Experiment title: Sequence and topological imprinting of nucleotide derivatized polydiacetylene (PDA) monolayer using complementary and partially complementary oligonucleotides.	Experiment number: SC-2341
Date of experiment: from 07/11/2007 to 12/11/2007	Date of report: 12/10/2008
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Report:

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 with complementary and partially complementary oligonucleotides.

It is better to observe lipid molecules on water - wet state, because when it deposited on solid substrates such as glass and mica the molecules are in a dried state. Since X-Ray specular Reflectivity (XRR) and Grazing Incidence X-ray Diffraction (GIXD) can be applied to the water surface, this technique can give us an answer to this demand.

The XRR and GIXD studies carried out directly at the air/water interface at the surface diffraction beamline ID10B. The incoming beam was monochromatized using a diamond (111) double crystal monochromator to the energy of 8.00 keV.

For the GIXD measurements the angle of incidence was chosen to be 0.11^0 and for XRR it was changed from 0^0 to 4^0. The 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 44.5°. In order to collect the reflected beams at higher than 44.5°, the detector was moved to a higher position, allowing an overlap between the two positions. The height calibrations of the trough and the channel calibrations of PSD detector were done always using attenuation to the primary beam.

Langmuir monolayers of 3:1 PDC/PDOH molar mixture[1] formed on Trizma buffer (pH 7.5) by compression on Langmuir trough to 10±2, 18±2 and 24±2mN/m. The monolayer was allowed to settle for 15 minutes before UV polymerization with pen-ray mini UV lamp (λ=254nm) for 20 seconds and GIXD/XRR measurements.

Likewise short, complementary and not complementary oligonucleotides ($G_{12}T_5$ and $C_{12}T_5$) were introduced to the subphase, under the polymerized film. The system was incubated for 30 minutes in room temperature.

All the measurements were conducted under helium atmosphere.

XRR and GIXD results

PDC75% film before and after UV photopolymerization:

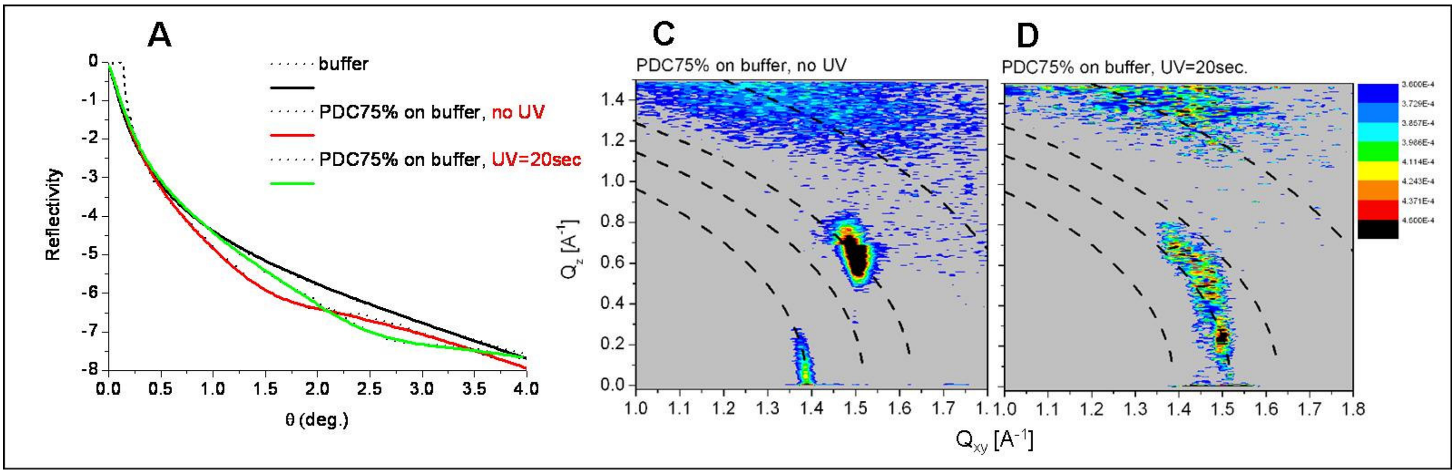

Figure 1. XRR profiles (A) and 2-D reciprocal space maps (C, D) obtained from PDC75% nucleolipid monolayer films created on buffer at 24±2mN/m before and after UV photopolymerization. Arcs are q_{tot}=1.92Å^{-1}, 1.63Å^{-1}, 1.52Å^{-1} and 1.39Å^{-1} which corresponds to d=3.27Å, 3.85Å, 4.13Å and 4.52Å, respectively.

