STRUCTURAL PARAMETERS OF MACROSCOPICALLY FLAT LIPID MULTILAYERS ON A SILICA SOL SUBSTRATES

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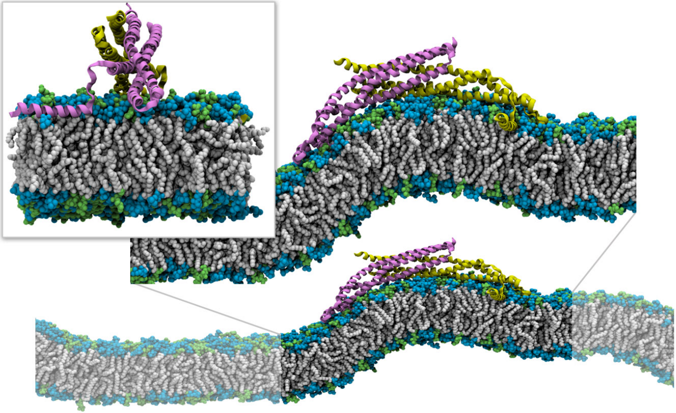
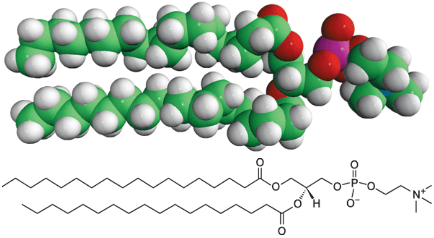
Cell membrane model — phospholipid bilayer

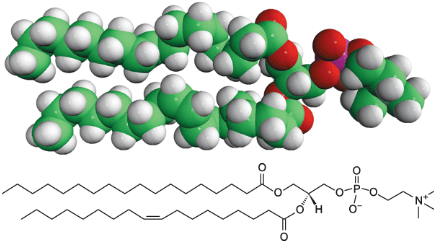


Model lipids

1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)



1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC)



Tabular properties:

Lipid	Formula	Mol. weight	Γ	T_c, °C	CAS Index
DSPC	$C_{44}H_{88}NO_8P$	790.145	438	55	816-94-4
SOPC	$C_{44}H_{86}NO_8P$	788.129	436	6	56421-10-4

D. M. Small, Physical Chemistry of Lipids, NY, Plenum Press, 1989.

How can one prepare a macroscopic lipid multilayer?

Langmuir-Blodgett technique

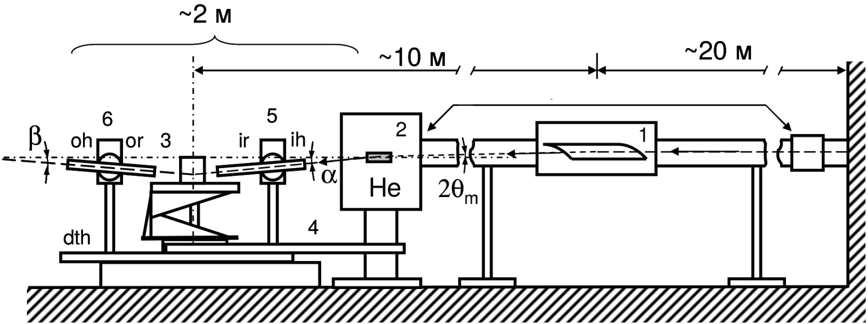
- **PRO**: widely used and relatively easy to implement;
- **PRO**: highly homogeneous lipid layers;
- **CON**: solid substrate does not correspond to actual cell body!
- **CON**: compression keeps lipid film homohenity up to the monolayer only.

"Free" film on liquid substrate

- Relatively low homogenity of the layers on clean water surface.
- Possibilities to use different water solutions as a substrates?

X19C beamline at NSLS, Brookhaven

$\Phi = 10^{14}$ photons/s, $E = 15000$ eV, $\Delta E/E \sim 10^{-3}$



1 – thoroidal X-ray mirror; 2 – single-crystal monochromator; 3 – vibro-damping platform and sample chamber; 4 – 2θ-rotation platform; 5 – two-slit collimator; 6 – β-rotation platform with detector slit.

