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Supporting Information for:

Highly Stable Oil-in-Water Emulsions with a Gemini Amphiphilic Pseudopeptide

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General experimental and synthetic procedures

Chemicals and starting materials: The solvents such as methanol, dichloromethane, 2-propanol, ethelyene glycol dimethyl ether, chloroform, acetonitirile, tetrahydrofuran, n-pentane, ethyl acetate, aqueous ammonia, conc HCl , borane pyridine complex and reagents such as NaCl, NaOH, $MgSO_4$ and deuterated solvents for NMR such as DMSO, CD_3OD, $CDCl_3$, were purchased from Scharlab. The N-Cbz-Valine, N-hydroxysuccinamide, N,N´dicyclohexylcarbodiimide, HBr/AcOH(33%), diethyl amine, diethyl triamine, dansyl chloride, citric acid and pyrene were purchased from Aldrich. 4-n-Decycloxybenzaldehyde was purchased from abcr GmbH&Co.KG Germany and tri-sodium citrate dihydrate was purchased from E-Merk Germany.
The reagents and solvents were used without further purification exception pyrene was recrystallized. The precursor bis(amido amine) was prepared as previously described [1]

Electron microscopy: Scanning Electron Microscopy was performed either in a LEO 440I or in a JEOL 7001F microscope with a digital camera. Samples were obtained by slow evaporation of a solution of the compounds (~1-2 mg/ml) directly onto the sample holder, and were conventionally coated previous to the measurement. Transmission Electron Microscopy was carried out in a JEOL 2100 microscope at 120 KV. The micrographs were obtained from ~1 mg/ml solutions onto a holey carbon copper grid. The samples were sonicated for 10 minutes previous to the measurement, one drop added onto the grid and collected directly without staining. Atomic Force Microscopy was carried out in a JEOL JSPM 5200 scanning probe microscope with a digital camera. The micrographs were obtained from ~1 mg/ml solutions, previously sonicated for 10 minutes. One drop of such solution was added onto a previously prepared smooth surface mica and left for 24hrs slow evaporation prior to the measurement.

NMR spectroscopy: The NMR experiments were carried out on a Varian INOVA 500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C). Chemical shifts are reported in ppm using TMS as a reference.

Steady-state fluorescence spectroscopy: Steady-state fluorescence spectra were recorded in a Spex Fluorog 3-11 equipped with a 450 W xenon lamp. Fluorescence spectra were recorded in the front face mode. All the samples were measured in aerated conditions. 30 μM 9,10 dimethyl anthracene was prepared by serial water dilution and its intensity was measured at λ excitation = 358 nm.

Optical fluorescence microscopy: Optical microscopy images were captured in Epi-fluorescence microscope N-400FL by a digital USB camera and using 100x / 1.25 oil immersion plan-achromatic objective.

Emulsion preparation: 1.26 mM A4NVal_a10obenz (1) was prepared in acidic 1:1 MeOH :H_2O which then was evaporated by rotavap at 45ºC. 99.4 :0.6 corresponding oil : water mixture was added to the completely dried (1) and was vigorously stirred at room temperature for 30-90 minutes in order to obtain smooth stable emulsion**.**

Calculation of the critical aggregation concentration (CAC) of compound 1: A series of aqueous solutions containing different concentrations of compound **1** (10^{-3} M – 1.5 10^{-7}M) were prepared. Each solution (2 mL) was mixed with a saturated recrystallized pyrene solution (2 mL) contained in small vials. The small vials were tightly sealed at 25 ºC for more than two hours to achieve the equilibrium. Finally, the measurements were carried out by steady state fluorescence spectroscopy and the emission spectra were recorded (λ_{exc} = 352 nm). The CAC was obtained by plotting the ratio between the first and third band emission of the pyrene (I_1/I_3) versus the overall concentration of **1** (in log scale) [2].

