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Local contact: Dr. Amarjeet Singh	Beamline: ID-10B
Names and affiliations of applicants (* indicates experimentalists): Dr. Amir Berman*, Dept. of Biotech. Engineering Inna Sigal*, Dept. of Biotech. Engineering Ben-Gurion University of the Negev, Beer-Sheva 84105, ISRAEL	Shifts: 24

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Abstract
A 2-dimensional bio/synthetic hybrid system at the air-solution interface made of polymerized diacetylene Langmuir film with nucleobase modified headgroups is presented. The polymerized film present a crystalline array of nucleobases, capable of specific binding of complementary mononucleoside or oligonucleotide sequences.
Monolayers of the linear polyconjugated polydiacetylene (PDA) films derivatized with Cytosine (10,12 pentacosadiyne-cytidyl, PDC) monomers[1], compressed and polymerized at the air-water interface with circular polarized light (CPL) or nonpolarized UV light.
Here we report a grazing incidence x-ray diffraction (GIXD) investigation of PDC films polymerized to different chirality and hybridized with complementary ssDNA strands. We have demonstrated for the first time enantioselective interactions on synthetic structured interfaces produced by Langmuir surface compression followed by polymerization with circular polarized UV light (CPL). The *left* and *right* CPL polymerized light exhibit the same well defined crystalline structure, closely related to the polydiacetylene (PDA) blue phase structure. The observed difference between *Left* and *Right* CPL polymerized PDC75% Langmuir films compressed over the complementary mononucleotide Guanosine or hybridized with fully complementary $ssG_{12}T_5$ oligonucleotide in subphase suggests that they are indeed enantiomeric structures, capable of enantioselective binding of their natural ligand, guanosine, solely as a result surface induced asymmetry in *"left"*, but not in *"right"* form. Our finding may also be related to the intriguing question of chiral selection during the early period of "Origin of Life". We show that a--chiral compounds, as a result of irradiation with circular polarized light, can organize in chiral surface structures capable of amplification biopolymers binding of particular handedness.

Results and discussion

1. PDC75% UV irradiated film structure

GIXD reciprocal maps obtained from PDC films that were polymerized with CPL UV light (**Fig. 5B**) are compared to PDA (blue phase), (**Fig. 1A)** and PDC75% films polymerized with non-polarized light (**Fig. 1**D) and to un-irradiated PDC75% film (monomer) (**Fig. 1C)**.

The reciprocal map depicted in **Figure 1B** was used to solve the PDC75% film structures polymerized with CPL (the PDC75%_L and PDC75%_R reciprocal maps are identical). Four reflections are observed; two with $q_z<0.5Å^{-1}$ corresponding to small inclination of the diffracting planes, and two highly inclined reflections with $q_z > 0.6$ $Å^{-1}$ (according to recently solved PDA blue phase structure (**Fig. 1A**)[1]. Accordingly, the low q_z reflections are attributed to the hydrophilic sublayer, and the high q_z correspond to the highly tilted hydrophobic sublayer (**Table 1**).

Miller indices	q_{xy}, Å^{-1}	q_z, Å^{-1}	q_{tot}, Å^{-1}	τ^0	d_{xy}, Å
$(11)_A$	1.38	0	1.38	0	4.55
$(11)_B$	1.38	0.41	1.44	16.55	4.55
(02)	1.27	0.94	1.58	36.51	4.95
$(1\bar{1})$	1.49	0.61	1.61	22.26	4.22

Table 1. Summary of the GIXD reflections in Figure 1B. q_{xy} and q_z are the peak coordinates in reciprocal space; $q_{tot}=(q_{xy}{}^2+q_z{}^2)^{1/2}$ is the reciprocal interplanar distance, τ is the tilt angle of the diffraction vectors with respect to the normal.

Considering the recently solved carboxylate terminated headgroup PDA structure[2] as a benchmark, we have indexed the reflections and deduced the structure of the CPL polymerized PDC films (**Table 2)**. We note that *left* and *right* CPL results are indistinguishable 2-D structures. Nevertheless they are of opposite handedness as was shown by circular dichroism (CD) measurements for PDA21 and for PDC (manuscript in preparation).

As is the case in PDA (blue phase), the main structural feature of PDC structure are the highly inclined alkyl chains in the [11] direction, manifested in the (11) reflection with q_z=0.

