Soft nanocontainers based on hydroxyethylated geminis: role of spacer in self-assembling, solubilization, and complexation with oligonucleotide

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Surface tension measurements

Surface tension measurements were performed using the du Nouy ring detachment method with the tensiometer K6 (Kruss). Each data point represented the average of ca. fifteen measurements of surface tension. Each of concentration dependence was obtained three times, and results were within 2%.

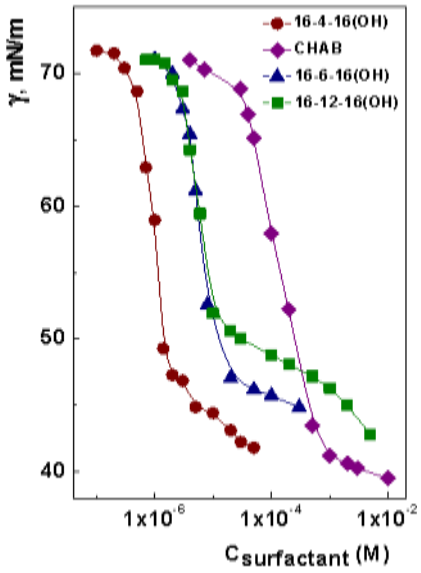

Fig. S1. Surface tension isotherms of hydroxyethylated surfactants.

Quantitative treatment of surface tension isotherms

Based on the surface tension isotherms, quantitative parameters characterizing the adsorption of surfactants at the air/water interface and their micellization were estimated. The surface excess Γ_{max} and the surface area per a molecule, A_{min}, have been calculated using the Gibbs equation:

$$\Gamma_{max} = \frac{1}{2.3nRT} \lim_{C \to cmc} (d\pi/d\log C) \qquad \text{(S1)}.$$

$$A_{min} = \frac{10^{18}}{N_A \Gamma_{max}} \qquad \text{(S2)},$$

were R = 8.31 JmolK^{-1} (gas constant), π is the surface pressure obtained from the surface tension of water minus the surface tension of the surfactant solution, and T is the absolute temperature in K. N_A is Avogadro number (6.02×10^{23} mol^{-1}). The parameter n represents the number of species at the interface the concentration of which changes with surfactant concentration. The constant n takes the value 2 for

an ionic surfactant where the surfactant ion and the counterion are univalent, while the value 3 is taken for the dicationic surfactants.

Fluorescence spectroscopy with the use of Pyrene

The fluorescence spectra of Pyrene (1 10^{-6} mol L^{-1}) in surfactant solutions in the absence of and the presence of a quencher (cetylpyridinium bromide) were recorded at 25 ^{0}C in a Varian Cary Eclipse spectrofluorimeter. Fluorescence intensities of the first peak at 373 nm (I_I), of the third peak at 384 nm (I_{III}), and of the peak at 394 nm were obtained from the spectra. The determination of aggregation numbers was performed by the most intense peak at 394 nm. The N values were calculated from the dependence (1):

$$\ln\frac{I_0}{I} = \frac{N}{C - CMC}[Q] \qquad \text{(S3)},$$

where I_0 and I are the intensities of the fluorescence of pyrene in the absence and presence of a quencher, respectively; C is the total surfactant concentration; [Q] is the concentration of the quencher. The cmc value was determined from the dependence of the intensity ratio of the first and third peaks on the surfactant concentration.

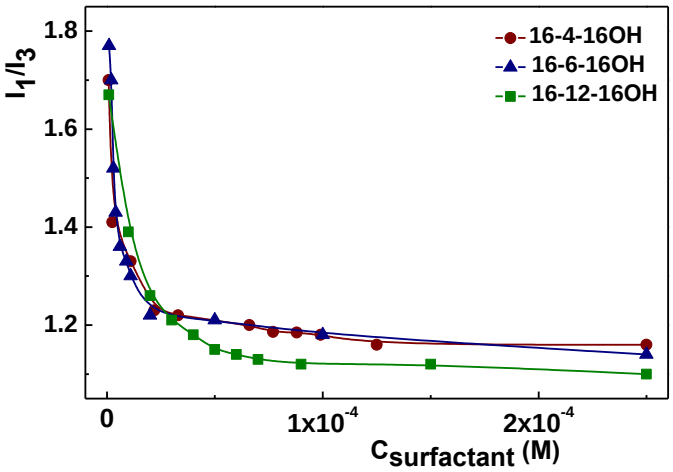

Fig. S2. The intensity ratio of the first and the third vibronic peaks of pyrene vs 16-s-16(OH) concentration; 25 ^{0}C.

