Experimental

Synthesis of SL. The sophorolipid molecule is synthesized according to a well-established procedure [14]. The yeast Candida bombicola (ATCC 22214) was provided by the Industrial Yeast Collection DBVPG (University of Perugia, Italy). Lyophilized yeast culture is grown in a standard Yeast Medium (YM: 3 g Yeast extract, 3 g Malt extract, 5 g peptone, 10 g glucose, 1 L MilliQ water) broth (5mL) at 25°C for 48h. Then, a loopful of the previous mixture is inoculated into 5 mL YM broth at 25°C for four days. An inoculum (~100 μL) of the broth stock cultures is added to a 400 mL pre-culture medium (1 g/L urea, 10 g/L yeast extract, 100 g/L glucose) in shake flask (2 L) at 30°C under stirring (100 rpm. After 35-39 hours of incubation, corresponding to the late-exponential phase of growth, the culture is harvested by centrifugation at 6000 rpm for 10 minutes at 20°C. The pellet consisting of the crude biomass is added into 70 mL of culture medium supplemented with 40 g/L of oleic acid (Aldrich) in a shake flask (500 mL). The flask is incubated at 30°C and 200 rpm. After four days, 100 g/L of glucose and 40 g/L of oleic acid are further added. After phase separation, the viscous brown liquid is then removed from the aqueous medium several times during the incubation (about 15 days) and, in order to maintain the total volume constant, an equivolumetric amount of culture medium is systematically introduced inside the shake flask. All fractions of the sophorose mixture are then put together and treated in order to remove traces of the oleic acid using ethyl acetate and hot hexane according to the published procedure [19]. Finally, hydrolysis of the mixture to obtain the acidic COOH form of SL is performed with 5M NaOH solution and extracted with pentanol according to procedure described in [20]. The product is characterized by Thin Layer Chromatography on silica gel 60 plates (Whatman) using a 15:65 Methanol:Chloroform mixture [9d] as eluent and stained with KMnO4. 13C NMR (D2O and CDCl3:CH3OD = 1:1) is used to check if the sophorose mixture (lactonic and acetylated species) has been hydrolyzed into the acidic form. An average downfield 5 ppm shift from 172 ppm to 177 ppm is used as reference to verify the deacetylation of the COOH group.

Synthesis of silica film by EISA. Mesoporous silica thin films are prepared by dip-coating via the evaporation-induced self-assembly (EISA) technique [3]. Tetraethyl orthosilicate (TEOS), ethanol (EtOH), concentrated HCl 37% and NaOH are purchased from Aldrich and used without further purification. An initial pre-hydrolyzed TEOS solution is prepared as follows: 0.53 mL of TEOS, 0.37 mL EtOH and 40 μL of a 0.77 M HCl/H2O solution. This solution is stirred for 45 min, then 1.86 mL of EtOH, 0.19 mL of water, 50 μL of 1 M HCl/H2O and 0.225 g of SL (10 w%) are added to the sol. This is stirred for 15 min before dip-coating. Silicon substrates (thickness 400 μm) are dip-coated at constant relative humidity (≈40%), the pulling rate is set at a variable speeds ranges from to 1.9 mm s⁻¹ to 1.4 mm s⁻¹. Finally, after deposition, films are calcined at 350 °C under air for 1 h. The pH value of the solutions is raised by adding about 180 μL of a [1] NaOH solution. Dip-coating is immediately perfomed before gelification of the sol.

Small Angle X-ray Scattering. SAXS experiments are carried out at the SWING beamline of the Soleil synchrotron (Saint-Aubin, France) at an energy of 12 keV using a PCCD170170 AVIEX...
two-dimensional detector. The sample-to-detector distance and the instrumental angle between X-ray beam and sample are set at 150 cm and 90° respectively, and the signal is recorded for a total time of 100 s. The SAXS signal is then normalized to get absolute intensities, and radial integration is performed on the 2D image using the local software (FOXTROT).

The morphology of the SL/silica samples is studied by TEM using a FEI Tecnai microscope equipped with a LaB₆ filament operating at 120 kV. Finely ground films scratched from the silicon substrate are dispersed in ethanol by sonication, then they are dropped on a carbon-coated copper grid and dried for TEM observations. Pore size and Fourier Transform analysis are obtained by using ImageJ [21] software on TEM micrographs.