In contrast, the non-CPL polymerized PDC film exhibit poor crystallinity, judged from its weak intensity reflections, packed in a different structure (**Fig. 1D**). The 3 reflections observed for non-CPL PDC have similar interplanar tilted spacings of approximately 4.16Å^{-1}. Their projections onto the plane correspond (11) and (11) reflections of PDA blue phase. The third reflection cannot be unequivocally be related to the PDA-blue (02) reflection, so although this structural variation cannot be unambiguously solved at this stage, it appears more disordered.

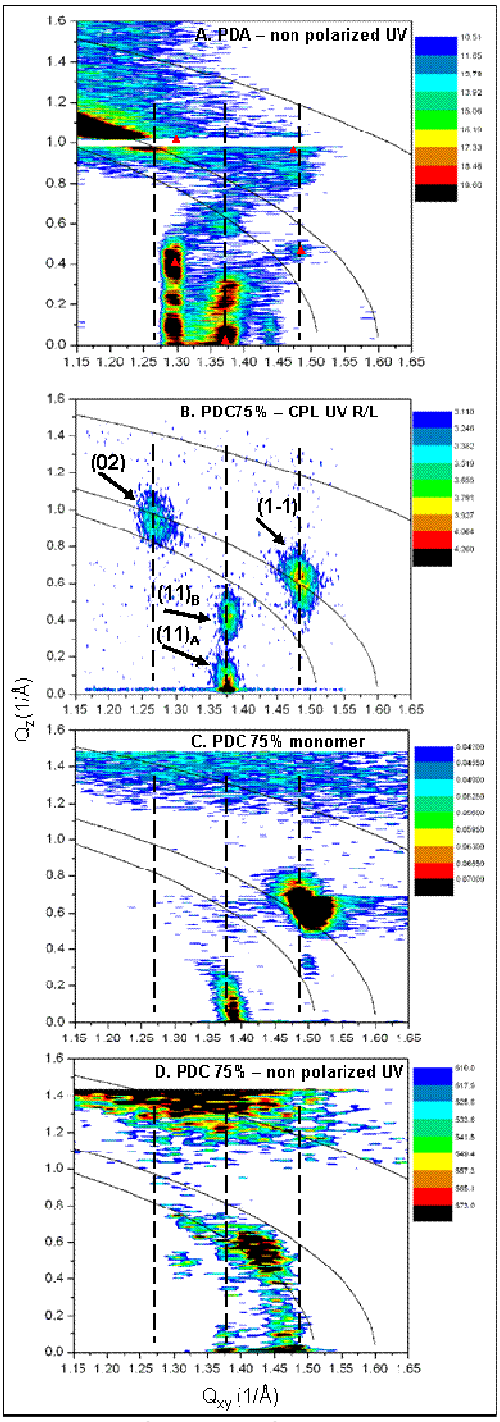

Figure 1. 2-D reciprocal space maps obtained from PDA film UV irradiated with non-polarized UV light[2] (A), PDC75% film UV irradiated with a *left* or *right* CPL (B), Un-irradiated PDC75% film (monomer) (C) and PDC75% film irradiated with non-polarized UV light (D). Arcs are q_{tot}=1.9 Å^{-1}, 1.60 Å^{-1} and 1.51 Å^{-1} which corresponds to d=3.31Å, 3.93 Å and 4.16 Å, respectively**.** The calculated positions for PDA based on the crystallographic model are marked by triangles in A.

Miller indices	d_{xy}, Å (PDA blue phase)	q_{xy} (PDC75%)	d_{xy}, Å (PDC75%)
(11)	4.55	1.375	4.57
(02)	4.83	1.44	4.86
$(1\bar{1})$	4.21	1.49	4.22

Table 2. Summary of the interplanar spacings in the 2D unit cells of PDA blue phase and PDC75% films.

