



<u>Experiment title:</u> Structural investigation of the conformational transitions of nucleotide derivatized polydiacetylene monolayer with its complementary mono- and oligonucleotide.	<u>Experiment number:</u> SC-1426
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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.

The purpose of this project was to reveal the mixed lipid monolayer structure of pentacosadiynoil-cytosinyl derivative (PDC) and pentacosadiynol (PDOH) at the air-water interface and its structural response upon specific recognition and base-pair formation. We used mixed lipid solution of PDC and PDOH in molar ratio of 65% and 80%, as precursors for the Langmuir monolayers. The solution is spread on Trizma buffer (pH 7.5) in the presence or absence of the complementary (Guanosine) base, compressed to 25 mN/m and UV polymerized ($\lambda=254\text{nm}$). The polymerized monolayer consists of linear polyconjugated molecules made of rigid chains that are organized in parallel in 2-D crystalline domains. In additional experiments where complementary oligonucleotides of Guanosine (dG_{16}) were injected into the subphase subsequent to the monolayer compression on buffer, DNA-like base-pair π -stacking is anticipated.

Grazing incidence diffraction data were collected in real-time and showed the following patterns:

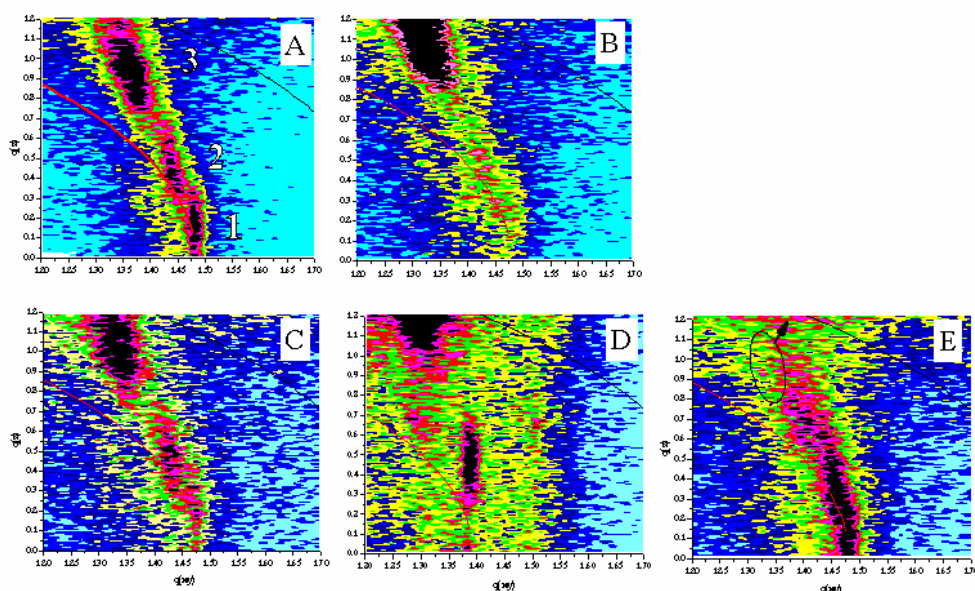


Figure 1: 2-D GIXD maps obtained from mixed films of PDC/PDOH. (A) 65% PDC on buffer, (B, D) 65% PDC on G before and after annealing in 40°C , (C) 80% PDC on buffer, (E) 65% PDC on buffer after incubation in presence of 16G-ssDNA. The arcs are $q_{101}=1.48\text{\AA}^{-1}$ (red) and $q_{101}=1.844\text{\AA}^{-1}$ (black) which corresponds to $d=4.24\text{\AA}$ and $d=3.4\text{\AA}$, respectively. The oval shape in E is where the prominent reflections in A-C were located. This reflection disappeared and instead increase in reflected intensity is observed at high q_z , with approximate spacing of 3.4\AA , in keeping with the known DNA stacking periodicity (arrow).

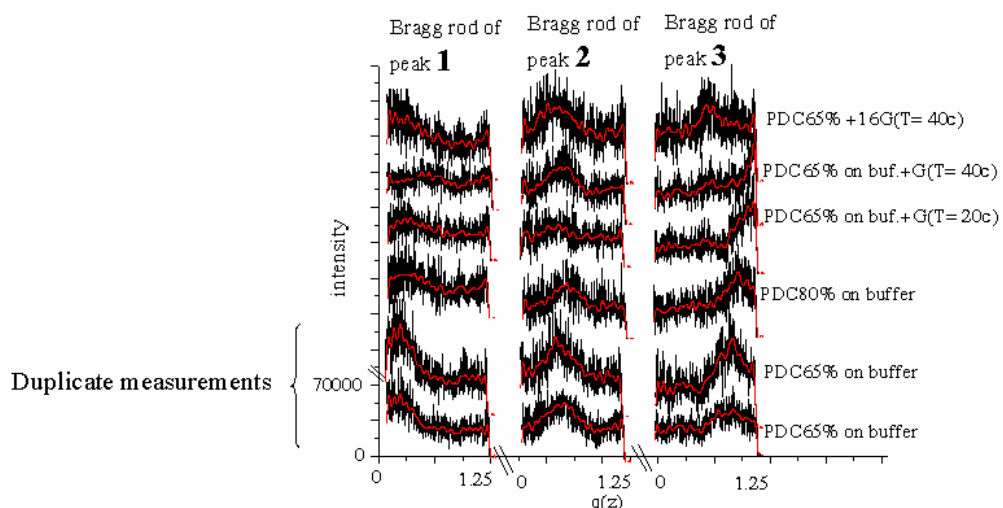


Figure 2: The vertical structure of the reflections is shown: Bragg rods are represented as intensity vs. q_z for a given q_{xy} value of the three observed diffraction peaks, as indicated in Fig. 1A.

