Experiment title: **Structural investigation of the conformational transitions of nucleotide derivatized polydiacetylene monolayer with its complementary mono- and oligonucleotide.**	Experiment no.: SC-1990 and SC-1711
Dates of experiments: from 21/6/2006 to 29/6/2006 (SC-1990) and 16/5/2005 to 21/5/2005 (SC-1711)	Date of report: 5/11/2006
Local contact: Dr. Oleg Konovalov	Beamline: ID-10B
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GIXD studies of the PDC film structure and the structural response of the PDC film upon specific recognition and base-pair formation

Monolayers of nucleolipids provide an organized 2-D structure that can serve as excellent model system to mimic biological processes such as DNA molecular recognition and can also find use in microfluidic devices, biosensing and biomolecular-based molecular electronics.

The purpose of this project was to study the structure of mixed lipid monolayer of pentacosadiynoil-cytosinyl derivative (PDC) and pentacosadiynol (PDOH) at the air-water interface and its structural response upon specific recognition and base-pair formation with complementary ssDNA mono- and oligonucleotides. By this recognition a new and stable complex of DNA-monolayer is created.
The study was carried out directly at the air/solution interface at the surface diffraction beamline ID10B. The incoming beam was monochromatized using a diamond (111) double crystal monochromator to the energy of 8.02 keV. For the grazing incidence diffraction measurements the angle of incidence was chosen to be 0.1004°. The diffracted intensity was measured using a linear position sensitive detector equipped with a Soller collimator. Angular aperture of the vertically oriented PSD detector in our experiments was 6.6º. In order to collect the reflected beams higher than 6.6º, the detector was moved to a higher position, allowing for some overlap between the two positions. Height calibration of the trough and the channel calibrations of PSD detector were routinely performed using the attenuated primary beam.

A turning circular trough was used in this experiment instead of standard Langmuir trough in order to obtain diffraction from discrete crystals with unknown orientation. Random "powder" orientation of the film's crystallites is a prerequisite for GIXD determination of Langmuir monolayer 2-D crystal structure. As we know from previous studies (BAM, optical microscopy and GIXD), PDC/PDOH film shows two types of "crystallinity": 1: random azimuthal orientation - "powder" (polycrystalline phase); 2: discrete large 2-D crystals in certain orientation ("single" crystal). This phenomenon was also observed using GIXD (Figure 1).

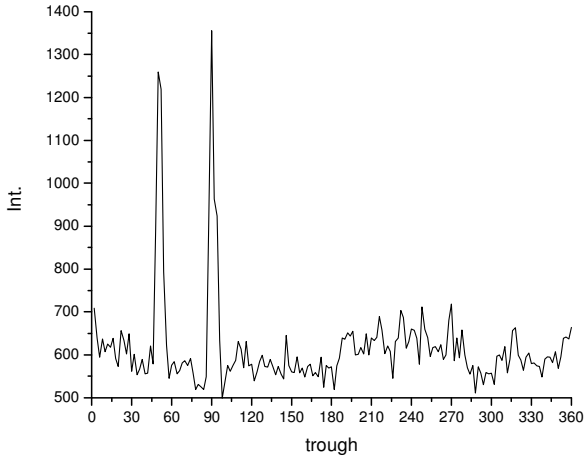

Figure 1. Representative GIXD signal from PDC75% on buffer subphase. The plot shows reflection intensity over trough angular position during 360º trough rotation. Two discrete crystalline domains are observed at 50º and 90º, whereas the background represent "random 2-D powder" of continuous polymer film (polycrystalline phase).

Since compression is not possible in the circular trough, 3:1 PDC/PDOH spreading solution (PDC75%) was spread drop-wise with constant time intervals, while monitoring π (with a tensiometer) until a uniform condensed monolayer was formed (at a surface pressure of 20 mN m^{-1}). The film was polymerized by UV lamp (λ=254 nm) for 60s, forming the robust polydiacetylene backbone.
The structural response of the PDC75% film upon specific recognition and base-pair formation was examined when complementary (16G-ssDNA, 8G-ssDNA) or partly complementary oligonucleotides (4GT-ssDNA, 8GT-ssDNA) were injected into the subphase. The system was incubated for 1 hour at 40ºC to allow for hybridization and then cooled to the initial temperature (R.T.).
All the measurements were conducted under helium atmosphere.
In another specific recognition experiments we used mononucleosides of Guanosine and Adenosine and mononucleobase Guanine that dissolved in warm (~40ºC) Trizma buffer pH=7.5 and filtered with Teflon filter 0.2μm.