XRR profiles of PDC75% nucleolipid monolayer films created on buffer (**Fig. 1, A red and green**) show some roughness that doesn't exist in the buffer XRR profile (**Fig. 1, A black**). That is to say XRR technique allows us to distinguish the PDC75% monolayer film.

The slope of the reflectivity profile is a "measure" of the surface roughness. Photopolymerized PDC75% film (**Fig. 1,A green**) has the slope of the reflectivity profile that decreases faster then that of not photopolymerized film (**Fig. 1,A red**) at high angles. That means that some roughness exists in the surface structure of the photopolymerized film.

When there is a thin film on the surface, we can observe the Kiessig fringe, the reflectivity maximum as a function of reflection angle. The clearness and sharpness of the fringe depend on the surface and interface roughness of the air/upper surface of the film and the lower surface of the film/substrate, in addition to the contrast (an electron density difference) between film and substrate. The fringe becomes unclear with increasing roughness and the slope of the profile itself increases at the same time. The fringe profile is also affected by the electron density differences. The fringe becomes undistinguishable when electron density difference between air, film and substrate becomes very small**[2]**.

According to 2-D reciprocal space maps not photopolymerizated PDC75% film (**Fig. 1,B**) reveal reflection positions at: q_{xy}=1.39Å^{-1}(q_z=0.0Å^{-1}) on q_{tot}=1.39Å^{-1} arc and q_{xy}=1.50Å^{-1} (q_z=0.66Å^{-1}) on q_{tot}=1.63Å^{-1} arc. Same reflections appear when PDC75% was compressed on buffer to 10±2 and to 20mN/m without UV photopolymerization (data not shown).

Photopolymerizated PDC75% film (**Fig. 1,C**) exhibit three reflections at q_{xy}=1.50Å^{-1}(q_z=0.25Å^{-1}), q_{xy}=1.47Å^{-1}(q_z=0.50Å^{-1}) and q_{xy}=1.40Å^{-1} (q_z=0.66Å^{-1}) - all on q_{tot}=1.52Å^{-1} arc.

PDC75% film compressed to 10±2, 18±2 and 24±2mN/m.:

