

Supplementary Information

Chameleonic Amphiphile: the Unique Multiple Self-Assembly Properties of a Natural Glycolipid in Excess of Water

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Supplementary Information Text

Methods

Table S1. Known structures, conditions and type of behavior of G-C18:1 in water (mQ-grade) at concentrations below 5 wt%. Unless otherwise state, temperature is always room temperature (T~23°C).

Structure	Conditions	Number	Reference	Behaviour
Spheroidal micelles	pH > 7.5	1	^{1,2}	Surfactant-like
Unilamellar vesicles	pH < 6.5	2	^{1,2}	Lipid-like
Lamellar	pH < 4	3a	¹⁻³	Lipid-like
Lamellar	pH > 4, T < 15°C	3b	^{1,2}	Lipid-like
Complex coacervates	pH > 7.5, polycations (poly-L-lysine, chitosan...)	4	^{4,5}	Surfactant-like
Multilamellar wall vesicles (MLWV)	pH < 7.5, pH reduced from complex coacervate phase	5	^{4,5}	Lipid-like
Vesicle aggregates	pH < 6.5, polycations	6	⁴	Lipid-like
Crystalline fibers	pH > 7.5, Ca ²⁺ , Ag ⁺ , Mn ²⁺ , Cr ²⁺	7	⁶⁻⁹	Gelator-like
Cylinders, wormlike micelles	pH > 7.5, Fe ²⁺ , Co ²⁺ , Al ³⁺ , Cu ²⁺	8a	^{7,9}	Surfactant-like
Cylinders, wormlike micelles	6.5 < pH < 7.5	8b	^{1,2}	Surfactant-like
Multilamellar vesicles (MLV)	4 < pH < 6.5, basification from pH < 4	9	³	Lipid-like

Small angle X-ray scattering (SAXS). SAXS experiments have been performed at Diamond (UK), Soleil (France) and ESRF (France) synchrotron radiation facilities. Please, refer to the references in Table 1 for more experimental details of each system.

Cryogenic-transmission electron microscopy (Cryo-TEM). All cryo-TEM images are recorded on an FEI Tecnai 120 twin microscope operating at 120 kV with an Orius 1000 CCD numeric camera. The sample holder is a Gatan Cryo holder (Gatan 626DH, Gatan). Digital Micrograph software is used for image acquisition. Cryo-fixation is done with low dose on a homemade cryo-fixation device. The solutions are deposited on a glow-discharged holey carbon coated TEM nickel grid (Quantifoil R2/2, Germany). Excess solution is removed and the grid is immediately plunged into liquid ethane at -180°C before transferring them into liquid nitrogen. All grids are kept at liquid nitrogen temperature throughout all experimentation. Cryo-TEM images are treated and analyzed using Fiji software, available free of charge at the developer's web site.

Solid-state nuclear magnetic resonance (ssNMR). ¹³C solid-state NMR experiments were carried out on an Avance III HD Bruker 7.05 T (ν_{1H} = 300 MHz) spectrometer and a 4 mm magic angle spinning (MAS) probe. The ¹³C NMR spectra were obtained by ¹H → ¹³C cross-polarization (CP)

under magic angle spinning (MAS). $\nu_{\text{MAS}} = 10$ kHz, number of transients, NS= 1024 except for the vesicle sample, for which NS= 7168 due to the less amount of product in the rotor; time domain size, TD= 2 k; pulse length, $p(^1\text{H}) = 7.00$ μs , $p(^{13}\text{C}) = 3.62$ μs , relaxation delay, D= 3 s; CP is performed under ramped (square) conditions with contact time during CP is $t_c = 500$ μs , 1000 μs and 3000 μs .

Dynamic Scanning Calorimetry (DSC). DSC is performed using a DSC Q20 apparatus from TA Instruments equipped with the Advantage for Q Series Version acquisition software (v5.4.0). Acquisition is performed on freeze-dried samples prepared from different phases of G-C18:1 solutions in water. All samples (about 16 mg) are sealed in a classical aluminium cup and using an immediate sequence of heating and cooling ramps at a rate of $10^\circ\text{C}\cdot\text{min}^{-1}$.