In the process of trough rotation at certain delta, we examine reflection intensity from delta 8 to δ 30 every degree (Figure 2). In all trough rotations one can see expression of single phase and polycrystalline phase. Peaks of single phase appear at defined angular positions on the trough at certain δ positions. For example in Figure 2, two main groups of peaks are evident: at trough position of 30º and 200º. Peaks at trough position 200º have the highest intensity at δ=8º and their intensity is weakening till δ=19º. This group of peaks belongs to the high and broad peak at the 2-D maps (see for example Figure 4B). Peaks at trough angular position 30º have the highest intensity from δ=19º to δ= 22º. These peaks belong to low q_z peaks at 2-D maps (see for example Figure 4B). **Their angular difference of app. 170º, corresponds directly to the film crystal structure and represent information that is inaccessible in a typical GIXD experiment.**

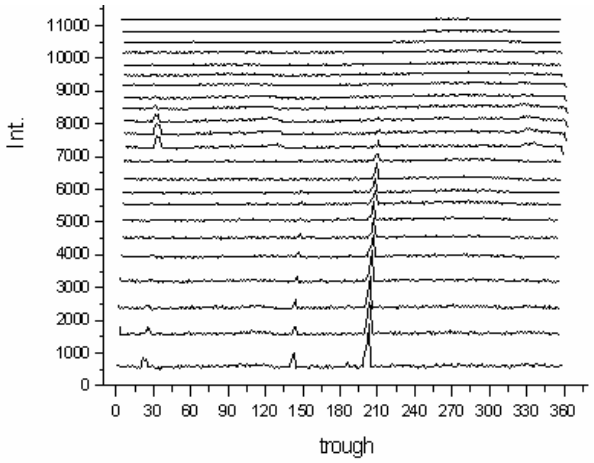

Figure2. GIXD signals from PDC75% on buffer after incubation with 16-G DNA oligomers. Plots show reflection intensity over trough position during full rotation of the trough (360º). Each plot represents rotation at one degree δ intervals from delta=8º (bottom) to 30º (top).

Polymerization of diacetylenes occurs by means of UV irradiation, X- (or γ) rays, electron beam or by thermal treatment. Upon polymerization, a linear, rigid backbone is formed with an extended π-conjugation. During the polymerization process the polydiacetylene backbone keeps a fully extended conformation without any interruptions of its conjugation, forming the “blue” form of the polymer. As polymerization proceeds, the regular planarity of the all-trans alkyl chain is converted into less regular pattern containing increasing amount of gauche conformations. The latter conformation causes interruptions in the fully extended conjugated backbone of the polymer and a reduction of the average conjugation length, resulting in the formation of the blue-shifted "red" form of the polymer [3].

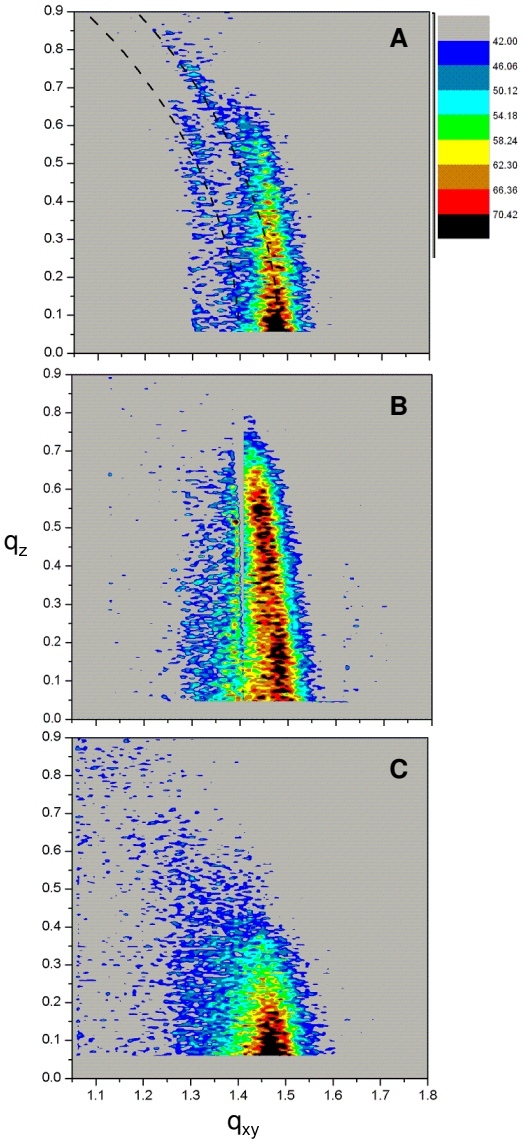

Figure 3. 2-D reciprocal space maps obtained from: (A) PDC75% on Trizma buffer pH=7.5 without photopolymerisation, Arcs are q_{tot}=1.485Å^{-1} and q_{tot}=1.42Å^{-1} which corresponds to d=4.23Å and d=4.42Å, respectively. (B) PDC75% on buffer after photopolymerisation for 1min. (C) PDC75% on buffer after photopolymerization for 2min.

