alignment does not occur at that stage in which β strands of **1** are isolated from each other, akin to what was deduced for PBLG and alamethicin.^[3] Thus, alignment is, in all likelihood, brought about in that state in which strong interactions exist involving the N–H $\cdots$ O=C bonds and the Glu, Lys, and Ser groups perhaps interlinked through H₂O molecules along the 4.8 Å axis, when a sufficient number of β strands have already formed proto- β -sheet crystals. In short, we invoke cooperative effects between neighboring β strands of the proto-crystals. We also suggest in a manner akin to what was concluded for PBLG and alamethicin, that the proto- β -sheets were forced by a sequence of pulses to align parallel to the electric field **E**, with the condition that alignment be sustained between the laser pulses. However, unlike what was found for the crystal rods of **1**, the alignment direction of the α helices was neither parallel nor perpendicular to the **E** field, suggesting that the “pulsed-kick” model to align the α helices parallel to **E** was still at play at the end of the process. Note that different laser sources^[13] were used in the present and previous experiments. It is also noteworthy that the experimental success rate was about 40 %, owing to the complexity of the process.^[14]

In contrast to what was found for the PBLG and alamethicin films, chloroform as the primary solvent did not hamper the formation of aligned films of **1**; for the α -helical films alignment was achieved with slower-evaporating toluene. A factor that might account for the observed difference is that the interaction between neighboring β strands of **1** is stronger than that between α -helical rods. Thus the onset of the “collective state” for the β sheet may occur at an earlier stage, providing a larger time window for induced alignment.

The approach described here and previously^[3] provides a route for thin-film engineering and additional means to monitor crystal nucleation and derive structural information for crystal films. The method might provide a solution to the problem of membrane protein crystallization. By contrast with globular proteins, fewer membrane proteins have been obtained as 3D crystals, although some form “2D crystalline powders” on water.^[15] Since membrane proteins comprise α helices, it may be possible to induce aligned 2D crystals of membrane proteins for structural characterization by GIXD.

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