Stability studies:

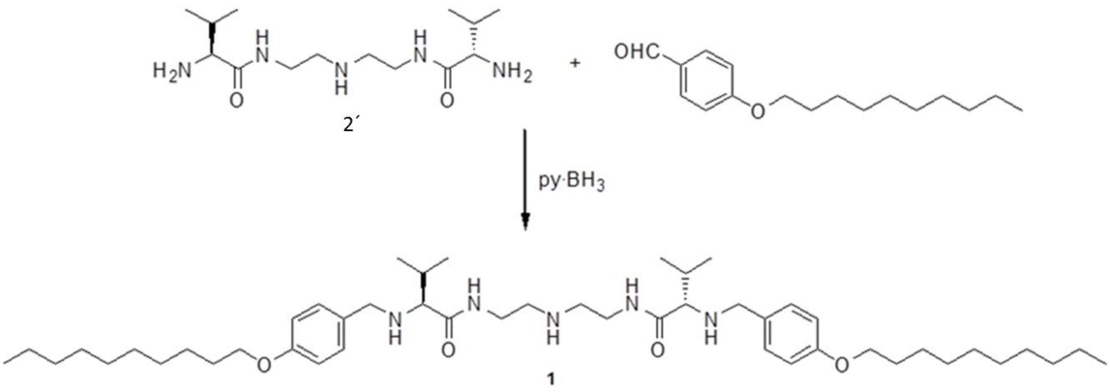
●**Long term effect:** the previously prepared GAPs emulsion was left for more than 8 weeks and every week, the sphere photos were captured by optical microscope**.**

●**Mechanical effect (centrifugal effect):** the previously prepared GAPs emulsion was centrifuged at 3000 rpm for 30 minutes. Every 5 or 15 or 30 minutes, an aliquot was taken for measurement under the optical microscope and its photos were captured

Thermal effect: the previously prepared GAPs emulsion was heated at 45º C for four months previous to its vesicles photos capture.

pH effect: the previously prepared GAPs emulsion was exposed to the acidic and basic effect by the addition of 1 M HCl and 0.1 M NaOH respectively.

Thermolysin effect: 200 µL of thermolysin (1.5 mg / mL) was added to the previously prepared GAPs emulsion. The enzymatically disassembled vesicles photos were captured.



Synthesis of 1. The precursor bis(amido amine) **2´** (298.4 mg, 0.990 mmol) was dissolved in 10 mL of $CHCl_3$ and the solution was placed inside a flask under nitrogen atmosphere. 4-Decyloxybenzaldehyde (563.7 µL, 519.5 mg, 1.980 mmol) was dissolved in 5 mL of $CHCl_3$, this solution was added to the solution of **2´** and then, 5 mL of $CHCl_3$ were added until a final volume of 20 mL (0.05 M final concentration each). The mixture was stirred overnight, then a large excess of py·BH_3 complex, 95% (1052.7 µL, 968.5 mg, 9.900 mmol) was carefully added at 35 ºC, and the mixture was allowed to react for 24 h before being hydrolysed (conc. HCl, to acidity) and evaporated to dryness. The residue thus obtained was dissolved in water, basified with 1N NaOH, and extracted with $CHCl_3$. The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuum*. The product was purified by flash chromatography on silica gel using CH_2Cl_2 as eluent, increasing slowly the polarity with MeOH and several drops of aqueous ammonia.

Yield (535 mg, 68%); mp 78-81 ºC; $[\alpha]_D^{25}$ = -32.4 (c = 0.01, $CHCl_3$); IR (ATR) 3314, 2955, 2920, 2869, 2851, 1640, 1622, 1613, 1546, 1510 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.52 (t, *J* = 5.6 Hz, 2H), 7.18 (d, *J* = 8.6 Hz, 4H), 6.84 (d, *J* = 8.6 Hz, 4H), 3.92 (t, *J* = 6.6 Hz, 4H), 3.68 (d, *J* = 12.9 Hz, 2H), 3.54 (d, *J* = 12.9 Hz, 2H), 3.38 (dd, *J* = 5.9, 11.9 Hz, 4H), 2.94 (d, *J* = 4.6 Hz, 2H), 2.79 (t, *J* = 6.0 Hz, 4H), 2.08 (dtd, *J* = 4.8, 6.9, 13.8 Hz, 2H), 1.76 (m, 4H), 1.44 (m, 4H), 1.27 (m, 24 H), 0.94 (d, *J* = 6.9 Hz, 6H), 0.86 (m, 12H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0, 158.4, 131.7, 129.4, 114.5, 68.2, 67.8, 52.9, 48.9, 38.7, 31.9, 31.4, 29.5, 29.5, 29.4, 29.3, 29.3, 26.0, 22.6, 19.6, 17.7, 14.1 ppm; HRMS (ESI-TOF)$^+$ calc for $C_{48}H_{83}N_5O_4$ (M + H)$^+$: 794.6523; found 794.6530; Anal. calcd. for $C_{48}H_{83}N_5O_4$: C, 72.6; H, 10.5; N, 8.8. Found: C, 72.5; H, 10.7; N, 8.9.