Grazing incidence diffraction data were collected in real-time and showed the following patterns: The observed reflections of PDC 75% on buffer (Fig. 3) are outcome of different photopolymerization time, reveal similar reflection positions at q_{xy}=1.47Å^{-1} and low q_z values. PDC75% film without photopolymerization, (i.e monomeric PDC, Fig. 3A) exhibit enhanced arced reflections, probably due to some disorder of its alkyl chains compared with the alkyl chains of photopolymerized films (Fig. 3B,C). Photopolymerization for 1 min. (Fig. 3B) exhibit two well defined peaks that are less tilted and therefore indicate higher order and "uprightness" of molecules. It is assumed that this peak can be assigned to the alkyl chains of the head-groups layer in an orthorhombic (distorted hexagonal) lattice, whose spacing is typically d=$2\pi/q_{xy}$≈4.3Å. [1]. Photopolymerized film for 2 min. (Fig. 3C) results in one broad reflection that is probably the result of damage to the film from UV over exposure.

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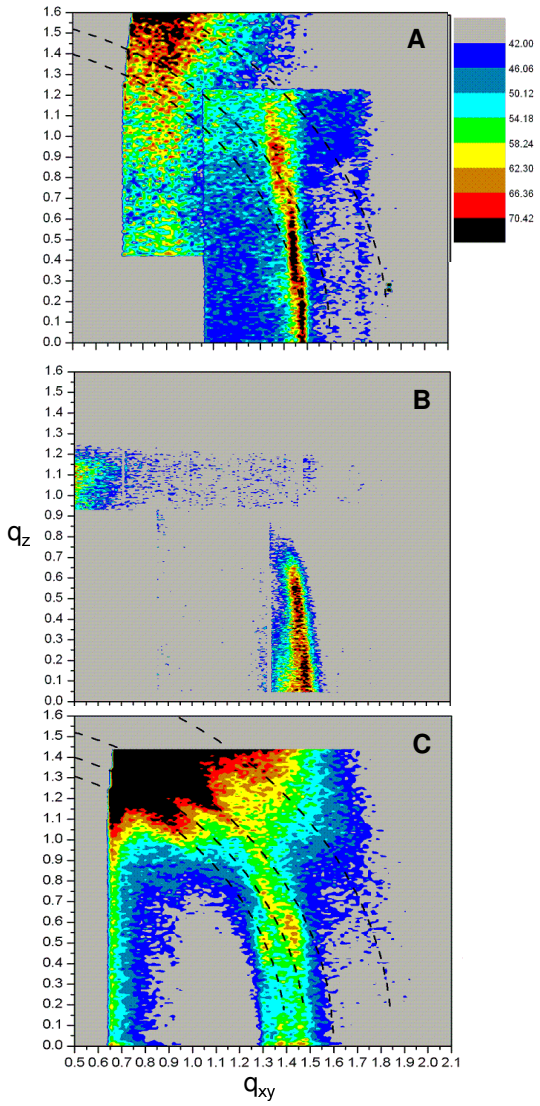

Figure 4. 2-D reciprocal space maps (q_z vs. q_{xy}) obtained from mixed films of PDC/PDOH: (A) PDC65% on buffer Trizma pH=7.5 after compression on Langmuir trough to 20±1mN/m (see report SC-1426). Arcs are q_{tot}=1.85Å^{-1} , 1.60Å^{-1} and 485Å^{-1} which corresponds to d=3.40Å, 3.93Å and 4.23Å respectively, (B) PDC75% spread on buffer drop after drop in certain time spacing during π measurement (final π=20±1mN/m) to give "solid" phase without compression, (C) PDC75% spread on buffer drop-after-drop to give "liquid-expanded" phase without compression. Arcs are q_{tot}=1.85Å^{-1}, 1.60Å^{-1}, 1.485Å^{-1} and 1.40Å^{-1} which correspond to d=3.40Å, 3.93Å, 4.23Å and 4.49Å, respectively.