Basic assumptions:

- Let $\delta(z) = \mathrm{Re}\,1 - \varepsilon(z)$ distribution contains m special points of n-th order, where function $\delta(z)$ and its $n-1$ derivatives $\delta'(z)\ldots\delta^{(n-1)}(z)$ are continuous but n-th derivative suffers a step-like variation.
- Dielectric permittivitiy of external medium equals to vacuum $(\delta(z < z_{min}) \equiv 0)$ while permittivity of substrate is constant $(\delta(z > z_{max}) \equiv \delta_+ \neq 0)$.
- All discontinuity points $z_1 < z_2 < \cdots < z_m$ are of the same order.
- Absorption of the X-rays in a matter is neglected: $\mathrm{Im}\,\varepsilon(z) \equiv 0$.

Reflectivity asymptotic in the frames of first-order Born approximation:

$$r\left(q\right) \cong -k^2 \left(\frac{i}{2q}\right)^{2+n} \sum_{j=1}^{m} \Delta^{(n)}\left(z_j\right) \exp\left(2iqz_j\right),$$

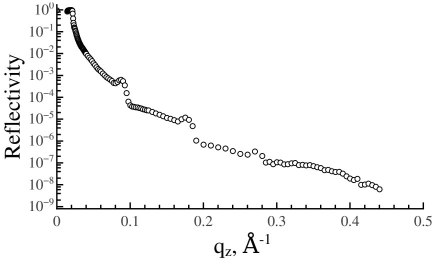
$$\Delta^{(n)}\left(z_j\right) \equiv \frac{d^n\delta}{dz^n}\left(z_J + 0\right) - \frac{d^n\delta}{dz^n}\left(z_J - 0\right),$$

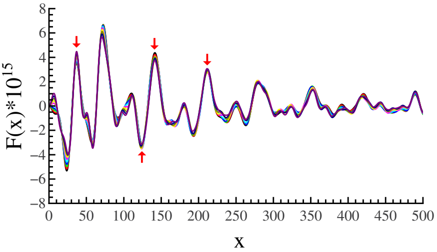
I. V. Kozhevnikov, Nucl. Instruments and Methods in Phys. Res. A (2003) 508:519

Regularized Fourier-fransform of the reflectivity curve

$$F(x) = \frac{2^{2n+4}}{k^4 (q_{max} - q_{min})} \int_{q_{min}}^{q_{max}} \left[q^{2n+4} R(q) - C \right] \cos(2qx)\, dq$$

$$C = \frac{1}{q_{max} - q_{min}} \int_{q_{min}}^{q_{max}} q^{2n+4} R(q)\, dq, \qquad q = k \sin\theta$$





Model-independent reconstruction of electron density profile

Regularized merit function for numerical optimization:

$$\mathrm{MF} = \frac{1}{N}\sum_j \left[\frac{R_{exp}\left(\theta_j\right) - R_{calc}\left(\theta_j\right)}{R_{exp}\left(\theta_j\right)}\right]^2 + Q_1 \sum_{\substack{i=2,\dots,N \\ i\neq i_1,\dots,i_m}} \left(\delta_{i+1} - 2\delta_i + \delta_{i-1}\right)^2 + Q_2\left[\sum_{i=i_2,\dots,i_m} \left(\delta_{i+1} - \delta_i\right)^2 + \delta_1^2\right]$$

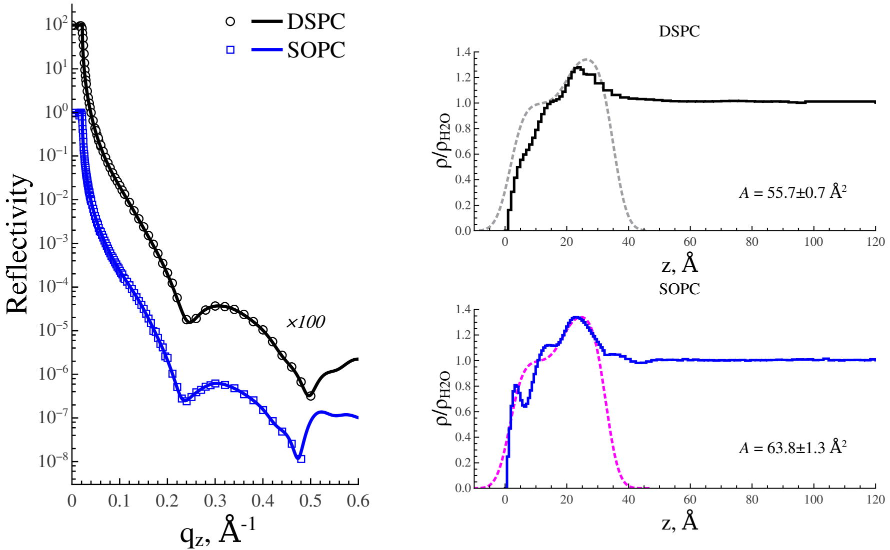
Extraction of structural parameters

$$\rho_e(z) = \frac{\pi\delta(z)}{r_0\lambda^2}, \qquad A = \frac{\Gamma}{\int\limits_{z1}^{z2}\rho(z)dz}$$