Figure S1. Spectral characterization of compound **1**: (A) ^{1}H NMR spectrum (500 MHz, 303 K, 20 mM in CDCl$_3$), (B) ^{13}C NMR spectrum (125 MHz, 303 K, 20 mM in CDCl$_3$), (C) Simulated (top) and experimental (down) HRMS (ESI^{+}-MS).

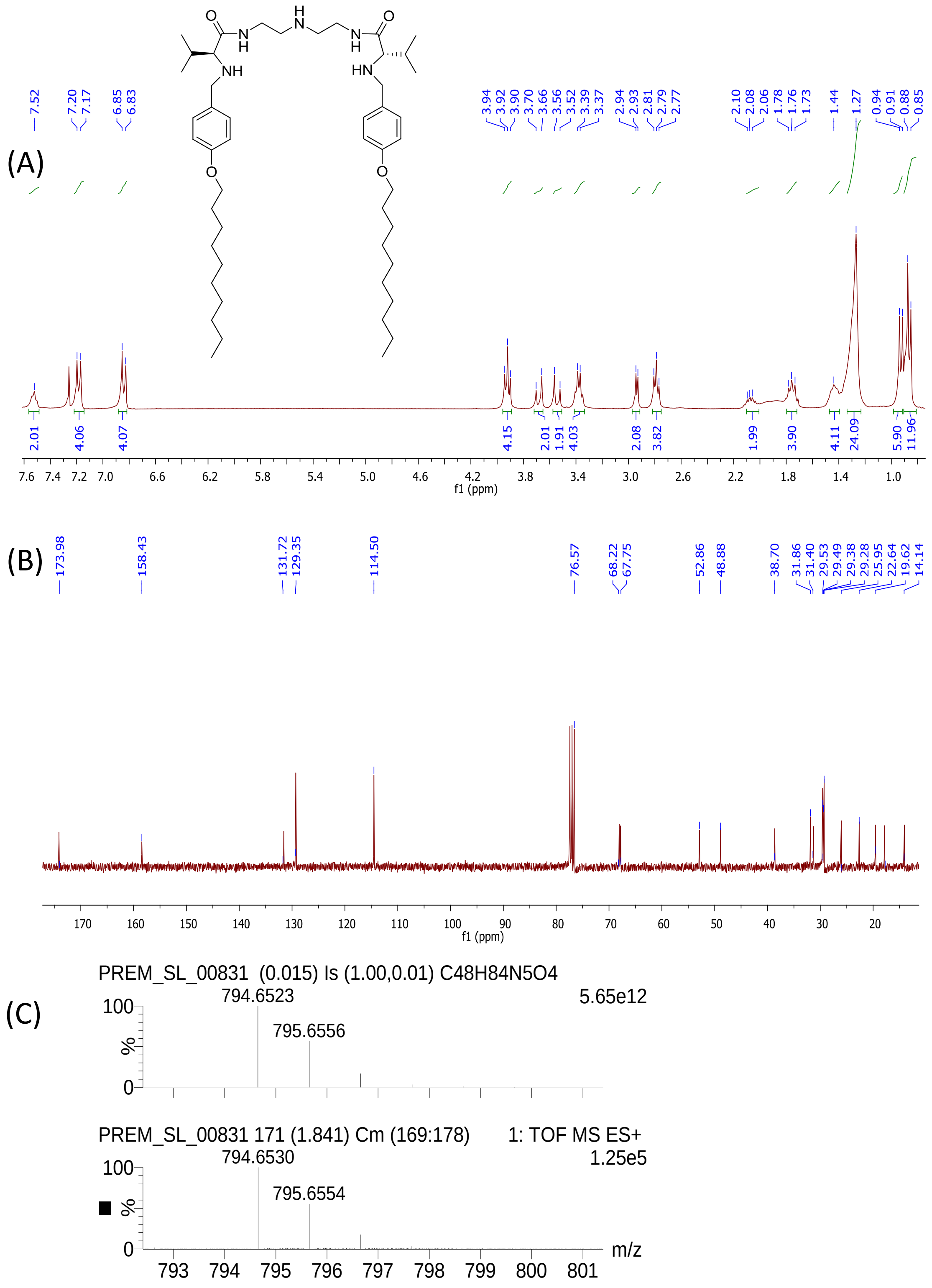
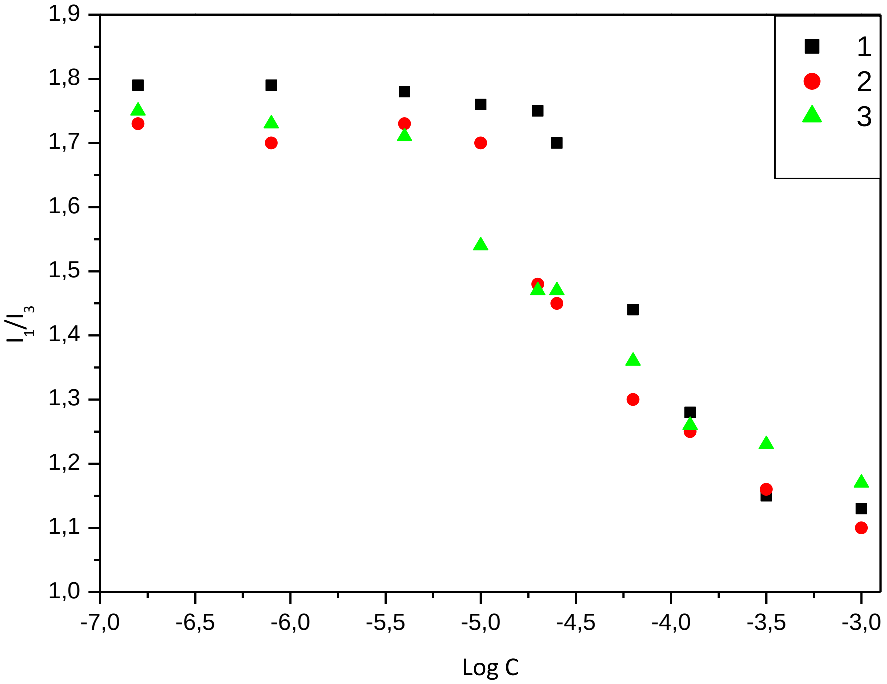
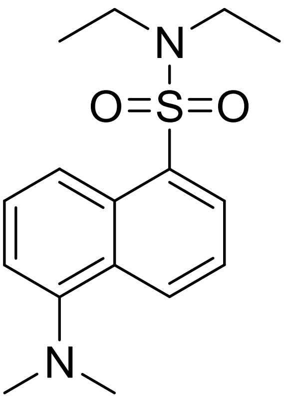