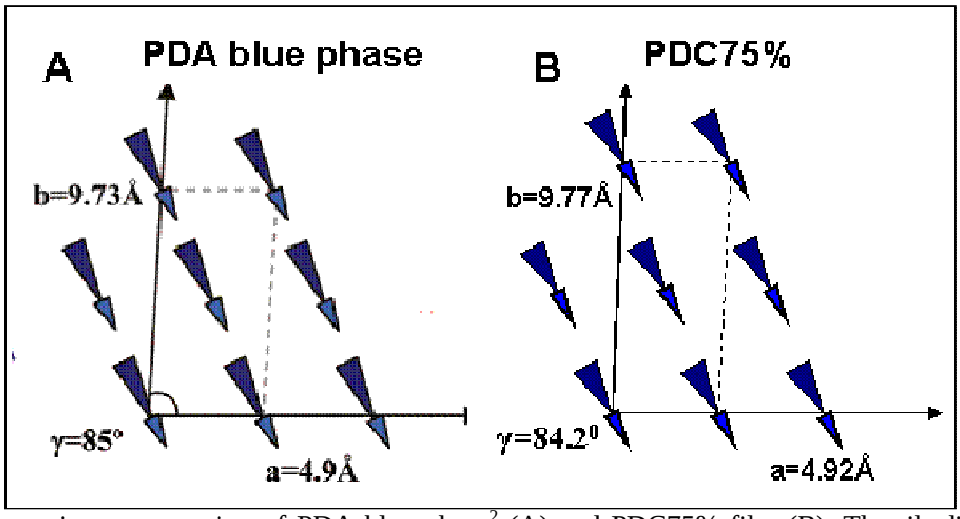

Figure 2. Schematic representation of PDA blue phase[2] (A) and PDC75% film (B). The tilt direction of the alkyl chain tilt is denoted by triangles. The triangle sizes represent the chain projection on the plane and, hence, their tilt. The tapered shape of the alkyl chains representation in A reflects their curved shape[2]. The *a* and *b* axes correspond to the centered cell. The structural information is summarized in **Table 2**.

2. Interactions of CPL-polymerized PDC75% films with complementary guanosine mononucleotides.

PDC75% Langmuir films were compressed over subphase containing the complementary mononucleoside, guanosine. The resultant film is organized in a structure that is related to that of PDC monomer (**Figure 1C**), though with several important differences, **(Figure 3A)**. The prominent observation is the appearance of a series of reflections at q_{xy}=1.31 and q_z = 0.0, 0.4, 0.5 and 0.7Å^{-1}. Shift of the reflection to higher q_{xy} values, indicating smaller, more compact unit cell as the result of base-pair formation.

It is plausible to index the reflections as follows q_{xy}=1.31 – (02); q_{xy}=1.42 – (11); q_{xy}=1.50 – (11), according to their relative order in PDC CPL irradiated structure (**Figure 1B**). The absence of the (02) reflection in PDC monomer (**Figure 1C),** and its appearance in the presence of G indicate the increase in the film's order induced by basepairing.