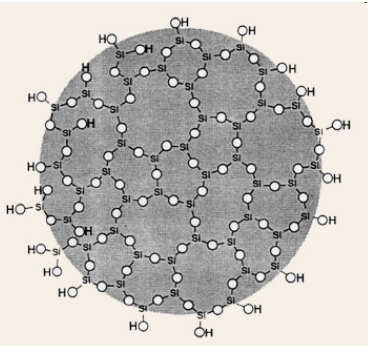
where $r_0 = 2.814 \times 10^{-5}$ Å, Γ – quantity of electrons per structure unit.

Lipid monolayer on a water substrate

Density profiles normalized by $\rho_{H_2O} \approx 0.333\,\text{Å}^{-3}$



Colloidal silica nanoparticles solutions

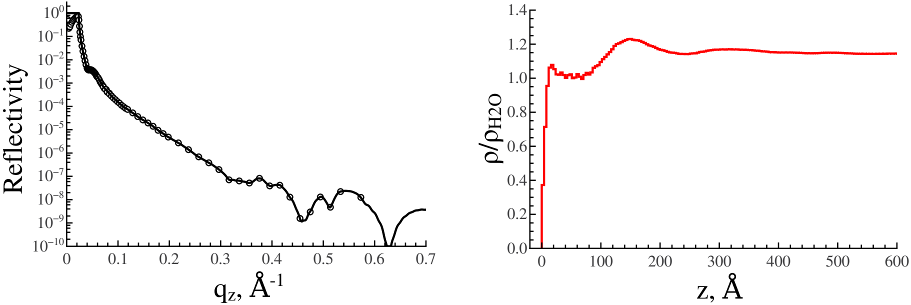


	D, nm	ρ, g/cm^3	C_{SiO2}, %	C_{NaOH}, %	pH
FM	5	1.1	16	0.3	9
SM-30	10	1.22	30	0.5	10
HS-40	12	1.27	40	0.3	9.5
TM-40	22	1.30	40	0.03	9

J. Depasse, A. Watillon, J. Colloid. Interface Sci. 33:430 (1970)

Surface effects on a clean silica sol

Example: sol SM-30, $\rho_{H_2O} \approx 0.333\,\text{Å}^{-3}$

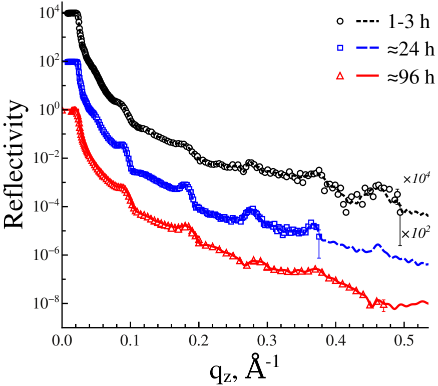


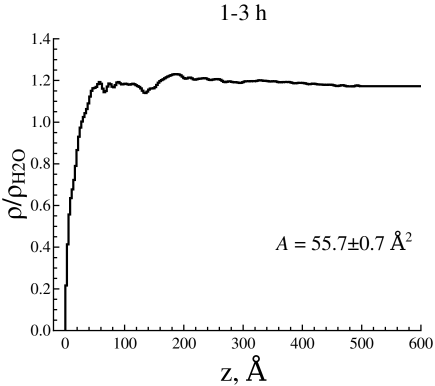
- image-charge effect influences the particles near air/sol interface
- Debye screening length $\Lambda_D = \sqrt{\frac{\varepsilon_0 \varepsilon_2 k_B}{c^- N_A e^2}} \sim 300 \ldots 500$Å.
- air/"levitating ions"/depleted layer/SiO_2 particles/sol volume
- Approximate Na^+ surface density $(4.8 \pm 0.3) \times 10^{18} m^{-2}$

V. E. Asadchikov, Yu. O. Volkov, A. M. Tikhonov et al. // JETP Lett. 94(7):625 (2011)

Phospholipid layer on a sol substrate

DSPC on SM-30, measurements over different time, $\rho_{H_2O} \approx 0.333\,Å^{-3}$





- 1–3 hrs: single layer, $d \approx 132\,Å$; SiO_2 particles are disappeared!