Figure 4 represents PDC/PDOH films in different stages of compression on Langmuir trough.
Figure 4A shows PDC/PDOH film compression to 20±1mN/m. Figure 4B- PDC/PDOH film spread drop after drop in certain time intervals during π measurement (final pressure π=20±1mN/m). Both figures (Figure 4A and B) represent nearly incompressible "2D solid" phase, where regular crystalline order of the molecules dominates the structure [2].
PDC/PDOH film compressed on Langmuir trough (Figure 4A) performs three sharp and prominent peaks that indicate molecules axis are perpendicular to the water surface. Two lower peaks can be assigned to the alkyl chains of the head-groups layer in an orthorhombic (distorted hexagonal) lattice, whose spacing is typically d=2π/q_{xy}≈4.3Å. [1]. Third reflections with q_{tot}=1.60Å^{-1} at high q_z originate from –CH_3 terminated alkyl chains [SI-1016 report & publication in preparation]. The diffused arced reflection around q_{tot} = 1.85Å^{-1} (d=3.4Å) was assigned to base π-stacking according to the expected spacing.
PDC/PDOH film created by drop spreading till final π=20±1mN/m (Figure 4B) performs two peaks that identical to two lower peaks of compressed PDC/PDOH film (Figure 4A). This experiment

confirms that it is possible to create “2D solid" phase PDC/PDOH film by drop spreading without compression on Langmuir trough.
PDC/PDOH film spread on buffer drop after drop without compression to give "liquid" phase represented by Figure 4C. This stage is characterized by weak intermolecular interactions, mainly between the hydrophobic tails, and short-range order in the arrangement of the molecules [2] that manifested by arced reflections. The position of two lower peaks matches to the position of these two peaks of “2D solid" phase PDC/PDOH film but them are weaker and duller. Arced reflections with q_{tot}=1.60Å^{-1} at high q_z that originate from –CH_3 terminated alkyl chains, match to the third reflection of compressed Langmuir PDC/PDOH film (Figure 4A).