Transmission Electron Microscopy under cryogenic conditions (Cryo-TEM) were run on a Jeol JEM 2010F at the microscopy center of the Institut Pasteur (Paris, France). Filament operates at 200 kV and magnification was set to 60,000 times. A Gatan ultrascan 4000 camera was used to acquire the image and DigitalMicrograph™ as software. Liquid sample at desired concentration was deposited on a TEM copper grid covered with a porous (10 μm) carbon membrane. The grid was firstly dried and then immediately dipped into liquid ethane using a home-made cryo-fixation device. Sample was successively transferred into a liquid nitrogen bath until measuring was completed. Any contact with air moisture was accurately avoided.

**Small Angle Neutron Scattering** SANS is performed at the Léon Brillouin Laboratory (Orphée Reactor, Gif sur Yvette, France) on the PACE beamline. The spectrometer configuration is adjusted to cover a q-range between 0.01 and 0.2 Å⁻¹ (neutron wavelength is λ= 5 Å; sample-to-detector distance is d= 350 cm; beam dimension= 0.7 cm x 0.7 cm), where q is defined as 4π/λ sin θ/2, where θ is the scattering angle between the incident and the scattered neutron beams. Different amounts of the SL are added to D₂O (Aldrich) solutions (c= 0.05, 0.5, 1, 2, 5, 10 and 17 w%). The pH of these solutions is acidic and it ranges between 4.5-5. To increase the pH value to about 6.5, 0.5 M and 1 M NaOH solutions (15 to 50 μL) are further added.

All samples are introduced in a 2 mm quartz cell and studied at room temperature. The blank sample is 99.9% D₂O, whose signal is subtracted to the measurements. Data treatment is done with the Paresu and Regiso software provided at the beamline. Absolute values of the scattering intensity (I(q) in cm⁻¹) are obtained from the direct determination of the number of neutrons in the incident beam and the detector cell solid angle. The 2-D raw data were corrected for the ambient background and empty cell scattering and normalized to yield an absolute scale (cross section per unit volume) by the neutron flux on the samples. The data were then circularly averaged to yield the 1-D intensity distribution, I(q). The incoherent scattering was approximated from the high q intensity plateau and subtracted from the corresponding reduced data.

Analysis of SANS data in Fig.1 is done using typical fitting procedures at low (Guinier regime, eq.S1) and high (Porod regime, eq. S2) q-values [22] according to the well-established formulas below using the Origin© software (version 6.1).

\[
\ln[I(q)] = \ln[I(0)] - \frac{Q^2R_G^2}{3} \quad \text{(S1)}
\]

\[
\lim_{q \to \infty} I(q) = 2\pi\Delta\rho^2 \frac{S_v}{q^4} \quad \text{(S2)}
\]

Here, q is the scattering vector [Å⁻¹], R_G is the radius of gyration, Δρ is the difference in Scattering Length Densities (SLD) between the object (for the SL, SLD= 5.58 10¹⁰ cm⁻²) and the medium (for D₂O, SLD= 6.39 10¹⁰ cm⁻²); S_v is the overall surface of the scattering objects per volume of irradiated sample.

A spherical and cylindrical models (mathematical expressions are provided in Fig.S1 below) have been used to fit the form factor of the SANS data sets by mean of the software Scatter [23].
Figure S1 – SANS data and corresponding fits for a) 0.5 and b) 5 w% solutions (pH=4.5) of SL in D₂O. Fits have been done with the Scatter software (see SANS in experimental part above for more details) using the form factors (given below) for a full sphere (Ps) in (a) and a cylinder (Pc) in (b).

In (a): R is the radius of the sphere; in (b): R is the radius of the cylinder, L is its length and θ is the angle between the normal to the cylinder and the wave vector q. σ accounts for the polydispersity.

\[ P_s(q, R) = \left[ \frac{3 \sin(qR) - (qR) \cos(qR)}{(qR)^3} \right]^2 \]

\[ P_c(q, L, R) = \left[ \int_0^{\pi/2} \sin \theta d\theta \frac{\sin(Lq \cos \theta)}{Lq \cos \theta} \frac{2J_1(Rq \sin \theta)}{Rq \sin \theta} \right]^2 \]
Cryo-TEM experiments

Cryo-TEM experiments (Fig.2S) have been done on the 0.5 w%, 5 w% and 17 w% samples. Imaging is performed inside the pore of the carbon grid containing amorphous ice and the sample at the desired concentration value. At 0.5 w% (Fig.2S A), micelles are spherical objects with an average diameter of 3 nm. At increasing concentration values, spherical objects turn into elongated rods (Fig.2S A and B) whose size is several tens of nanometers. At 17 w%, micelles seem to be more flexible objects than at 5 w%.

In all cases, concentration effects can be observed on the carbon grid giving origin to various micellar textures.
References

For references, see main article.