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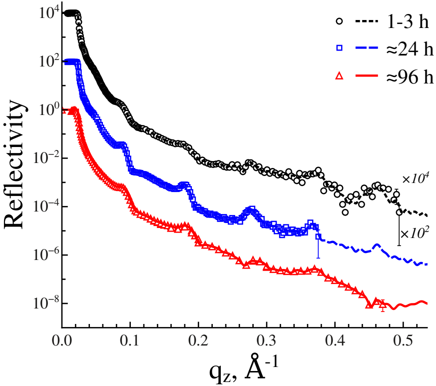

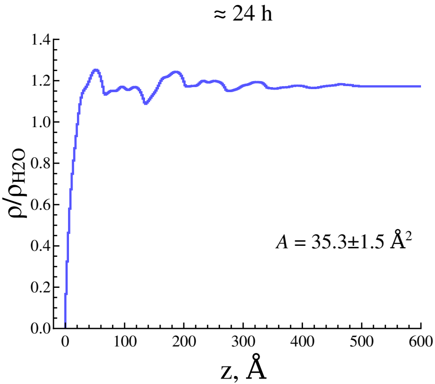

- 1–3 hrs: single layer, $d \approx 132\,\text{Å}$; SiO_2 particles are disappeared!
- $\approx$ 24 hrs: periodic structure, $L = 68.0 \pm 2.1\,\text{Å}$; $\Phi \approx 8$ Na^+ ions/lipid molecule.

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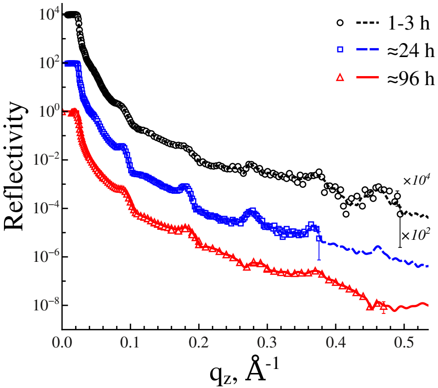
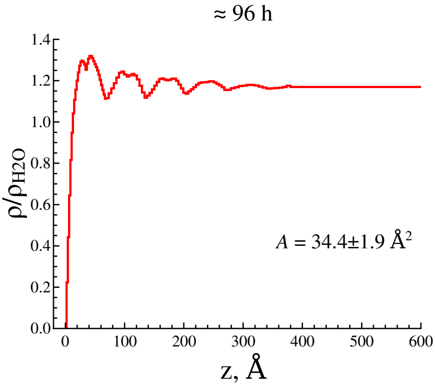
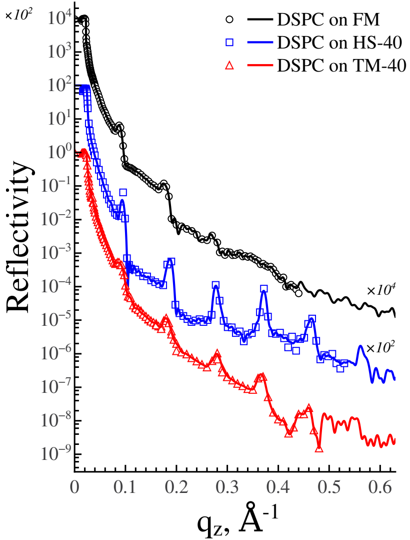
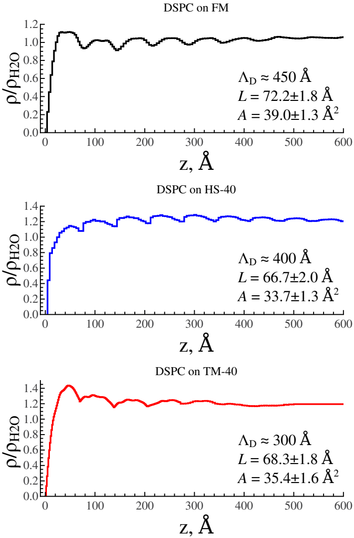




- 1–3 hrs: single layer, $d \approx 132\,\text{Å}$; SiO_2 particles are disappeared!
- ≈ 24 hrs: periodic structure, $L = 68.0 \pm 2.1\,\text{Å}$; $\Phi \approx 8$ Na^+ ions/lipid molecule.
- ≈ 96 hrs: multilayer, $L = 68.1 \pm 0.9\,\text{Å}$; $\Phi \approx 10$ Na^+ ions/lipid molecule.