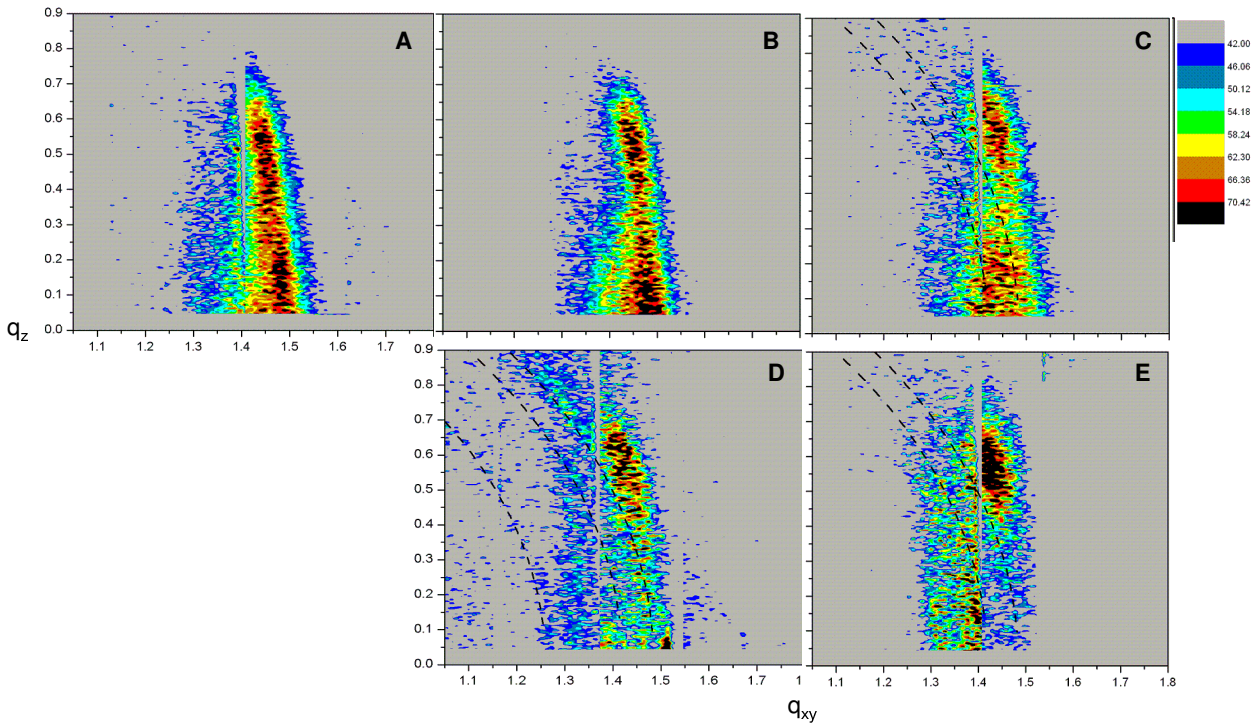

Figure 5. 2-D reciprocal space maps obtained from: (A) PDC75% on buffer Trizma pH=7.5, (B) PDC75% on buffer after incubation with 8-GT DNA oligomers, (C) PDC75% on buffer after incubation with 8-G DNA oligomers. Arcs are q_{tot}=1.485Å^{-1} and q_{tot}=1.42Å^{-1} which corresponds to d=4.23Å and d=4.42Å respectively, (D) PDC75% on buffer after incubation with 16-G DNA oligomers. Arcs are q_{tot}=1.485Å^{-1}, q_{tot}=1.42Å^{-1} and q_{tot}=1.26Å^{-1} which corresponds to d=4.23Å, d=4.42Å and d=4.99Å, respectively. (E) PDC75% on buffer after incubation with 4-GT DNA oligomers. Arcs are q_{tot}=1.485Å^{-1}and q_{tot}=1.42Å^{-1} which corresponds to d=4.23Å and d=4.42Å respectively.

In presence of complementary 8-G DNA (Figure 5C), 16-G DNA (Figure 5D) and 4-GT DNA (Figure 5E) oligomers in the subphase, defined peaks (typical Bragg-rods like in Figure 5A) turn into arced reflections. This is in agreement with our expected twisted PDC/DNA assembly.
Furthermore, the lowest peak at q_{xy}=1.47Å^{-1} (Figure 5A) became mach weaker or disappeared. The second peak at q_{xy}=1.45Å^{-1}, q_z=0.55Å^{-1} (Figure 5A) moves to q_{xy}=1.43Å^{-1}, q_z=0.60Å^{-1} (Figure 5C-E). In other words, the second peak moves from angle of 47^0 to angle of 50^0 as a result of incubation with complementary DNA oligomers. These imply that the specific interactions between the Cytosine moiety on the PDC74% film interface and the Guanosine in Trizma buffered solution take place.

In presence of complementary 8-G DNA (Figure 5B) oligomers in the subphase, defined peaks remain without any change probably because hybridization unsuitability between PDC/PDOH film and these oligonucleotides.

According to our previous GIXD studies, we determine arced reflections in presence of complementary 16-G DNA (Figure 6B), 8-GT DNA (Figure 6C) and 8-G DNA (Figure 6D) oligomers in the subphase in comparison to defined peaks of the same film without presence of these oligonucleotides (Figure6A).