	#	q_{xy} [\AA^{-1}]	q_z^0 [\AA^{-1}]	q_{tot} [\AA^{-1}]	$Dq_{xy,FWHM}$ [\AA^{-1}]	l_{xy} [\AA]	d_{xy} [\AA]	d_{tot} [\AA]	d_{xy}^* [\AA^{-1}]	d_{tot}^* [\AA^{-1}]	tilt angle ϕ^0
PDC 65% on buffer (T=20c)	1	1.481	0.159	1.489	0.044	126	4.244	4.220	0.236	0.237	83.88
	2	1.442	0.437	1.507	0.047	118	4.357	4.169	0.229	0.240	73.16
	3	1.362	0.949	1.660	0.093	59	4.612	3.785	0.217	0.264	55.15
PDC 80% on buffer (T=20c)	1	1.478	0.184	1.49	0.033	168	4.250	4.217	0.235	0.237	82.90
	2	1.431	0.472	1.51	0.031	178	4.392	4.161	0.228	0.240	71.75
	3	1.337	1.024	1.68	0.053	104	4.701	3.740	0.213	0.267	52.54
PDC 65%+16G (T=40c)	1	1.479	0.130	1.48	0.028	197	4.247	4.245	0.235	0.236	84.97
	2	1.456	0.310	1.49	0.038	146	4.315	4.217	0.232	0.237	77.97
	3	1.410	0.667	1.56	0.055	101	4.456	4.028	0.224	0.248	64.67
PDC 65% +Guanos. (T=20c)	1	1.467	0.263	1.490	0.045	123	4.284	4.217	0.233	0.239	79.84
	2	1.423	0.525	1.517	0.051	111	4.416	4.142	0.229	0.226	69.74
	3	1.324	1.069	1.701	0.028	197	4.746	3.694	0.207	0.211	51.09
PDC 65% +Guanos. (T=40c)	1	1.384	0.029	1.38	0.049	113	4.542	4.55	0.220	0.220	88.79
	2	1.388	0.437	1.45	0.042	132	4.527	4.33	0.221	0.231	72.54
	3	1.288	1.192	1.75	0.036	154	4.880	3.59	0.205	0.279	47.21

Table 1: Summary of GIXD diffraction data obtained from the main peaks of PDC/PDOH on deferent subphases.

Several observed reflections of PDC 65% on buffer reveal similar reflection positions with three peaks: $q_{xy}=1.48 \text{ \AA}^{-1}$ that is close to $q_z=0$, $q_{xy}=1.44 \text{ \AA}^{-1}$ and $q_{xy}=1.37 \text{ \AA}^{-1}$ which their maximum is at high q_z values. The reflection data indicate that the PDC 65% structure on buffer subphase is close to that of PDC 80% and differ from the PDC 65% structure on Guanosine solution. Formation of the PDC 65% film on Guanosine containing subphase results in disappearance of the low q_{xy} reflection and increase in the relative reflected intensity of the high q_z peak that shifted slightly to higher q_z and q_{tot} values, while retaining the same in-plane separation. Heating of PDC 65% on Guanosine solution to 40^oc affect the PDC structure (the peaks seem sharper) and significant shifts

in several reflection positions are observed. Distinct Bragg rod at $q_{xy}=1.38 \text{ \AA}^{-1}$ indicating that crystalline order different from what is observed for films in the absence of G or without annealing is formed. The structure of PDC 65% after long incubation in the presence of complementary 16G-ssDNA in the subphase differ from PDC 65% structure on buffer subphase. The prominent reflection at $q_{xy}=1.36 \text{ \AA}^{-1} / q_z=0.95 \text{ \AA}^{-1}$ ($q_{tot}=1.66 \text{ \AA}^{-1}$ $d=4.61 \text{ \AA}$) (ellipse, fig. 1E) has broadened and shifted (arrow) to $q_{xy}=1.41 \text{ \AA}^{-1} / q_z=1.20 \text{ \AA}^{-1}$ ($q_{tot}=1.84 \text{ \AA}^{-1}$ $d=3.4 \text{ \AA}$). Although the center of mass of this reflection is outside the frame, the general trend is clear. The structural differences in observed reflections of PDC film before and after annealing with complementary mono- and oligonucleotides can be explained as structural response of the PDC film upon specific recognition.