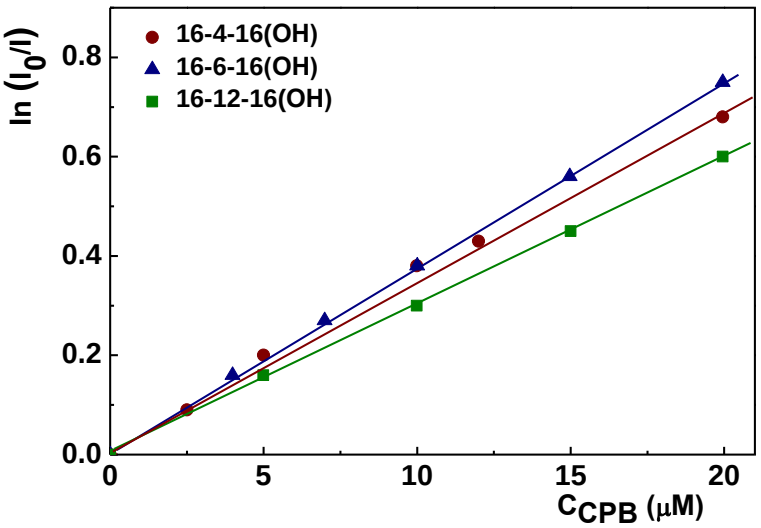

Fig. S3. Logarithm of the ratio of fluorescence intensity in the absence and presence of quencher (cetylpyridinium bromide, CPB) vs. quencher concentration plots for 16-s-16(OH) systems at concentration of 29, 20 and 40 μM for s=4, 6 and 12 respectively; 25 ^{0}C.

NMR experiment

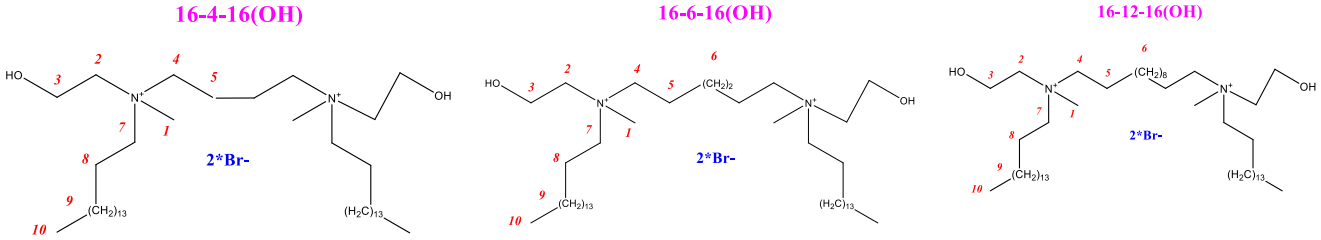

Fig.S4. Structural formulas of 16-s-16(OH), with the atoms numerated

The diffusion experiments were performed at least three times and only the data with the correlation coefficients of a natural logarithm of the normalized signal attenuation (ln I/I_0) as a function of the gradient amplitude $b=\gamma^2\delta^2g^2(\Delta-\delta/3)$ (γ is the gyromagnetic ratio, g is the pulsed gradient strength, Δ is the time separation between the pulsed-gradients, δ is the duration of the pulse) higher than 0.999 were included. All separated peaks were analyzed and the average values were presented. The temperature was set and controlled at 30°C with a 535 l/h airflow rate in order to minimize temperature fluctuations owing to sample heating during the magnetic field pulse gradients. After Fourier transformation and baseline correction, the diffusion dimension was processed with the Bruker Xwinnmr software package (version 3.5). The pulse programs for all NMR experiments were taken from the Bruker software library. The molecular structures for calculation of hydrodynamic radii were generated using molecular

modeling program CS Chem3D Ultra 6.0 (CambridgeSoft Corp http://www.camsoft.com.). Full geometry optimizations to a minimum energy were executed by Molecular Mechanics employing the MM2 force field.}

The Fourier transform pulsed-gradient spin-echo (FT-PGSE) [61-64] experiments were performed by BPP-STE-LED (bipolar pulse pair–stimulated echo–longitudinal eddy current delay) sequence. Data were acquired with a 50.0 or 120.0 ms diffusion delay, with bipolar gradient pulse duration from 2.2 to 6.0 ms (depending on the system under investigation), 1.1 ms spoil gradient pulse (30%) and a 5.0 ms eddy current delay. The bipolar pulse gradient strength was varied incrementally from 0.01 to 0.32 T/m in 16 steps.