Figure S2. Change in the I_1/I_3 fluorescence ratio of pyrene as a function of the concentration of compound **1;** (1) in 1 µM citrate buffer at pH 6.8, (2) in 0.1 M citric acid with 0.1M NaCl at pH 2.2, (3) in 0.1 M citric acid with 1M NaCl at pH 2.2.



[1] A) J. Rubio, I. Alfonso, M. Bru, M. I. Burguete and S. V. Luis, *Tetrahedron Lett.*, **2010**, 51, 5861. B) J. Rubio, I. Alfonso, M. I. Burguete and S. V. Luis, *Soft Matter*, **2011**, 7, 10737.

[2] A) K. S. Sharma, C. Rodgers, R. M. Palepu and A. K. Rakshit, J. Colloid Interface Sci., **2003**, 268, 482. B) T. Yoshimura, T. Ichinokawa, M. Kaji and K. Esumi, *Colloids Surf., A*, **2006**, 273, 208.



the *N*,*N*-diethyldansylamide **2**

Synthesis of 2. 0.275 g Dansyl chloride (yellowish solid, 1 mmol) was dissolved in 10 mL dried acetonitrile and added drop wise to an acetonitrile solution of diethyl amine (1 mmol).This mixture was reflux at 89 ºC over the night. A few drops 1N NaOH was added to basify the medium and thus the residue obtained was extracted with $CHCl_3$.The combined organic layers were evaporated by the rotavap and then dried *in vacuum* in order to get the semi-oily (pasty) greenish black compound **2** [3].

