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Electronic Supporting Information (ESI)

Bio-based glyco-bolaamphiphile forms a temperature-responsive hydrogel with tunable elastic properties

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Figure S 1 - HPLC-ELSD (top) and LC-MS (bottom) chromatograms of the symmetrical bola C16:0 sophoroside (SS) purified batch. The peak at 15 or 17.5 minutes (m/z value of 905) corresponds with the sBola C16:0 SS, for HPLC-ELSD and LC-MS respectively.



Figure S 2 – a) Time evolution of the storage and loss moduli with time at T= 25°C, C= 3 wt%, and stress, τ = 1 Pa. Full symbols refer to a sampling rate of 560 points/h under a frequency, f= 1 Hz; empty symbols refer to a sample at rest, sampled at t= 0 h, 1 h, 2 h and then every 2 h (f= 1 Hz during analysis). b) Time-dependent SANS experiments recorded at C= 3 wt% and T= 25°C.

The stability of the hydrogel over time has been tested by repeating the experiment under two different conditions: 1) the gel, kept at rest in the geometry, is subjected to a short solicitations, as shown on Figure S 2; 2) the gel is submitted to a continuous oscillating time sweep (f= 1 Hz and τ = 1 Pa). As shown on Figure S 2a, the two methods provide data points which are superimposable throughout the time-scale, indicating that the constant solicitation does not alter the gel formation/structuration during the experiment.



Figure S 3 – SANS experiments collected on sBola C16:0 SS at 0.3 wt% at T= 25°C at t= 0 h and t= 12 h.



Figure S 4 – Cryo-TEM micrographs collected on sBola C16:0 SS at 3 wt% at T= 25 $^{\circ}C$ and t= 0 h



Figure S 5 - Cryo-TEM micrographs collected on sBola C16:0 SS at 1 wt% at T= 25°C and t= 0 h. The righthand image shows the length distribution, fitted using a log-normal function. Data have been treated using the built-in function within the Origin Pro 2015 software package.



Figure S 6 - Cryo-TEM micrographs collected on sBola C16:0 SS at 1 wt% at T= 25°C and t= 15 h. Arrows point at twisted ribbons.

Figure S 4, Figure S 5 and Figure S 6 show that morphology changes are not responsible for the increase in the mechanical properties of the hydrogel. The low-magnification cryo-TEM images for the 3 wt% (Figure S 4) and 1 wt% (Figure S 5) samples at t= 0 h obviously show a higher density of the fibers in the 3 wt% sample, which can explain the higher storage modulus at t= 0 h for this sample. However, the time-dependent increase of G' can be explained by an increase in length of the fibers themselves over time, thus confirming from a visual point of view the analysis of the time-dependent evolution of G'(C). At t= 0 h, one can fit the length distribution of the 1 wt% sample with a log-normal function having mean and standard deviation respectively of μ = 436.6 ± 30.8 nm and σ = 0.6 ± 0.1 (Figure S 5). Despite the limited number of sampled population (N= 160), due to the strong aggregation of the fibers on the cryo-TEM grid, a factor which made it impossible to analyze the length distribution at 3 wt%, one should retain the presence of fibers strongly increases and it becomes "infinite" (with respect to the cross-section) after 15 hours (low-magnification image in Figure S 6. Cryo-TEM confirms the rheology assumptions: fiber growth and entanglement are responsible for the gel formation and its elastic properties.



Figure S 7 - Measurement of the time-dependent (f= 1 Hz, τ = 1 Pa) storage and loss moduli for sBola C16:0 SS at C= 3 wt% and T= 5°C



Figure S 8 – a) Guinier plot Log[I(q)] vs q² at q< 1 nm⁻¹ (Guinier approximation for the whole particle)^{1,2} corresponding to the SANS profile of sample at C= 3 wt% and T= 25°C, presented in Figure 3c in the main manuscript. b) Fit of the SANS profile corresponding to the same sample. The fit has been done with the SasView 3.1.2 software package (https://www.sasview.org).

The fit in Figure S 8b is intended to provide another way to estimate the radius of the micelles and it is not intended to precisely describe the structure of the micelle, most likely expected to be core-shell, as found in similar system.³ For this reason, we have used a simple homogeneous sphere form factor model with the following parameters:

Scattering Length Density (SLD) of the solvent (D₂O): $6.34 * 10^{-4} nm^{-2}$ (fixed).