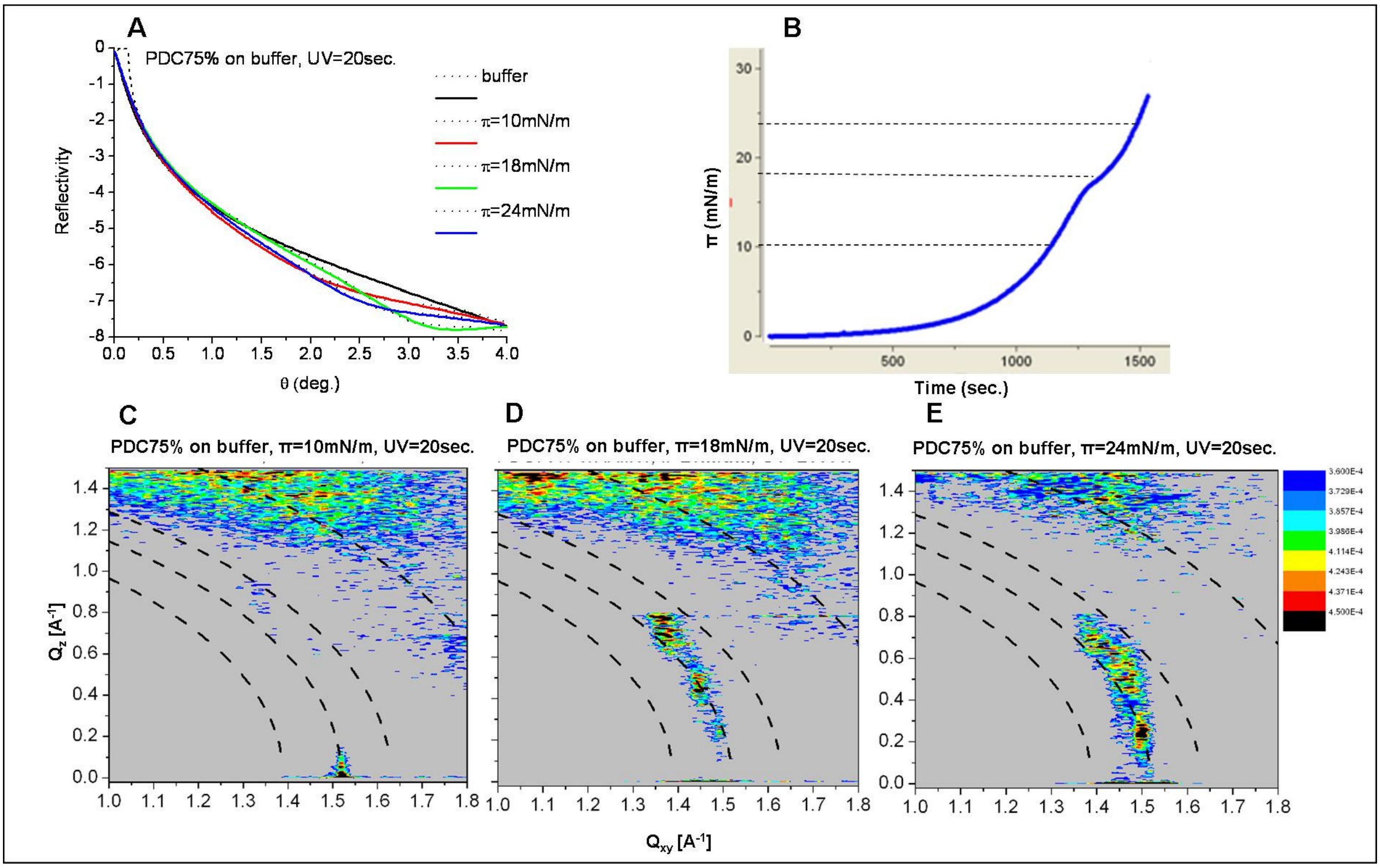

Figure 2. XRR profiles (A), typical π-A isotherm (B) and 2-D reciprocal space maps (C-E) obtained from PDC75% nucleolipid monolayer films created on buffer at 10±2mN/m (C), 18±2mN/m (D) and 24±2mN/m (E) and UV photopolymerized. Arcs are q_{tot}=1.92Å^{-1}, 1.63Å^{-1}, 1.52Å^{-1} and 1.39Å^{-1} which corresponds to d=3.27Å, 3.85Å, 4.13Å and 4.52Å respectively.

Figure 2, **A** show XRR profiles of PDC75% films created on buffer at 10±2mN/m (**red line**), 18±2mN/m (**green line**) and 24±2mN/m (**blue line**) in comparison to XRR profile of buffer (**black line**). The comparison rates are according to typical π-A isotherm shown in **Figure 1**, **B**.

XRR profiles of PDC75% films (**Fig. 2, A red, green and blue**) show some roughness that doesn't exist in the buffer XRR profile (**Fig. 2, A black**).

Significant difference could be observed around the reflection angle of 3 degrees: the slope of the reflectivity profile from PDC75% film at 18±2mN/m (**Fig. 2, A green line**) and 24±2mN/m (**Fig. 2, A blue line**) decreases faster than that of PDC75% film at 10±2mN/m (**Fig. 2, A red line**). That means the PDC75% film at 10±2mN/m (**Fig. 2, A red line**) is thinner and a less uniform and/or dense layer.

Figure 2, C-E show observed reflections of PDC75% on buffer in different stages of compression: 10±2, 18±2 and 24±2mN/m.

PDC75% compressed on buffer to 10±2mN/m (**Fig. 2,B**) reveal reflection positions at: q_{xy}=1.52Å^{-1}(q_z=0.0Å^{-1}) on q_{tot}=1.52Å^{-1} arc and q_{xy}=1.33Å^{-1} (q_z=0.84Å^{-1}) on q_{tot}=1.63Å^{-1} arc.

PDC75% compressed on buffer to 18±2mN/m (**Fig. 2,C**) reveal reflection positions at q_{xy}=1.50Å^{-1}(q_z=0.25Å^{-1}), q_{xy}=1.44Å^{-1}(q_z=0.44Å^{-1}) and q_{xy}=1.38Å^{-1} (q_z=0.70Å^{-1}) - all on q_{tot}=1.52Å^{-1} arc.

PDC75% compressed on buffer to 24±2mN/m (**Fig. 2,D**) reveal reflection positions at q_{xy}=1.50Å^{-1}(q_z=0.25Å^{-1}), q_{xy}=1.47Å^{-1}(q_z=0.50Å^{-1}) and q_{xy}=1.40Å^{-1} (q_z=0.66Å^{-1}) - all on q_{tot}=1.52Å^{-1} arc.