The GIXD results were interpreted into a crystallographic model describing the PDC/PDOH arrangement on the air-water interphase in a 2-D oblique structure:

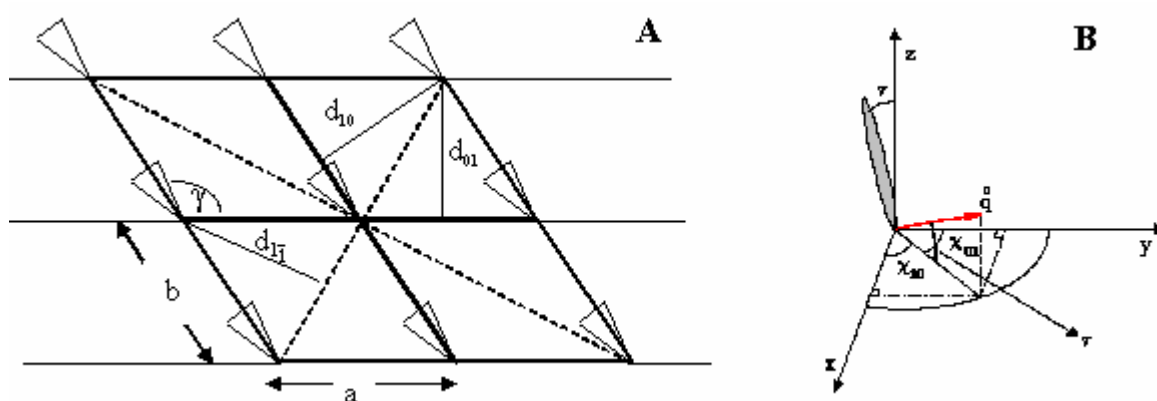


Figure3: (A) Schematic representation of PDC/PDOH unit cell deduced from the GIXD data. (B) Determination of molecular tilt (τ) in the orthogonal system.

	$d_{01}^* = a^* [\text{\AA}^{-1}]$	$d_{10}^* = b^* [\text{\AA}^{-1}]$	γ ($^\circ$)	a [\AA]	b [\AA]	τ ($^\circ$)
PDC 65% on buffer (T=20c)	0.217	0.230	116.35	4.86	5.15	36.71
PDC 80% on buffer (T=20c)	0.213	0.228	115.63	4.87	5.21	39.22
PDC 65%+Guanosine (T=20c)	0.211	0.226	115.52	4.89	5.26	40.11
PDC 65%+Guanosine (T=40c)	0.205	0.220	117.46	5.12	5.50	43.20
PDC 65%+16G (T=40c)	0.224	0.232	118.11	4.89	5.05	26.58

Table 2: Summarized data for mixed PDC/PDOH films at different conditions. The reciprocal lattice parameters (a^* , b^*), the 2-D cell parameters (a , b), the angle between a and b (γ) and the molecular tilt angle (τ) are shown. In particular, note the significantly different cell parameters of the film that underwent interaction with the complementary 16G oligomer.

Based on the GIXD results obtained with this work, the scheme of the 2-D unit cell of the mixed PDC/PDOH film structure on different subphases is shown in Figure 3. This scheme shows the oblique primitive cell. Basic conclusions that can be made:

- (1) PDC mixed films (2:1 PDC/PDOH) are crystalline and diffract grazing incidence x-rays (Figure1).

- (2) PDC/PDOH 80% structure on buffer subphase is close to that of PDC/PDOH 65% but the molecular tilt increase from 36.7° to 39.2° in PDC/PDOH 80% structure. The crystallinity of other PDC/PDOH 80% is lower, as expected, due to high strain in the layer.
- (3) Specific binding of the complementary base (Guanosine) is established and results in a different diffraction pattern (for PDC 65% $a=4.86\text{\AA}$, $b=5.15\text{\AA}$, $\tau=36.7^\circ$ and for PDC 65% on G. solution $a=4.89\text{\AA}$, $b=5.26\text{\AA}$, $\tau=40.1^\circ$).
- (4) Elevated temperature (40°C) during the association of Guanosine with the PDC films results in a narrower reflection and increase in 2-D cell parameters (a , b , γ) and the molecular tilt angle (τ) due to crystal ripening.
- (5) Binding of complementary 16G-ssDNA oligomer resulted in a major shift in one reflection from $q_{tot}=1.66\text{\AA}^{-1}$ to $q_{tot}=1.84\text{\AA}^{-1}$, which corresponds to $d=3.4\text{\AA}$, which is the expected spacing for π -stacked bases. The shifted reflection is broad and centered at high q_z , suggesting a possible agreement with the expected twist of the PDC/DNA assembly.

In order to better understand the complex structure of PDC mixed films and 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) obtain better quality crystallographic data sets of the mixed film of PDC/PDOH. (2) Monitoring the formation of base-pairs at the monolayer solution interface. (3) Base-pairs π -stacking is expected in experiments where oligonucleotides are used. (4) Development of twisted structure is anticipated in case when π -stacked base-pairs deform the original conformation.

References:

Chen, J. and Berman, A. (2004) Formation of nucleotide base-pairs at the interface of polydiacetylene cytosine derivatized monolayers. *Nanotechnology*, **15**, S303.