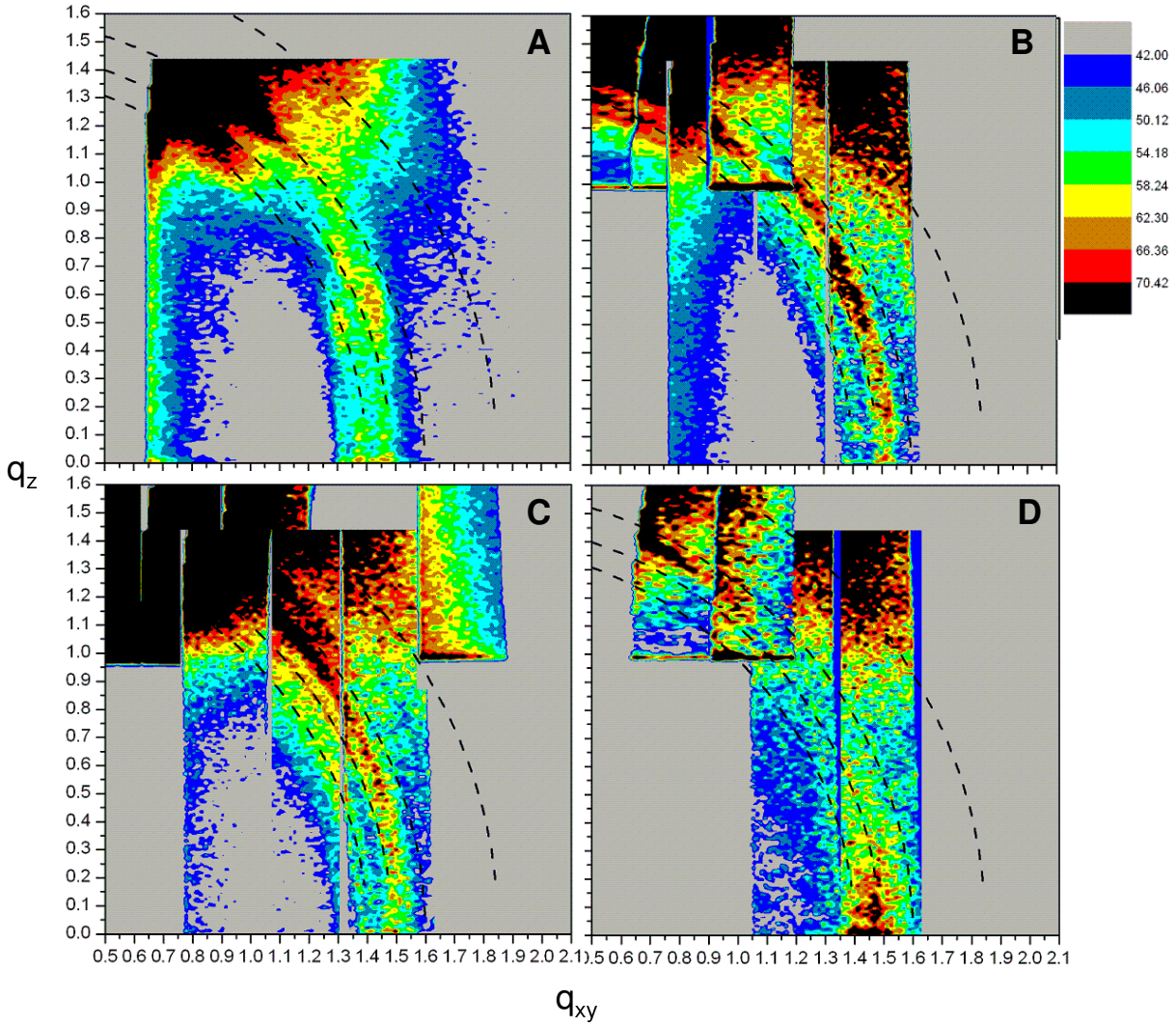

Figure 6. 2-D reciprocal space maps obtained from: (A) PDC75% spread on buffer drop-after-drop to give "liquid" phase without compression. (B) PDC75% on buffer after incubation with 16-G DNA oligomers, (C) PDC75% on buffer after incubation with 8-GT DNA oligomers, (D) PDC75% on buffer after incubation with 8-G DNA oligomers. Arcs are q_{tot}=1.85Å^{-1}, 1.60Å^{-1}, 1.485Å^{-1} and 1.40Å^{-1} which correspond to d=3.40Å, 3.93Å, 4.23Å and 4.49Å, respectively.

These 2-D maps obtained from PDC75% "liquid" phase without compression. The shape and intensity of the arcs indicate that PDC75% "liquid" phase film (Figure4C) creates more specific binding than PDC75% "solid" phase film (Figure4A,B). Weak interactions without long range order between molecules in "liquid" phase permit more freedom to move and interact with complementary mono- and oligonucleotides.

GIXD q-space reciprocal map obtained from PDC/PDOH monolayer films of 3:1 mixture ratio (PDC 75%) are presented in Figure 7A in order to compare it to PDC 75% monolayer films on buffer Trizma pH=7.5 containing complementary and not complementary DNA monomers (Figure 6B-D).