Yield (65%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.23 (d, *J* = 8.7 Hz, 1H), 8.11 (d, *J* = 7.3 Hz, 1H), 7.44 (m, 2H), 7.10 (d, *J* = 7.5 Hz, 1H), 3.30 (d, *J* = 7.1 Hz, 4H), 2.81 (s, 6H), 1.02 (d, *J* = 7.2 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.6 (s), 135.6 (s), 130.1 (m), 129.4 (s), 127.8 (d, *J* = 14.4 Hz), 123.1 (d, *J* = 15.3 Hz), 119.7 (s), 115.1 (d, *J* = 12.7 Hz), 45.4 (q, *J* = 10.0 Hz), 40.9 (t, *J* = 8.3 Hz), 13.7 (q, *J* = 8.9 Hz); ESI-MS *m*/*z* 307.3 ($M+H^+$), 329.2 ($M+Na^+$), 345.2 ($M+K^+$).

[3] R. R. Naredla, D. A. Klumpp, *Tetrahedron Lett*, **2013,** 54, 5945.

Figure S3. SEM micrographs of **1** grown from 1:1 MeOH : H_2O at acidic pH values. The corresponding scale bars are shown for every picture

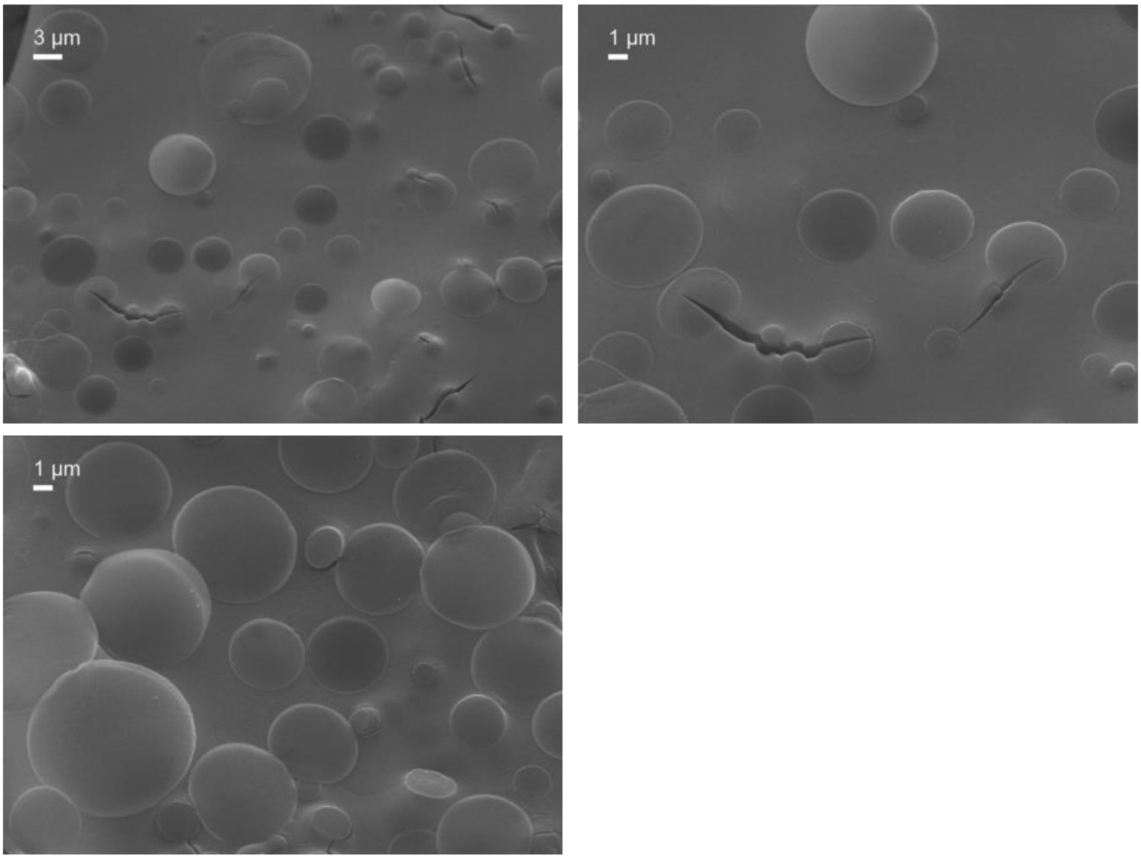


Figure S4. TEM micrographs of **1** grown from 1:1 MeOH : H_2O at acidic pH values. The corresponding scale bars are shown for every picture.