2D NOESY experiments were performed with the mixing time of 200 ms with pulsed filtered gradient techniques.

The aggregation numbers were estimated from the simple ratio:

$N_{agr}=V_{agr}/V_{mon}=(R_{H,agr}/R_{H,\,mon})^3$ (S4),

where V_{agr} and V_{mon} are the volumes of the aggregate and monomer surfactant molecule, respectively; $R_{H,agr}$ refers to the experimental hydrodynamic radius calculated by the Einstein - Stokes equation:

$$R_H = k_B T/6\pi\eta D_s$$

where R_H is hydrodynamic radius, k_B is Boltzmann constant, T is the temperature (K), η (Pa·s) is dynamic viscosity of the solvent, and D_s is self-diffusion coefficient. In this case $R_{H,\,mon}$ is theoretically calculated value.

The use of the highly hydrophobic probes such as hexamethyldisiloxane (HMDSO) which is water insoluble and completely solubilized by micelles makes it possible to model and reliable ascribe the D_S values for aggregated surfactant.

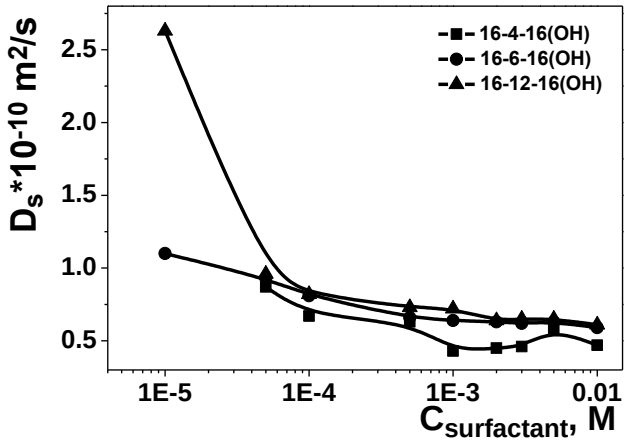

Fig. S5. Self-diffusion coefficient versus surfactant concentration plot for 16-s-16(OH) amphiphiles (s = 4, 6, 12); 30 °C.

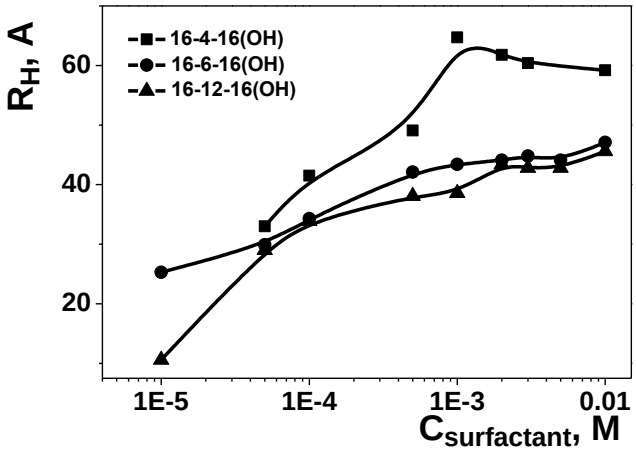

Fig. S6. Effective hydrodynamic radius versus surfactant concentration plot for 16-s-16(OH) amphiphiles (s = 4, 6, 12).

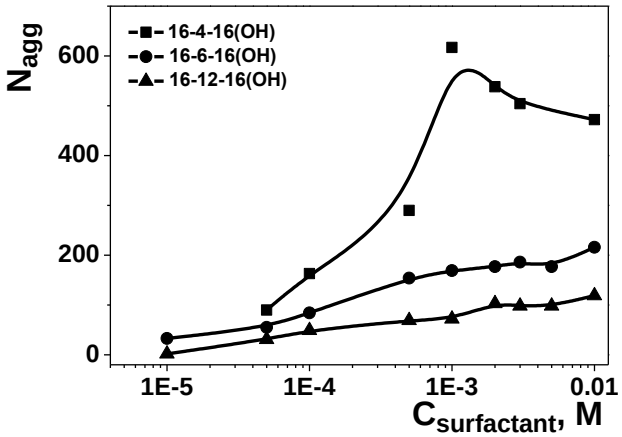

Fig. S7. Aggregation numbers versus surfactant concentration plot for 16-s-16(OH) amphiphiles (s = 4, 6, 12).