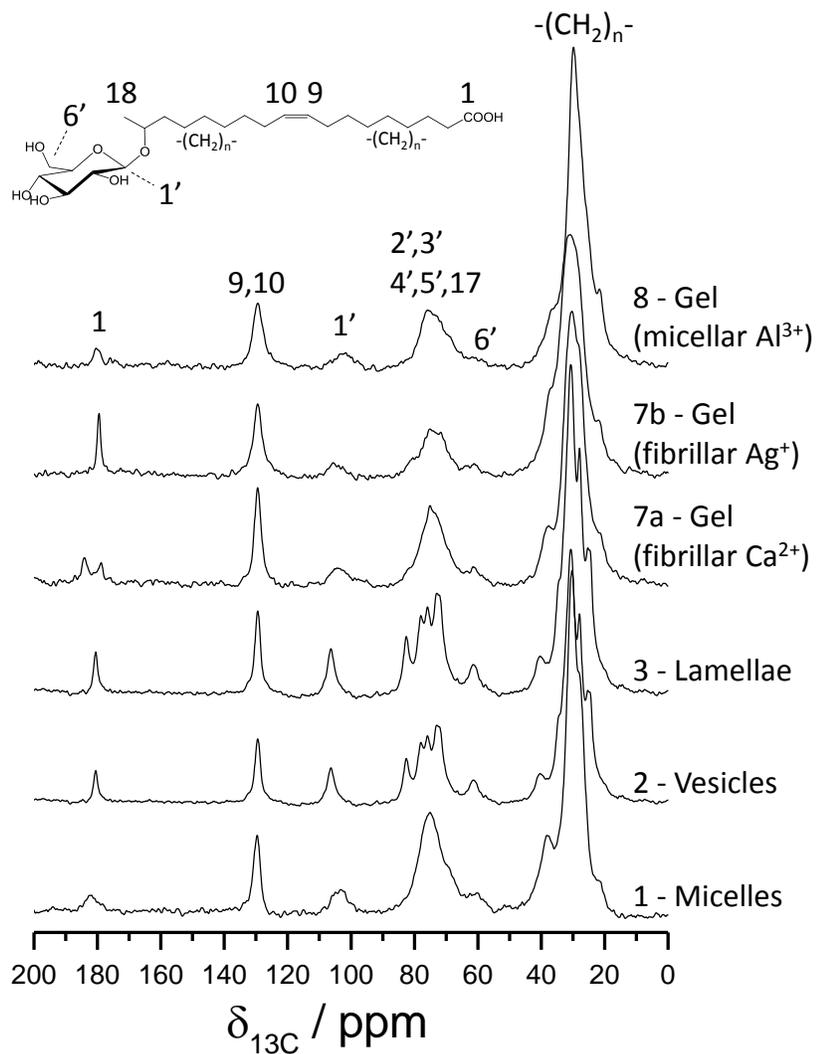


Fig. S1. Solid-state ^{13}C CP MAS NMR spectra recorded on freeze-dried samples prepared from solutions of (1) micelles ($t_c = 1$ ms), (2) vesicles ($t_c = 3$ ms), (3) lamellar precipitate ($t_c = 3$ ms), (7a) Ca^{2+} fiber gel ($t_c = 3$ ms), (7b) Ag^{+} fiber gel ($t_c = 1$ ms), (8) Al^{3+} micellar gel ($t_c = 1$ ms). t_c stands for the contact time related to the magnetization transfer from ^1H to ^{13}C during cross polarization.

Table S2. ^{13}C chemical shift values related to the peaks corresponding to the spectra of various G-C18:1 phases. Spectra are given in Figure S 1. $^{\circ}$: The ^{13}C

	C1	C9,10	C1'	C17	C3'	C5'	C2'	C4'	C6'	C16	C2	-(CH₂)_n-	C8,11	C15	C3	C18
Liquid^o	177.1	130.2	102.7	77.3	76.9	76.3	74.2	70.7	62.2	36.8	34.5	29-30	27.5	25.7	25.3	21.5
8 - Micellar gel Al³⁺	180.1	129.5	102	75.1				69.1	61.2	36.5		29.8	-	-	-	21.5
7b - Fiber gel Ag⁺	179.5	129.5	104.9	75.1				69.1	61.2	37.3		30.7	-	-	-	21.8
7a - Fiber gel Ca²⁺	184.1 178.7	129.5	104.2 96.5	75.1				69.1	61.2	37.3		30.7	28	-	-	21.9
3 - Lamellae	180.5	129.5	106.3	82.5	78.1	75.9	72.6	61.2	40.6	34.4	30.7	27.8	25.3	25.3		
2 - Vesicles	180.6	129.5	106.3	82.5	78.1	75.9	72.6	61.2	40.6	34.4	30.7	27.8	25.3	25.3		
1 - Micelles	182.1	129.5	103.9	75.1				69.1	61.2	38.3		30.7	27.8	-	-	21.6

are reported from ref. ¹⁰.