The reflections of PDC75% compressed to 18±2 and 24±2 mN/m (**Fig. 2,C and D**) have the same coordinates but not the same intensity. In the case of PDC75% compressed to 18±2 mN/m (**Fig. 2,C**) the most intense peak is the uppermost but in PDC75% compressed to 24±2 mN/m (**Fig. 2,D**) the most intense peak is the lowest one.

PDC75% film incubated with complementary and not complementary olygonucleotides:

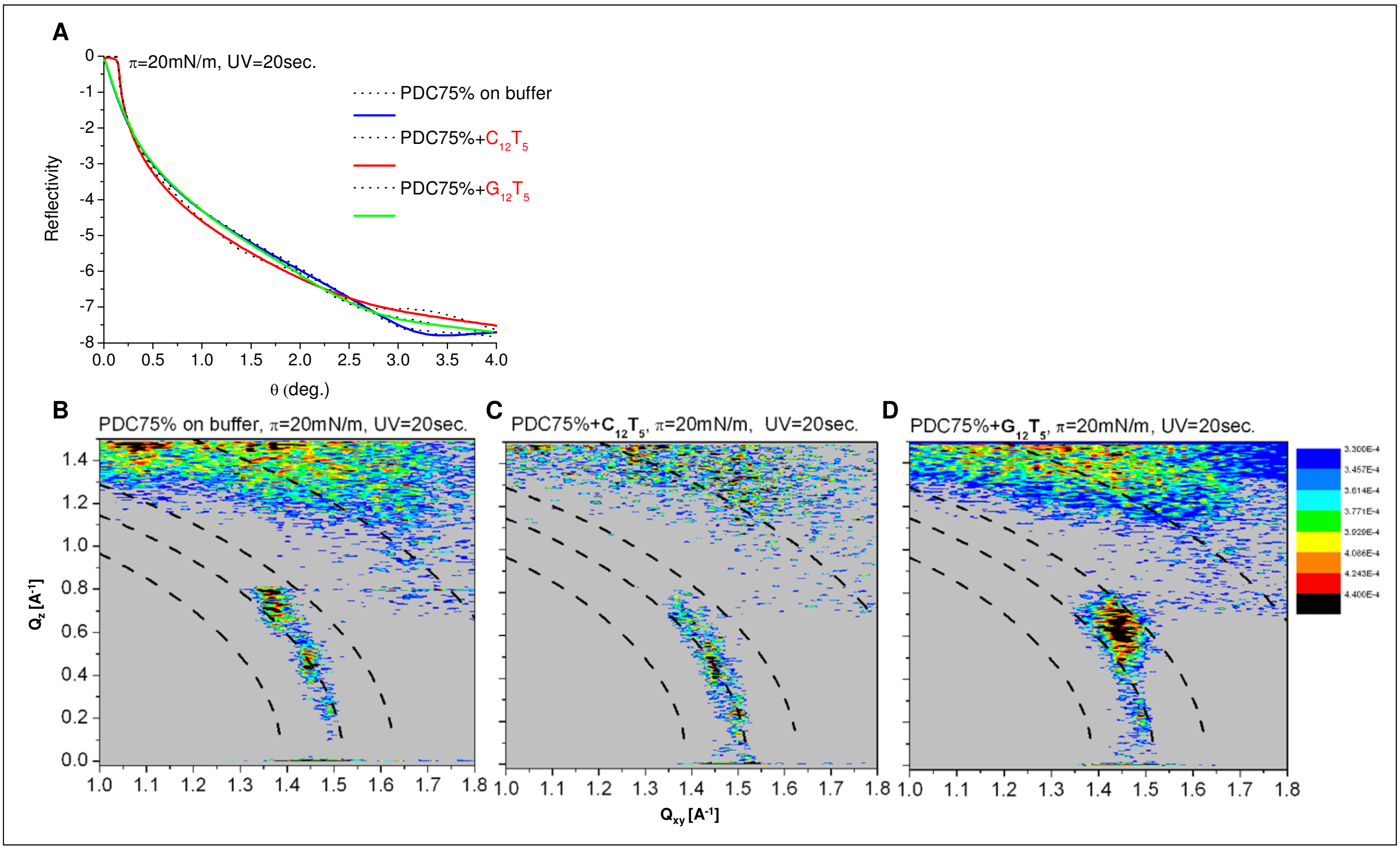

Figure 3. XRR profiles (A) and 2-D reciprocal space maps (B-D) obtained from PDC75% nucleolipid monolayer films created on buffer at 20±2mN/m and UV photopolymerized before (B) and after incubation with not complementary $C_{12}T_5$ (C) and complementary $G_{12}T_5$ (D) oligonucleotides. Arcs are q_{tot}=1.92Å^{-1}, 1.63Å^{-1}, 1.52Å^{-1} and 1.39Å^{-1} which corresponds to d=3.27Å, 3.85Å, 4.13Å and 4.52Å respectively.