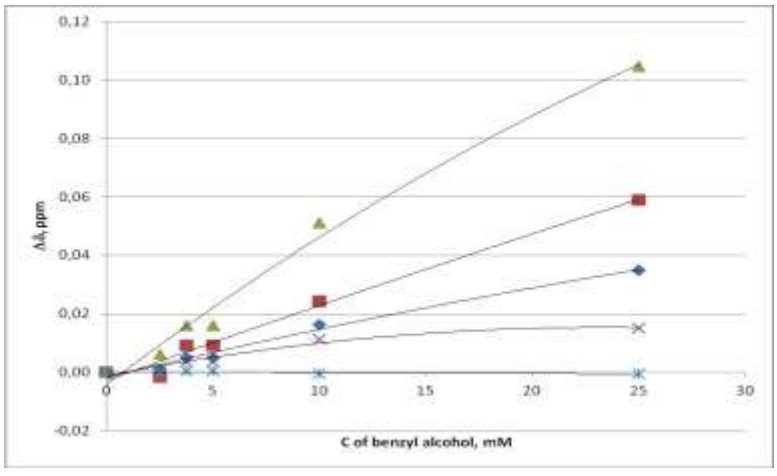

Fig.S8. Changes in ^{1}H chemical shifts of 16-4-16(OH) (5 mM) with an increase in concentration of benzyl alcohol (H3 is symbolled by diamond, H4, H7 by square, H5, H8 by triangle, H9 by cross, H10 by star); numeration of protons is given in Fig.S4.

Micropolarity study with the use of fluorescent dye Prodan

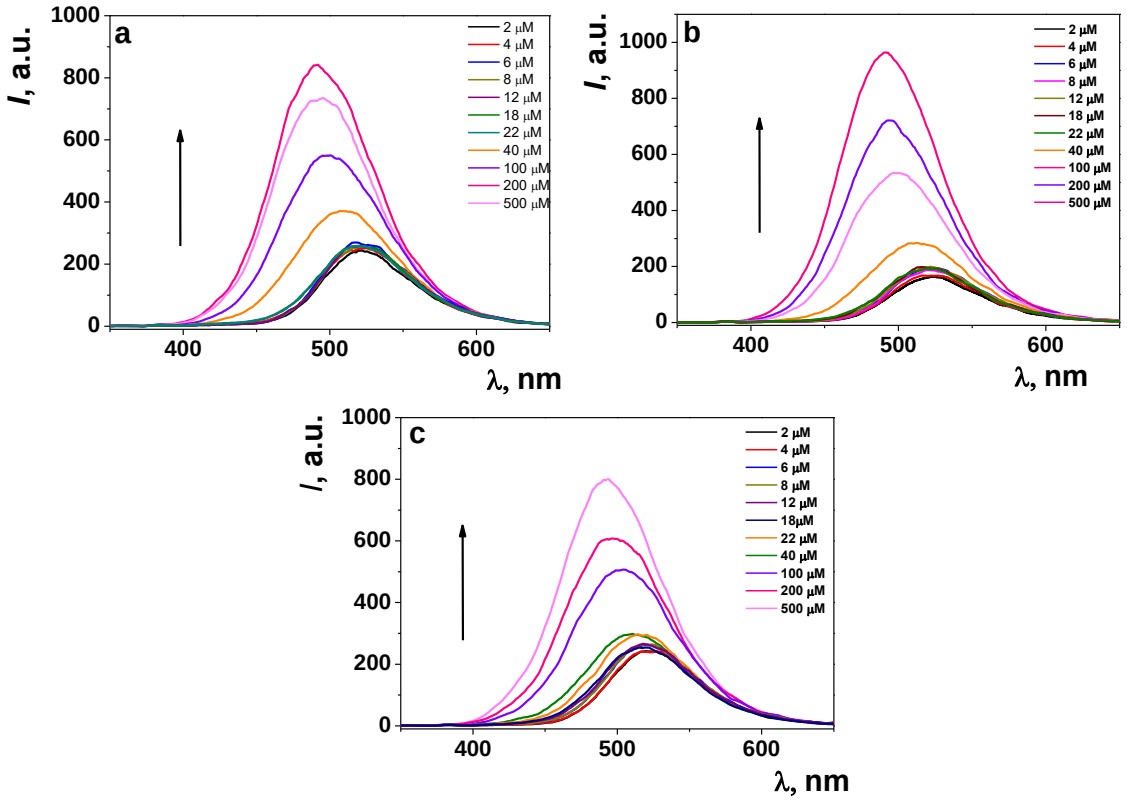

Fig. S9. Emission fluorescence spectra of prodan in the presence of various amounts of 16-s-16(OH) amphiphiles: a) 16-4-16(OH); b) a) 16-6-16(OH); c) 16-10-16(OH); C_{prodan} = 5 μM; 25 °C.