Scale: 0.03 (fixed). In the model proposed by SasView, this parameter is equivalent to the volume fraction, which corresponds to 3 wt%.

SLD of the sphere: $2.97 * 10^{-4} nm^{-2}$ (fitted). The SLD of the micelle is expected to be contained between $\sim 4 * 10^{-4} nm^{-2}$, corresponding to the SLD level of glucose in D₂O (including a full OH/OD exchange), and $\sim 10^{-5} nm^{-2}$, corresponding to the level of an aliphatic chain in water. The SLD values are estimated using the SLD calculator tool in the 3.1.2 version of Sasview software.

Radius of the sphere: 1.3 nm (fitted).

Background: 0.017 cm⁻¹ (adjusted through fitting).

^{1.} O. Glatter and O. Kratky, Small Angle X-ray Scattering, Academic Press, London, 1982

^{2.} A. Guinier, Ann. Phys. (Paris), 1939, 11, 161-237

^{3.} S. Manet et al., J. Phys. Chem. B, 2015, 119, 13113



Figure S 9 - Time-dependent evolution of the storage and loss moduli (f= 1 Hz, τ = 1 Pa) recorded on a sBola C16:0 SS hydrogel (C= 3 wt%) heated at T= 35°C for 10 min and brought to T= 25°C. Time scale starts at T= 25°C. a) and b) are two different samples, recorded on the same system so to show the fluctuations in terms of t_{gel}.



Figure S 10 – a) Measurement of T_{gel} at C= 3 wt% by following the Full Width at Half Maximum (FWHM) and integrated normalized area of the ¹H NMR signal of the alkyl chain of sBola C16:0 SS at δ = 1.3 ppm. b) Measurement of C_{gel} at T= 25°C by following the FWHM of the ¹H NMR signal of the alkyl chain of sBola C16:0 SS at δ = 1.3 ppm. Linear regression is used in both experiments to determine the intersection point. For C_{gel}, two points have been retained. c-d) Measurement of T_{gel} at C= 3 wt% by rheology (τ = 0.6 Pa, f= 1 Hz). In c), a hydrogel is formed at T= 20°C during 1h at rest. Next, temperature is increased with a rate of 0.5°C/min; methodology for the evaluation of T_{gel} are shown in the figure and the corresponding inset, representing the data in a log-lin scale. In d), the solution is heated at T= 40°C for 5 min and temperature is subsequently lowered at a rate of 0.5°C/min; T_{gel} is evaluated both at the G'/G'' intersection point and at the inflection point, as shown in the figure.



Figure S 11 – a) Time and temperature-dependent evolution of the Full Width at Half Maximum (FWHM) of the C16 alkyl resonance at 1.3 ppm in the ¹H NMR experiments. The data are collected after after a sol-gel transition from T= 40°C to the indicated value of the temperature (25°C, 15°C, 5°C). b) Avrami plots showing the dependency of the ln{-ln[(1- X_F)]} on ln(t), based on NMR signal decay of the alkyl resonance at 1.3 ppm (Figure 6b). c) Avrami plot against ln(t-t_g) based on the rheological property G* (Figure 4). For the definition of X_F in NMR and rheology, please refer to main text. Both data in b) and c) are recorded at T= 25°C after a sol-gel transition from T= 40°C.



Figure S 12 – a-d) Time and temperature evolution of X_F and FWHM under cooling and heating conditions and measured from the signal of the alkyl chain of sBola C16:0 SS at 1.3 ppm by ¹H NMR. In a), the system at t= 0 min is meant to be at T= 40°C and X_F = 0.



Figure S 13 – a) Avrami plots for the micelle-to-fiber phase transitions corresponding to the sBola C16:0 SS at C= 3 wt%. The plots are based on the data presented in Figure S 12b and they correspond to the ¹H NMR signal of the sBola C16:0 SS alkyl chain at δ = 1.3 ppm. *X_M* and *X_F* are, respectively, the fraction of micelles and the fraction of fibers corresponding to the A_{1.3} and [(1-A_{1.3})], where A_{1.3} is the normalized integrated area of the NMR aliphatic peak at δ = 1.3 ppm. b) Arrhenius plot from which the activation energy, E_a, is estimated. The Avrami plots which are used to obtain the values of ln(k) are given in a).