values
 recorded in the
 liquid
 state
 (solvent
 :
 CDCl₃/
 CD₃OD
 : 7/3)

References

- (1) Baccile, N.; Selmane, M.; Le Griel, P.; Prévost, S.; Perez, J.; Stevens, C. V.; Delbeke, E.; Zibek, S.; Guenther, M.; Soetaert, W.; et al. PH-Driven Self-Assembly of Acidic Microbial Glycolipids. *Langmuir* **2016**, *32*, 6343–6359.
- (2) Baccile, N.; Cuvier, A.-S.; Prévost, S.; Stevens, C. V.; Delbeke, E.; Berton, J.; Soetaert, W.; Van Bogaert, I. N. A.; Roelants, S. Self-Assembly Mechanism of PH-Responsive Glycolipids: Micelles, Fibers, Vesicles, and Bilayers. *Langmuir* **2016**, *32*, 10881–10894.
- (3) Renterghem, L. Van; Guzzetta, F.; Le Griel, P.; Selmane, M.; Messaoud, G. Ben; Teng, T. T. S.; Lim, S.; Soetaert, W.; Roelants, S.; Julián-López, B.; et al. Easy Formation of Functional Liposomes in Water Using a PH-Responsive Microbial Glycolipid: Encapsulation of Magnetic and Upconverting Nanoparticles. *ChemNanoMat* **2019**, *5*, 1188–1201.
- (4) Seyrig, C.; Griel, P. Le; Cowieson, N.; Perez, J.; Baccile, N. Synthesis of Multilamellar Walls Vesicles Polyelectrolyte-Surfactant Complexes from PH-Stimulated Phase Transition Using Microbial Biosurfactants. *J. Colloid Interface Sci.* **2020**, *580*, 493–502.
- (5) Seyrig, C.; Kignelman, G.; Thielemans, W.; Griel, P. Le; Cowieson, N.; Perez, J.; Baccile, N. Stimuli-Induced Non-Equilibrium Phase Transitions in Polyelectrolyte-Surfactant Complex Coacervates. *Langmuir* **2020**, *36*, 8839–8857.
- (6) Poirier, A.; Griel, P. Le; Hoffmann, I.; Perez, J.; Pernot, P.; Fresnais, J.; Baccile, N. Ca²⁺ and Ag⁺ Orient Low-Molecular Weight Amphiphile Self-Assembly into Fibrillar Hydrogels with Unusual β -Sheet-like Structure. *Submitted* **2022**, <https://hal.archives-ouvertes.fr/hal-03576359v1>.
- (7) Poirier, A.; Griel, P. Le; Perez, J.; Hermida-Merino, D.; Pernot, P.; Baccile, N. Metallogels from Glycolipid Biosurfactant. *ACS Sustain. Chem. Eng.* **2022**, DOI: 10.1021/acssuschemeng.2c01860.
- (8) Poirier, A.; Bizien, T.; Zinn, T.; Pernot, P.; Baccile, N. Shear Recovery and Temperature Stability of Ca²⁺ and Ag⁺ Glycolipid Fibrillar Metallogels with Unusual β -Sheet-like Domains. *Soft Matter* **2022**, DOI: 10.1039/d2sm00374k.
- (9) Poirier, A.; Griel, P. Le; Perez, J.; Baccile, N. Cation-Induced Fibrillation of Microbial Glycolipid Biosurfactant Probed by Ion-Resolved In Situ SAXS. *J. Phys. Chem. B* **2022**, DOI: 10.1021/acs.jpcc.2c03739.
- (10) Rau, U.; Heckmann, R.; Wray, V.; Lang, S. Enzymatic Conversion of a Sophorolipid into a Glucose Lipid. *Biotechnol. Lett.* **1999**, *21*, 973–977.