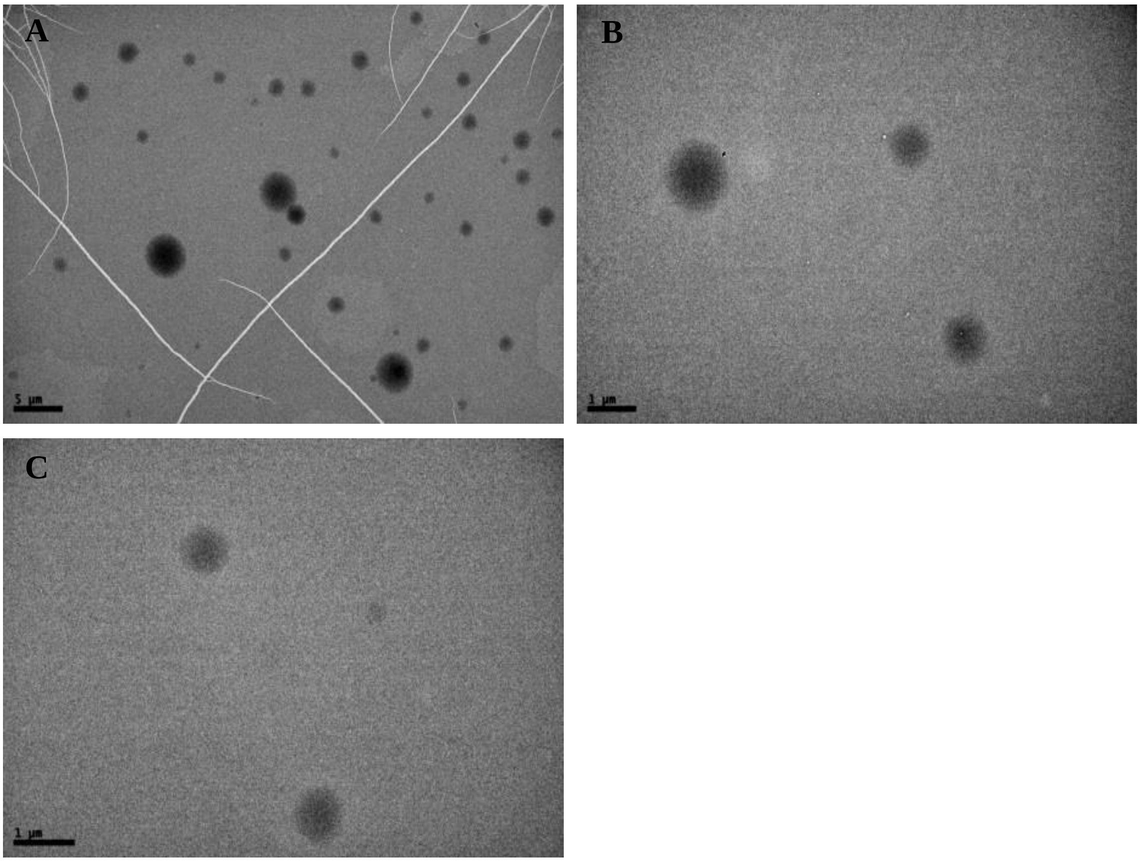


Figure S5. AFM micrographs of **1** grown from 1:1 MeOH : H_2O at acidic pH values. The corresponding scale bars are shown for every picture