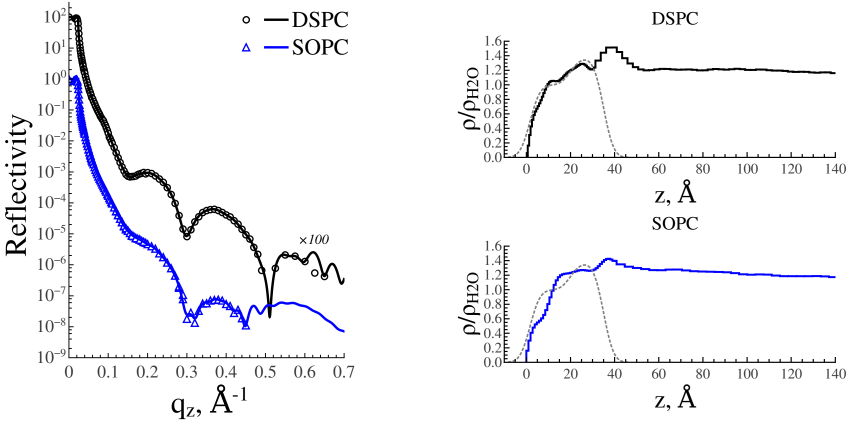
Influence of the substrate on multilayer properties





Substrate dopation with alkali ions

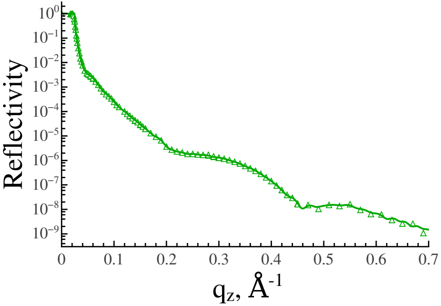
Sol FM-16 doped with Na^+, pH≈11.5

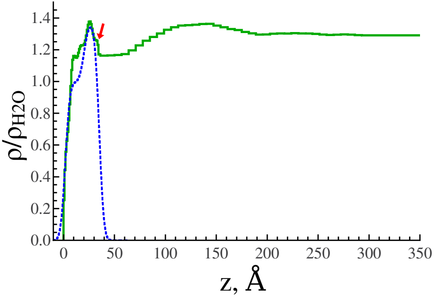


- $\Delta\mathrm{pH} = 1$ decreases Λ_D by $\sqrt{10}$ times;
- $L_1 \approx 35$Å, $A_{DSPC} = 44.9 \pm 1.7$Å^2 — crystalline monolayer;
- $A_{SOPC} = 65.3 \pm 2.5$Å^2 — liquid film;
- $d_2 \approx 20$Å$< d_{SiO2}$ — probably lipid vesiculae with Na^+ ions?

Ion screening effect: heavy alkali ions

DSPC on sol SM-30 doped with Cs^+, pH≈ 11



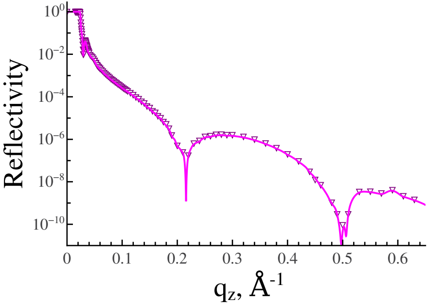


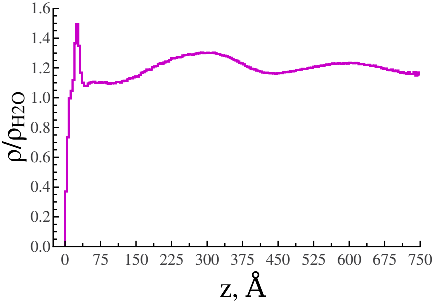
- SiO_2 particles layer is present!
- $L_1 = 35 \pm 0.7$Å, $A = 40.2 \pm 2.0$Å^2 — almost ideal lipid moonolayer.
- Approximate Cs^+ surface density $(4.4 \pm 0.2)\times 10^{17} m^{-2}$