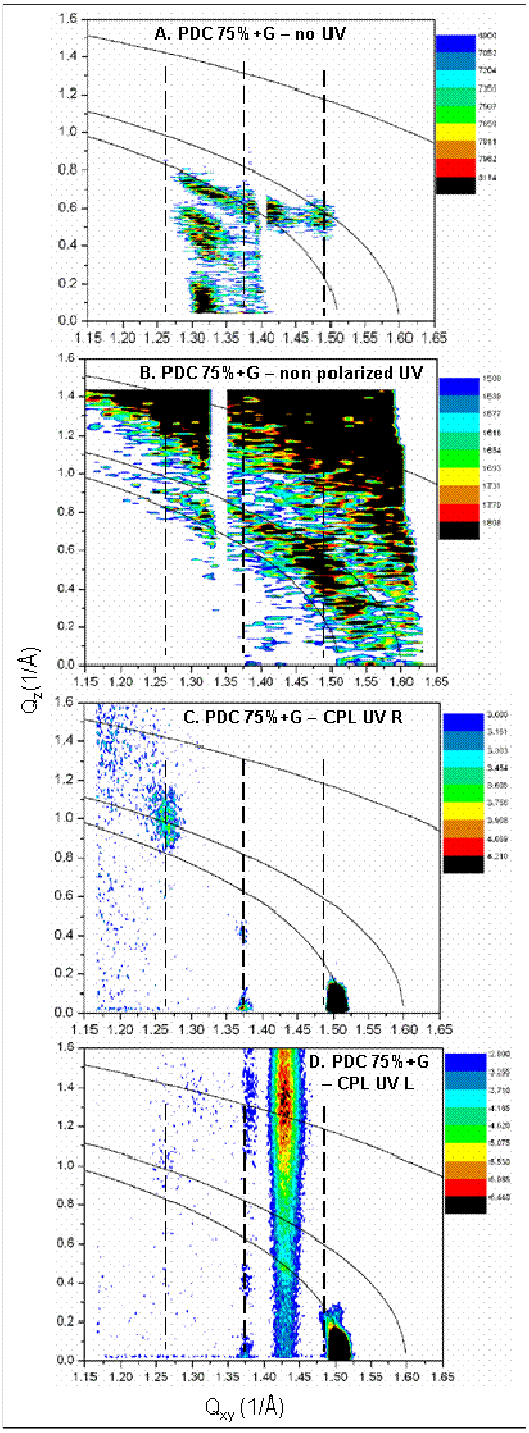

Figure 3. 2-D reciprocal space maps obtained from PDC75% films compressed on guanosine mononucleoside buffered solution and un-irradiated film, (A). Irradiation with non-polarized UV light (B), Irradiation with a *right* (C) and *left* (D) CPL UV light. Arcs are q_{tot}=1.9 Å^{-1}, 1.60 Å^{-1} and 1.51 Å^{-1} which corresponds to d=3.31Å, 3.93 Å and 4.16 Å, respectively

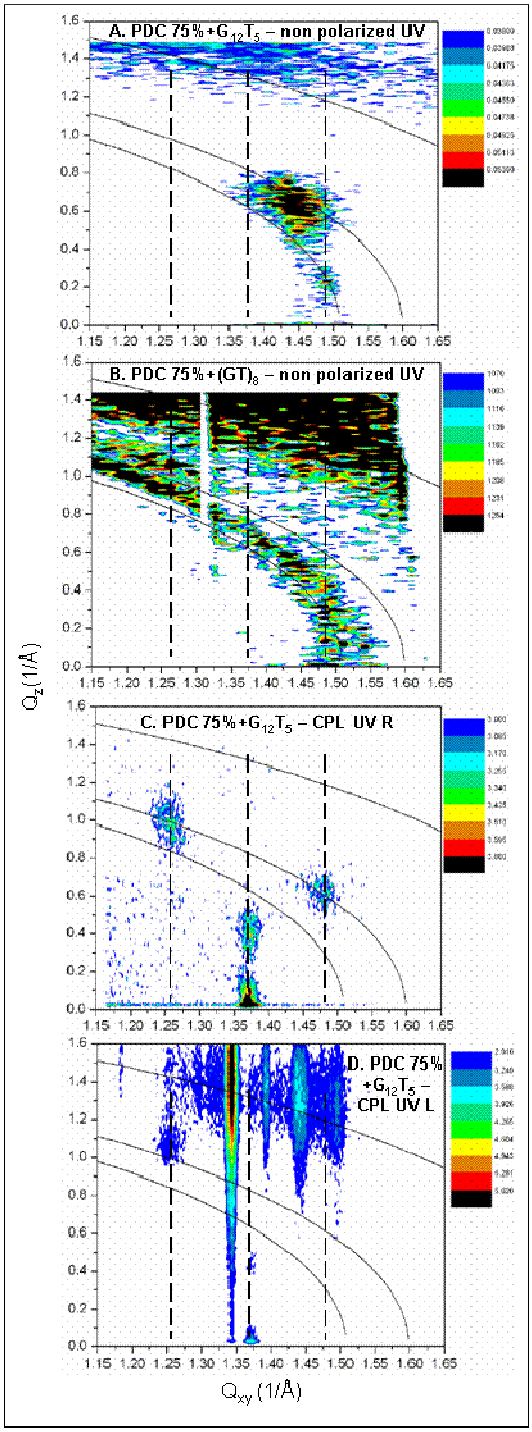

Figure 4. 2-D reciprocal space map obtained from PDC75% film UV irradiated with a non polarized UV light (A) and with a right (C) or left (D) CPL and incubated with $G_{12}T_5$-ssDNA in comparison to PDC75% film UV irradiated with a non polarized UV light and incubated with $(GT)_8$-ssDNA. Arcs are q_{tot}=1.9 Å^{-1}, 1.60 Å^{-1} and 1.51 Å^{-1} which corresponds to d=3.31 Å, 3.93 Å and 4.16 Å, respectively

It may also hint that the base-pairs planes are oriented along the polydiacetylene conjugated direction, (hence parallel to [02] direction). Polymerization of PDC film compressed on G subphase with non-CPL UV light for 20 sec. results in distorted reflections along equal q_{total} arcs, **(Figure 3B)**. The massive reflection spot centered at q_{tot} = 1.9 $Å^{-1}$ corresponds to d=3.3Å, the typical π-stacking distance of DNA basepairs.