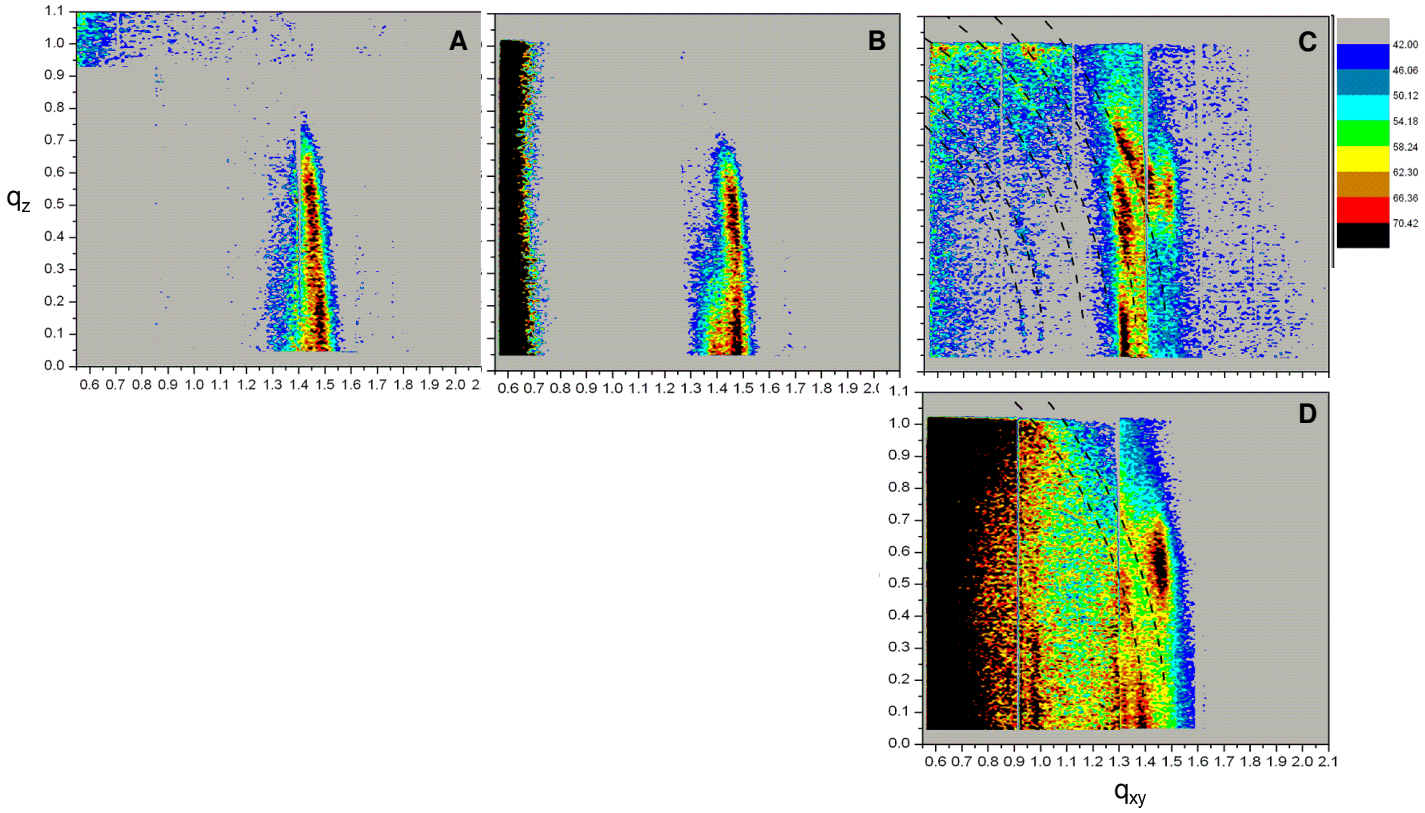

Figure 7. 2-D reciprocal space maps obtained from: (A) PDC75% on buffer Trizma pH=7.5, (B) PDC75% on buffer with Guanine mononucleobase. (C) PDC75% on buffer with Guanosine mononucleosides. Arcs are q_{tot}=1.485Å^{-1}, q_{tot}=1.37Å^{-1}, q_{tot}=1.27Å^{-1}, q_{tot}=1.17Å^{-1}, q_{tot}=1.015Å^{-1} and q_{tot}=0.94Å^{-1} which correspond to d=4.23Å, d=4.59Å, d=4.95Å, d=5.37Å, d=6.19Å, d=6.19Å and d=6.68Å, respectively. (D) PDC75% on buffer with Adenosine mononucleosides. Arcs are q_{tot}=1.485Å^{-1}and q_{tot}=1.4Å^{-1} which correspond to d=4.23Å and d=4.49Å, respectively.

The reflections of PDC 75% monolayer film in presence of complementary Guanosine mononucleosides in the subphase (Figure 7C) turn into arced reflections. This is in agreement with our expected twisted PDC/DNA assembly and in agreement with our previous GIXD study (Figure 8).
Furthermore, the lowest peak at q_{xy}=1.47Å^{-1} (Figure 7A) disappeared. The second peak at q_{xy}=1.45Å^{-1}, q_z=0.55Å^{-1} (Figure 7A) moves to q_{xy}=1.48Å^{-1} (Figure 5C). At q_{xy}=1.40Å^{-1}, q_z=0.55Å^{-1} appear new peak that was not existent in PDC 75% monolayer film. At q_{xy}=1.32Å^{-1} very weak Brag rod in PDC 75% film turn to four peaks: q_z=0.10Å^{-1}, q_z=0.40Å^{-1}, q_z=0.50Å^{-1} and q_z=0.65Å^{-1}.
This implies that the specific interactions between the Cytosine moiety on the PDC75% film interface and the Guanosine in Trizma buffered solution take place.
The reflections of PDC 75% monolayer film in presence of Guanine mononucleotides in the subphase (Figure 7B) remain identical to that of PDC 75% monolayer film (Figure 7A) probably because hybridization unsuitability between PDC/PDOH film and these mononucleotides.
The reflections of PDC 75% monolayer film in presence of not complementary Adenosine mononucleosides in the subphase (Figure 7D) turn into arced reflections but amount and intensity of arcs are smaller. PDC 75% with complementary Guanosine mononucleosides perform a lot of arcs in small q_{xy} values.
Furthermore, like in presence of Guanosine mononucleosides, the lowest peak at q_{xy}=1.47Å^{-1} (Figure 7A) disappeared. The second peak at q_{xy}=1.45Å^{-1}, q_z=0.55Å^{-1} (Figure 7A) remain identical to that of PDC 75% monolayer film. At q_{xy}=1.40Å^{-1}, q_z=0.05Å^{-1} appear new peak that was not existent in PDC 75% film and appears in presence of Guanosine mononucleosides at high q_z position. At q_{xy}=1.32Å^{-1}