A. M. Tikhonov, J. Chem. Phys. 130:024514 (2009)

Ion screening effect: saturated lipid salt

DSPC-Na salt on TM-40





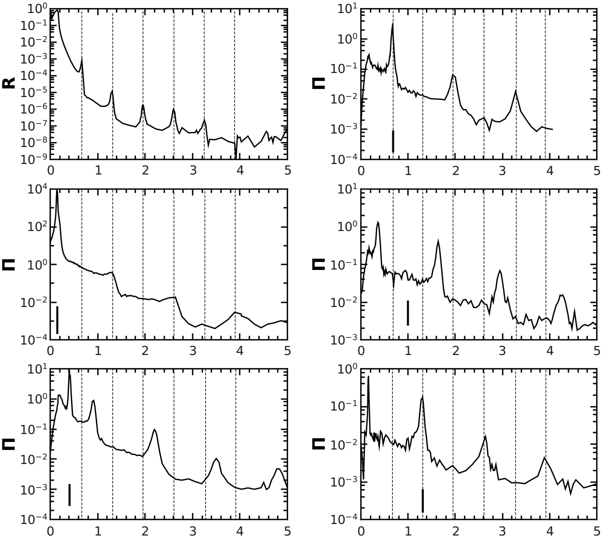
- SiO_2 particles layer is present;
- $d_1 \approx 64$Å — lipid bilayer.
- $A = 33.1 \pm 1.6$Å — 2-dimensional lipid crystal.

Cumulative table of lipid layer parameters

Approx. structure	substrate	pH	phospholipid	L (Å)	A (Å^2)
monolayer	water	7	DSPC	37 ± 2	55.7 ± 0.7
monolayer	water	7	SOPC	36 ± 2	63.8 ± 1.3
bilayer in stack	FM	9	DSPC	72 ± 2	39.0 ± 1.3
bilayer in stack	SM-30	10	DSPC	68 ± 1	34.4 ± 0.9
bilayer in stack	HS-40	9.5	DSPC	67 ± 2	33.7 ± 1.3
bilayer in stack	TM-40	9	DSPC	68 ± 1	35.4 ± 1.6
monolayer	FM-16	11.5	DSPC	≈ 35	44.9 ± 1.7
monolayer	FM-16	11.5	SOPC	≈ 35	65.3 ± 2.5
monolayer	TM-40/Cs^+	11	DSPC	35 ± 0.7	40.2 ± 2.0
bilayer	TM-40	9	DSPC-Na	64 ± 5	33.1 ± 1.6

Off-specular grazing incidence diffraction from DSPC multilayer

Preliminary data taken with laboratory source ($E = 8048$ eV), 25–30 June 2016



Results and discussion

A. Tikhonov, V. Asadchikov, Yu. Volkov // JETP Lett. 2015, 102(7):536–541, arXiv:1511.03543v1

- Phospholipid layer on the surface of silica sol compensates mirror charge effect, presumably due to the absorption of Na^+ ions by lipid molecules from sol volume.
- Lipid molecules within thick multilayer undergo spontaneous ordering effect under the influence of a surface electric field, leading to the formation of regular bilayer stack after a few days.
- Quantity of bilayers depends on Λ_D at the surface and, consequently, substrate pH. Thus one can reduce bilayer number by increasing of sol pH.
- Bilayer uniformity depends on surface electric field gradient and, consequently, size of SiO_2 nanaparticles in solution.
- Layer of “levitating ions” on the air/sol interface prohibits the ion absorption from sol volume, effectively screening lipid layer from the substrate. This leads to the formation of thin monolayer instead of bilayer stack.

Further goals and perspectives

- Off-specular and off-plane GIXRD, X-ray fluorescence experiments (fine determination of lateral crystalline structure);
- Phospholipid mixtures and in-situ variation of substrate composition;
- Theoretical interpretation of spontaneous lipid stratification process (biophysicist needed);
- In situ membrane-protein conformation processes;
- Phospholipid layers at liquid/liquid interface.

THANK YOU FOR YOUR ATTENTION!