Table S1. Cmc values for 16-s-16(OH) amphiphiles (s = 4, 6, 10) obtained by fluorescence spectroscopy using prodan as a probe

Amphiphile	16-4-16(OH)	16-6-16(OH)	16-10-16(OH)
C_{cr}, μM	22	23	13

Solubilization of Orange OT

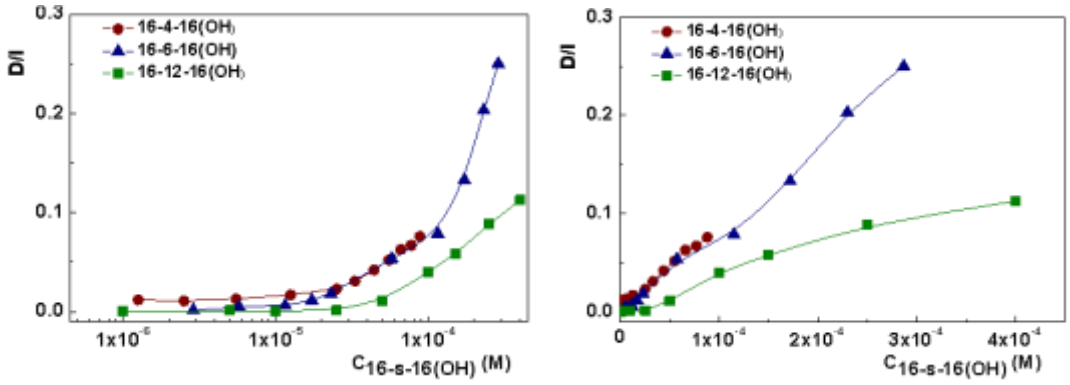

Fig. S10. Absorbance of Orange OT reduced to a 1 cm cuvette (*D/l*) vs surfactant concentration for 16-s-16 aqueous solutions, 25 ^{0}C, λ = 495 nm (s = 4, 6, 12) (the low concentration range is covered); the plot used logarithmic X-coordinate is on the left side, while normal concentration scale is used in the plot on the right.

Table S2. The values of aggregation number N, molecular mass of micelles (MM_m) and solubilization power S obtained from analysis of solubilization data in terms of linear equation D = a + b× C, where C is surfactant concentration above cmc (g/L), b is the slope of dependence, a is the X-axis intercept and D is the absorbance of saturated solution of Orange OT at 495 nm.

Surfactant	C_{cr}, M (from solubilization data, Fig. 2)	Linearization (in normal coordinanates)			S	N^a	N^b
		a	b	correlation coefficient R			
16-4-16	0.00021	0.0161	1410	0.998	0.075	12 (MM_m 8954)	14
16-6-16	0.00017	0.0485	809	0.987	0.043	18 (MM_m 14988)	21
16-12-16	0.00014	0.027	534	0.994	0.029	28 (MM_m 27595)	30

[a]calculated using C_{cr} value obtained from solibilization data, Fig.2;

[b]calculated using cmc values obtained from tensiometry data.

FTIR spectroscopic characterization of gemini surfactants

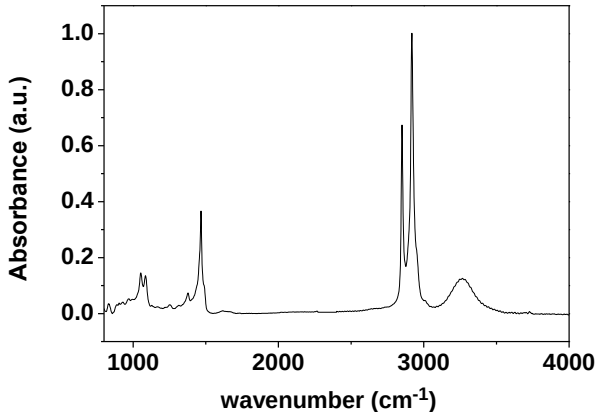

Fig.S11. FTIR spectrum of solid 16-12-16(OH) film.