appear two weak peaks at q_z=0.10Å^{-1}and q_z=0.45Å^{-1}. This data maybe indicate that the recognition between PDC 75% films and Guanosine mononucleosides is more specific then the possible recognition between PDC 75% films and Adenosine mononucleosides.

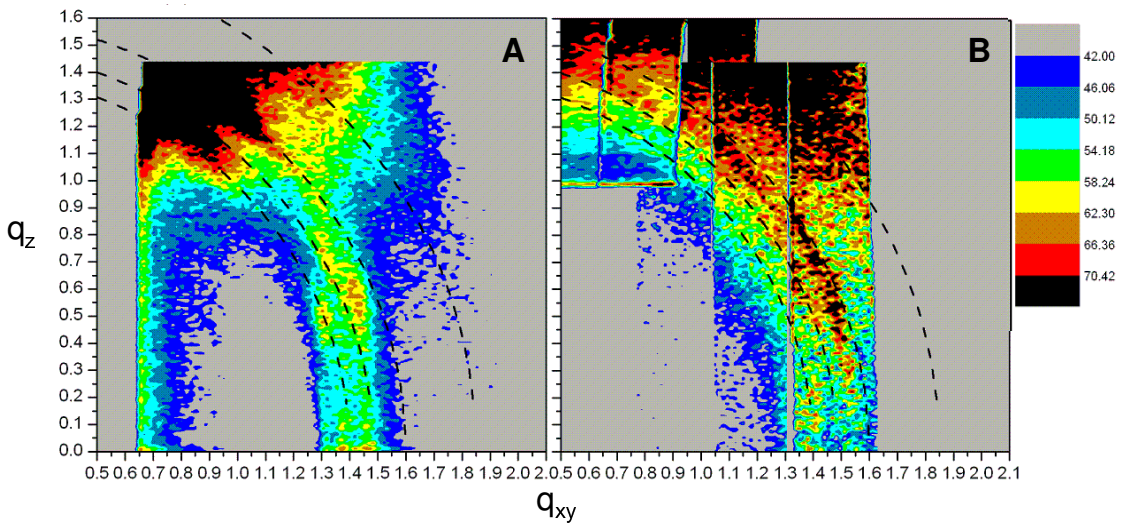

Figure 8. 2-D reciprocal space maps obtained from: (A) PDC75% spread on buffer drop after drop to give condensed monolayer coverage without compression. (B) PDC75% on buffer with Guanosine mononucleosides. Arcs are qtot=1.85Å-1, 1.60Å-1, 1.485Å-1 and 1.40Å-1 which corresponds to d=3.40Å, 3.93Å, 4.23Å and 4.49Å respectively.

In order to better understand the effect of complementary binding, we hereby propose to carry out further experiments. Currently we further request beam time on Troika beamline 10B in order to continue this exciting project in following directions:

1) To perform set of experiments by compressing PDC75% to give films in different phases. Each phase will be introduced to complementary mono- and oligonucleotides and specific binding will be examined.

(2) As a continuation to our successful progress in this theme we will integrate another nucleolipid film (pentacosadiynoil-tyminile derivative-PDT) and will try to reveal his monolayer structure at the air-water interface and its structural response upon specific recognition and base-pair formation with complementary ssDNA mono- and oligonucleotides. Yingqiu Liang fined out that formation of base pairs at the air/water interface occur between Langmuir–Blodgett (LB) films of octadecanoyl ester of 1-(2-carboxyethyl) thymine and its complementary adenosine in subphase.[2] From the FT-SERS spectra results he deduced that the orientation of nucleobase in the headgroup is different before and after the recognition effect occurred.

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