Yet, its broad nature suggest highly disordered structural feature. PDC polymerization on G containing subphase with ***Right*** CPL UV light results in slightly different structure (**Figure 3C)**, compared with the PDC film in G absence (**Figure 1B)**. The (11) and the (02) reflections appear at the same position and tilt as in CPL polymerized PDC in G absence. However, the (11) reflection has shifted towards higher q_{xy} value (from q_{xy} =1.48 to q_{xy} =1.51$Å^{-1}$, indicating a more condensed packing in this direction. As a result, this reflection intensified and appears at q_z=0, indicating that these planes are not tilted outside the [11] diection. The significant shift in position and reflected intensity suggests that the (11) reflecting planes are directly affected by the subphase; hence it may be associated with the planar Cytidyl groups.

Very distinct reflections centered on the q_{total} = 1.9$Å^{-1}$ arc were observed when PDC film was polymerized with ***Left*** CPL UV light **(Figure 3D)**. The two observed reflections projection onto the q_{xy} axis are at q_{xy} = 1.43 (very strong) and q_{xy} = 1.375$Å^{-1}$ (weak). The observed difference between ***Left*** and ***Right*** CPL suggests that they are indeed enantiomeric structures, capable of enantioselective binding of their natural ligand, guanosine, solely as a result surface induced asymmetry.

3. Interactions of CPL-polymerized PDC75% films with complementary ssDNA GT oligonucleotides.

PDC75% monolayers were compressed on buffer and polymerized with non-CPL, ***left*** or ***right*** CPL UV light. After polymerization, 16 or 17-mer oligonucleotides, fully or partially complementary to the PDC film were carefully injected under the film and given 30 min. to hybridize. **Figure 4A** depicts the 2-D diffraction map obtained when fully complementary $ssG_{12}T_5$ oligonucleotide was injected under a film polymerized with non-CPL light. The diffraction pattern resembles that of the same film without the oligonucleotide, **(Figures 4A and 1D**, respectively) and reflects a poorly crystalline assembly.

Injecting the partially complementary, alternate $(GT)_8$ 16-mer nucleotide, severe deformation of the film is observed **(Figure 4B)** – the well localized diffraction spot transformed into an arc of equal q_{total} value. This can be interpreted as the result of a planar film undergoes out-of-plane deformation, possibly due to the significant mismatch that cannot be accommodated between the regular presentation of cytidyl moieties in PDC film, and the doubly spaced, partial complementary nucleotide.

The fully complementary oligonucleotide $ssG_{12}T_5$ did not induce any observable change also when hybridized with the right-CPL light, **Figure 4C**. The 2-D diffraction map resembles that of similarly polymerized PDC film without oligonucleotide (**Figure 1B**).

A very different effect results when using ***left***-CPL. A series of high *q*z Bragg rods centered at q_{total} = 1.9$Å^{-1}$ (d=3.31Å) are observed, (**Figure 4D**). Notably, the PDC film reflections appear at the same positions as for CPL polymerized bare PDC. This observation suggests that complementary base-pairs are formed between the Cytidyl headgroups of the film and the complementary $G_{12}T_5$ oligomers at the subphase, without significantly disrupting or deforming the film order. The q_{xy} positions of these reflection correspond to their in-plane spacings, between 1.17 $Å^{-1}$<q_{xy}<1.50 $Å^{-1}$, corresponding to

in-plane spacing of 5.37 to 4.19Å. The observation of these reflections may suggest nonuniform orientation with respect to the PDC unit cell. It follows that basepair π-stacking may take place between neighboring Cytidyl moieties, not necessarily on the same polydiacetylene backbone.

4. Interactions of PDC75% films with non-complementary ssDNA $C_{12}T_5$.

Interaction of non-Watson-Crick complementary oligomers $C_{12}T_5$, resulted in similar effect to what was observed for the complementary oligomer – a series of intense reflections with typical π-stack spacing, tilted at about 45°±7° with respect to the surface plane, **Figure 5**. Surprisingly, this non-standard hybridization took place on ***right***-CPL polymerized PDC, but not on the ***left***-CPL, as is the case for hybridization of the mononucletide G and the fully complementary oligonucleotides. We cannot account at this stage for the exact stereochemical details of these structured enantioselective surface interactions.