PDC75% films incubated with complementary $G_{12}T_5$ and not complementary $C_{12}T_5$ oligonucleotides show a similar reflectivity profiles that are higher than that of PDC75% film around the angle of 3.3 degree. Furthermore PDC75%-$G_{12}T_5$ film has significantly lower reflectivity at higher angles than that of PDC75%-$C_{12}T_5$.

Figure 3, B-D show observed reflections of PDC75% films created by compression on buffer to 20±2mN/m in comparison to the same film incubated with complementary $G_{12}T_5$ and not complementary $C_{12}T_5$ oligonucleotides.

PDC75% film on buffer (**Fig. 3,B**) reveal reflection positions at $q_{xy}=1.50Å^{-1}(q_z=0.25Å^{-1})$, $q_{xy}=1.44Å^{-1}(q_z=0.44Å^{-1})$ and $q_{xy}=1.38Å^{-1}$ $(q_z=0.70Å^{-1})$ - all on $q_{tot}=1.52Å^{-1}$ arc.

PDC75% film incubated with not complementary $C_{12}T_5$ oligonucleotides (**Fig. 3,**C) reveal reflection positions at $q_{xy}=1.50Å^{-1}(q_z=0.24Å^{-1})$, $q_{xy}=1.46Å^{-1}(q_z=0.45Å^{-1})$ and $q_{xy}=1.38Å^{-1}$ $(q_z=0.67Å^{-1})$ - all on $q_{tot}=1.52Å^{-1}$ arc.

PDC75% film incubated with complementary $G_{12}T_5$ oligonucleotides (**Fig. 3,D**) reveal reflection positions at $q_{xy}=1.50Å^{-1}(q_z=0.22Å^{-1})$ and $q_{xy}=1.45Å^{-1}$ $(q_z=0.62Å^{-1})$. The higher reflection is very intense and sharp; indicate more order and uprightness of molecules as a result of structural response of the PDC75% film upon specific recognition.

The reflections of PDC75% film (**Fig. 3,B**) and PDC75%-$C_{12}T_5$ film (**Fig. 3,C**) have the same coordinates but the intensity of high peaks is dominant in PDC75% film in comparison to the intensity of lower peaks that it is dominant in PDC75%-$C_{12}T_5$ film.

PDC75% film incubated with complementary and not complementary mononucleotides:

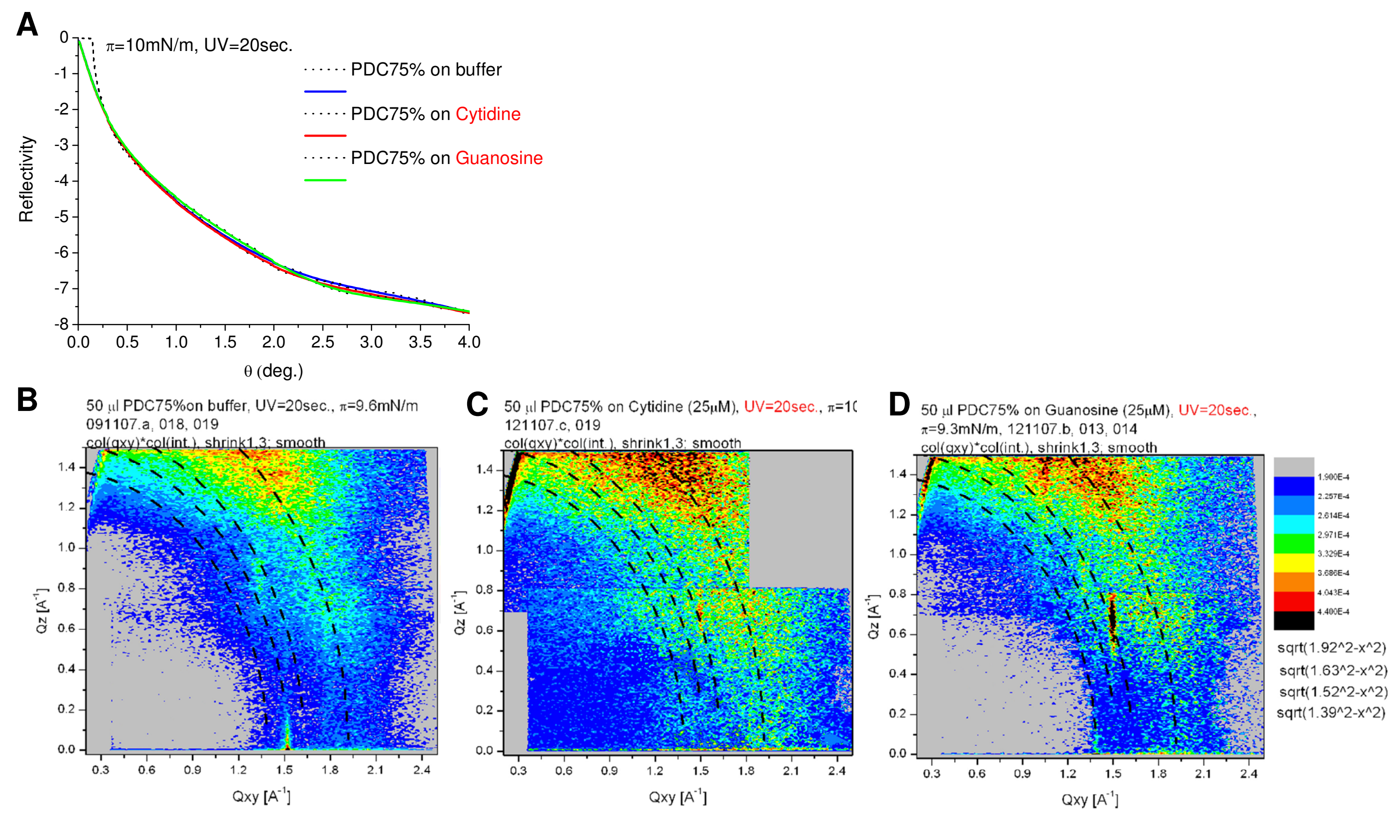

Figure 4. XRR profiles (A) and 2-D reciprocal space maps (B-D) obtained from PDC75% nucleolipid monolayer films created on buffer at 10±2mN/m and UV photopolymerized before (B) and after incubation with not complementary Cytidine (C) and complementary Guanosine (D) mononucleotides. Arcs are $q_{tot}=1.92Å^{-1}$, $1.63Å^{-1}$, $1.52Å^{-1}$ and $1.39Å^{-1}$ which corresponds to d=3.27Å, 3.85Å, 4.13Å and 4.52Å respectively.

PDC75% film incubated with complementary 16G oligonucleotides:

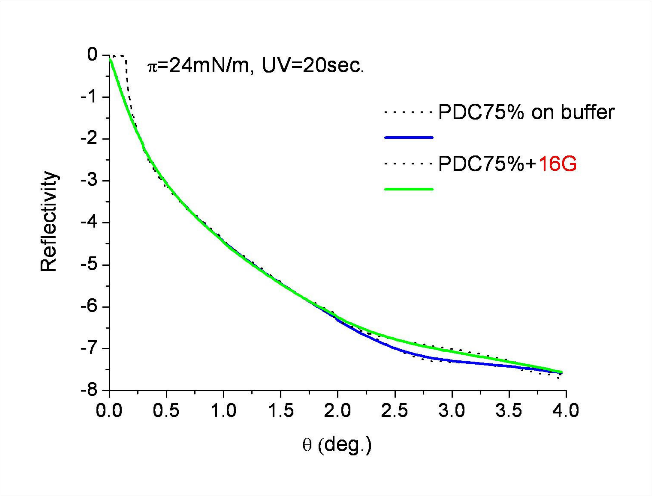

Figure 5. XRR profiles obtained from PDC75% nucleolipid monolayer films created on buffer at 24±2mN/m and UV photopolymerized before (blue line) and after incubation with complementary 16G oligonucleotides (green line).

PDC75%-16G films show higher reflectivity profile than that of PDC75% film at higher angles. That resembles the reflectivity profile behavior of PDC75%-$G_{12}T_5$ film (**Fig. 3A, green line**).

References:

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

[2] J. Daillant, A. Gibaud eds. "X-ray and Neutron Reflectivity: Principles and Applications", Springer, 1999; M. Tolan, "X-ray Scattering from Soft-Matter Thin Films", Springer, 1999; J. Als-Nielsen, D. McMorrow, Elements of Modern X-ray Physics", Wiley, 2000.