Interaction of surfactants with lipid bilayer

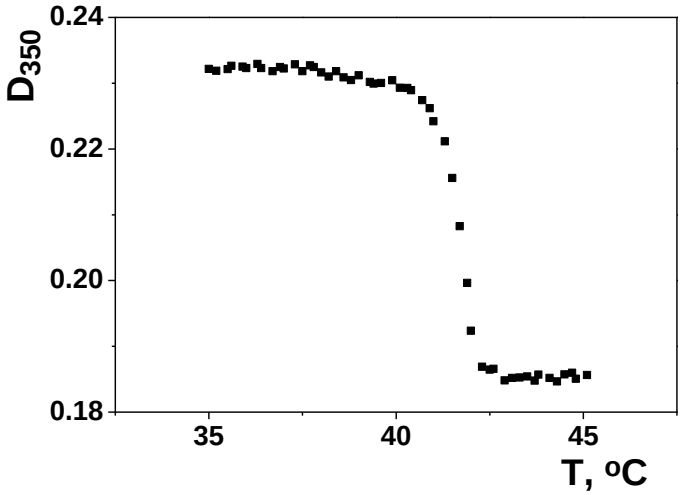

Fig. S12. Typical turbidimetric plot for DPPC individual liposomes.

Detailed description of sample preparation and the EB exclusion technique.

A 0.7 ml sample containing the 0.5 μM concentration of EB and the 10 μM concentration of oligonucleotide (counting on 1 base pair) in 4 mM tris-HCl buffer at pH 8.0 was prepared directly in the cuvette and equilibrated for 10 min at 25 °C. In several experiments a 1:5 EB/ONu ratio was used for comparison. After that increasing amounts of the surfactant of the required concentration were added into the mixture. The concentrations of surfactant stock solutions were prepared in such a way that the maximum dilution of oligonucleotide-EB complex was maintained within 10% due to the surfactant addition. The dilution factors were considered for the calculation of final charge ratios. Each of concentration dependence was performed twice, and the results were within 2%.

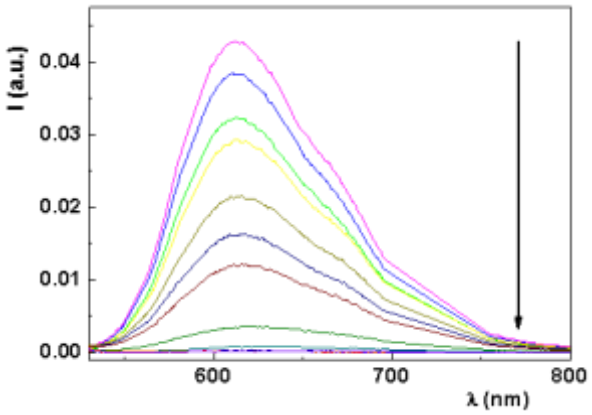

Fig. S13. Ethidium bromide fluorescence spectra for 16-12-16(OH)/oligonucletide at different surfactant/ONu (10 μM) molar ratio: 0.14; 0.29; 0.43; 0.57; 0.71; 0.86; 1.14; 1.29; 1.43; 2.86; 5.71. Arrow direction indicates the increase in surfactant/ONu molar ratio).

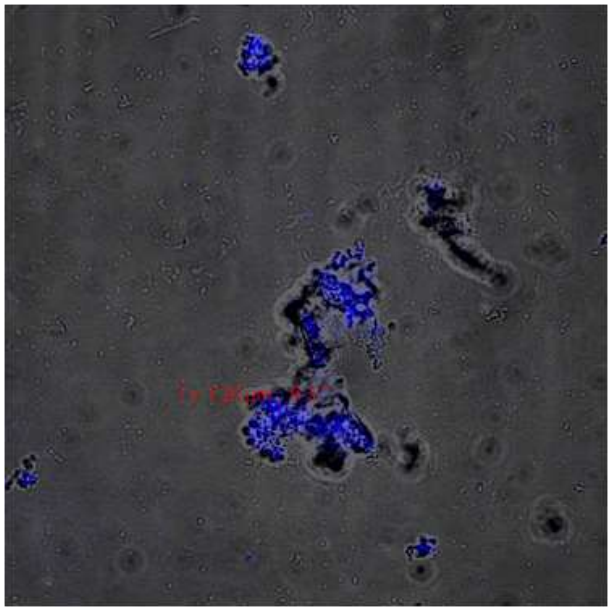

Fig. S14. The confocal microscope images of lipoplexes formed in ONu-16-12-16(OH) system above the charge neutralization point; $C_{16\text{-}12\text{-}16(OH)}$ = 2.83 мМ; C_{ONu}=1мМ. The merged images representing a combination of two channels (Hoechst33342-blue and transmitted light channel).