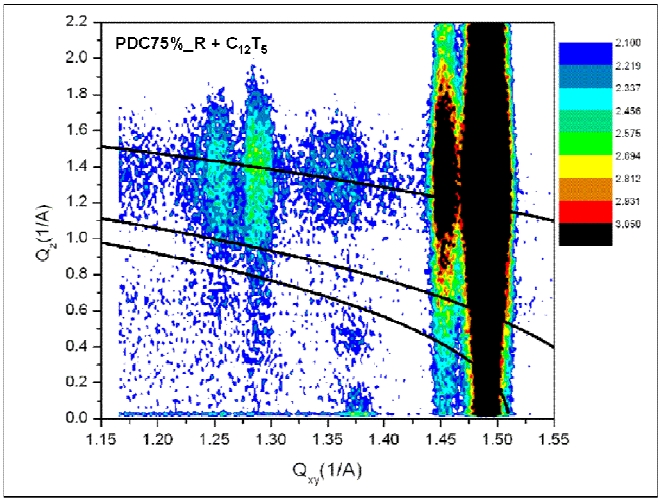

Figure 5. 2-D reciprocal space map obtained from PDC75% film UV irradiated with a right CPL and incubated with $C_{12}T_5$-ssDNA. Arcs are q_{tot}=1.9 $Å^{-1}$, 1.60 $Å^{-1}$ and 1.51 $Å^{-1}$ which corresponds to d=3.31 Å, 3.93 Å and 4.16 Å, respectively. Dotted circles correspond to the reflections observed for CPL polymerized film in the absence of oligonucleotide in figure 1B. These reflections are partially masked due to the intense reflections.

The somewhat disturbing observation of hybridization of cytosine rich oligonucleotides with the cytidyl headgroups in an ordered manner can possibly be explained on the basis of observations on cytosine-cytosine base pairing[3] or cytosine quartets[4, 5].
Similarly, it may well be that the observed hybridization of the G-rich oligonucleotides are related to non-Watson-Crick base pairing such as Hoogsteen basepair or G/C quartet formation[6].

Conclusions

In this work we have demonstrated for the first time enantioselective molecular recognition interactions on synthetic structured interfaces produced by Langmuir surface compression followed by polymerization with circular polarized UV light (CPL). The ***left*** and ***right*** CPL polymerized light exhibit the same well defined crystalline structure that is closely related to the polydiacetylene (PDA) blue phase structure. The main difference is that PDC organized in a monolayer while PDA tends to orderly collapse into a stable trilayer. The considerable difference in the limiting area between PDA and PDC likely stems from the domain morphology of the two films (PDA: 27$Å^2$/molecule *vs.* ~60$Å^2$/molecule for PDC). Minor differences include: straight alkyl chains in PDC compared to bend in PDA. The slightly different unit cell dimensions and structural transformations in PDC nevertheless do not lead to the stable PDA red-phase structure type. As is the case in blue phase PDA, the main structural feature of PDC structure are the highly inclined alkyl chains in the [11] direction, manifested in the (11) reflection with q_z=0. In contrast, the non-CPL polymerized PDC film exhibit poor crystallinity, judged from its weak intensity reflections, and probably represents a racemic structure.

Since the monomer is a-chiral, this notion is of particular interest and indicates that the chiral surface structures originate during UV irradiation by deformation of symmetric packing.
The observed difference between *Left* and *Right* CPL polymerized PDC75% Langmuir films compressed over the complementary mononucleotide G or hybridized with fully complementary $ssG_{12}T_5$ oligonucleotide in subphase suggests that they are indeed enantiomeric structures, capable of enantioselective binding of their natural ligand, guanosine, solely as a result surface induced asymmetry in the *"**left**"*, but not in *"**right**"* form.
Our finding may also be related to the intriguing question of chiral selection during the early period of "Origin of Life"[7]. We show that a-chiral compounds, as a result of irradiation with circular polarized light, or otherwise stabilized at the interface, can organize in chiral surface structures capable of selective amplification of biopolymers binding of particular handedness.

References

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