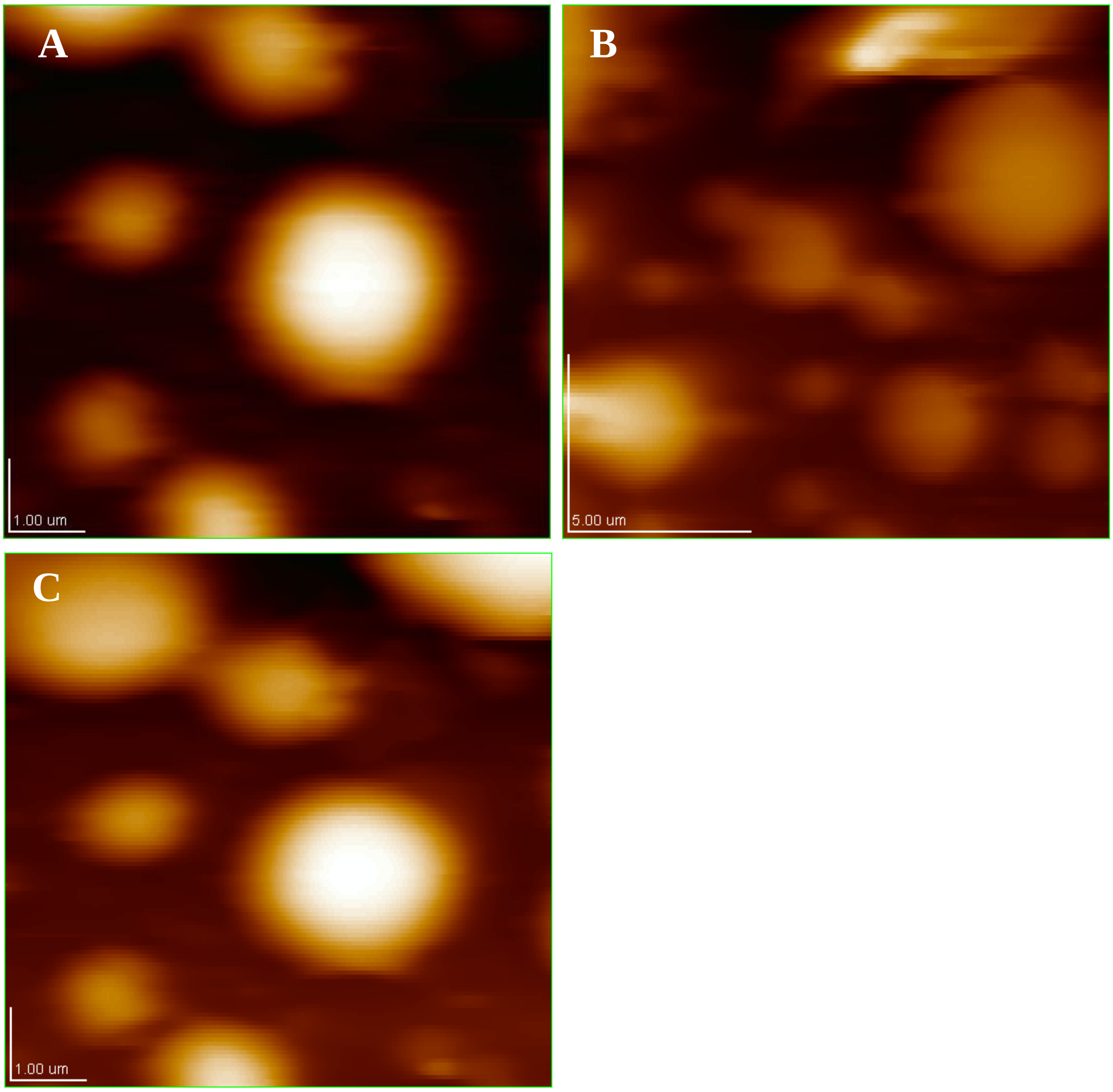
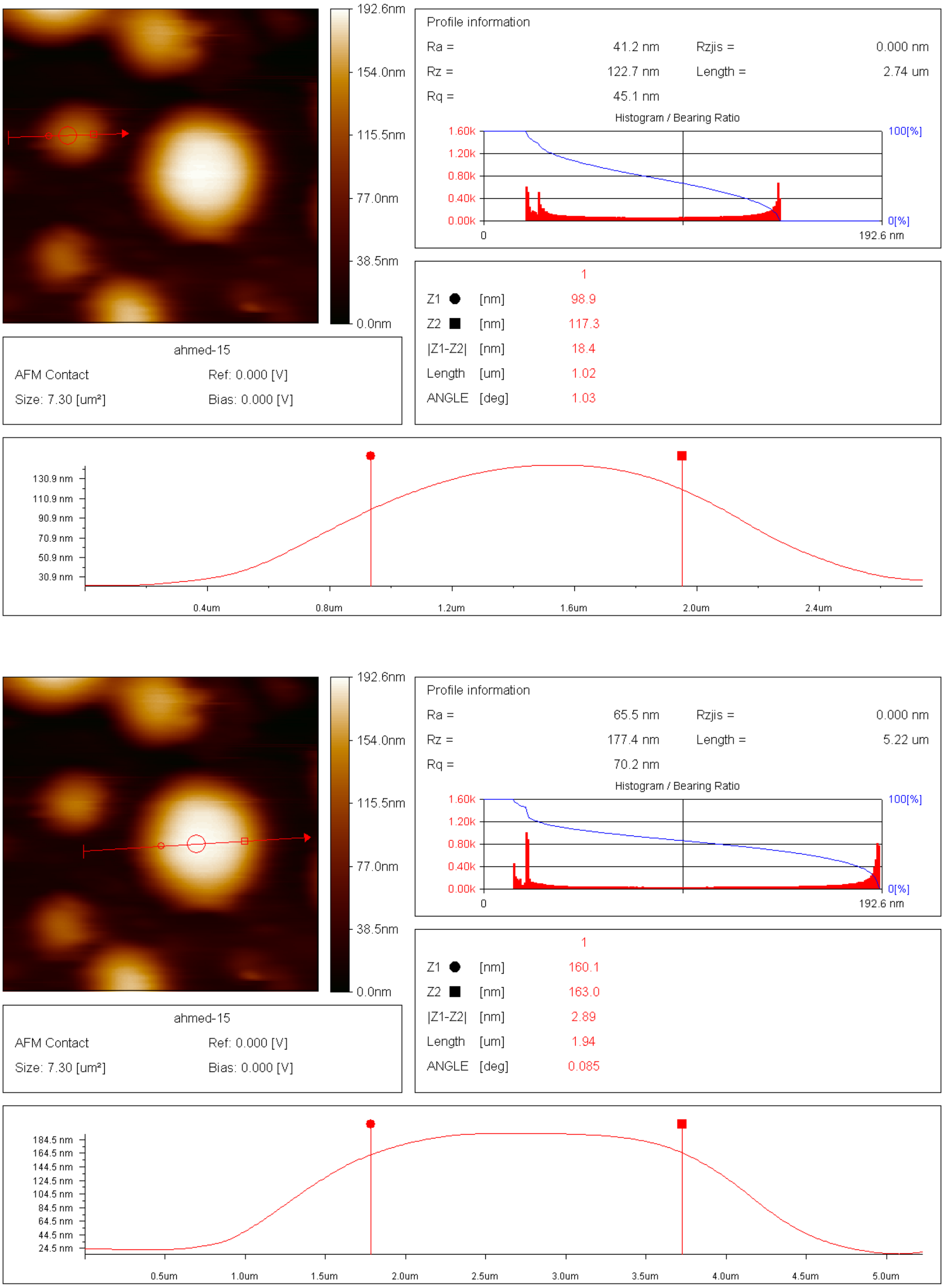


Figure S6. AFM profile analysis of two selected vesicles showing the width and height of the microstructures



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AFM Contact Ref: 0.000 [V]

Size: 7.30 [um²] Bias: 0.000 [V]

Profile information

Ra = 41.2 nm Rzjis = 0.000 nm

Rz = 122.7 nm Length = 2.74 um

Rq = 45.1 nm

		1
Z1 ●	[nm]	98.9
Z2 ■	[nm]	117.3
|Z1-Z2|	[nm]	18.4
Length	[um]	1.02
ANGLE	[deg]	1.03

ahmed-15

AFM Contact Ref: 0.000 [V]

Size: 7.30 [um²] Bias: 0.000 [V]

Profile information

Ra = 65.5 nm Rzjis = 0.000 nm

Rz = 177.4 nm Length = 5.22 um

Rq = 70.2 nm

		1
Z1 ●	[nm]	160.1
Z2 ■	[nm]	163.0
|Z1-Z2|	[nm]	2.89
Length	[um]	1.94
ANGLE	[deg]	0.085

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Figure S7. Optical microscopy pictures of the oil/water (o+w) mixtures alone (A) and after the addition of the GAP **1** and vigorous stirring for 30-90 minutes (B)

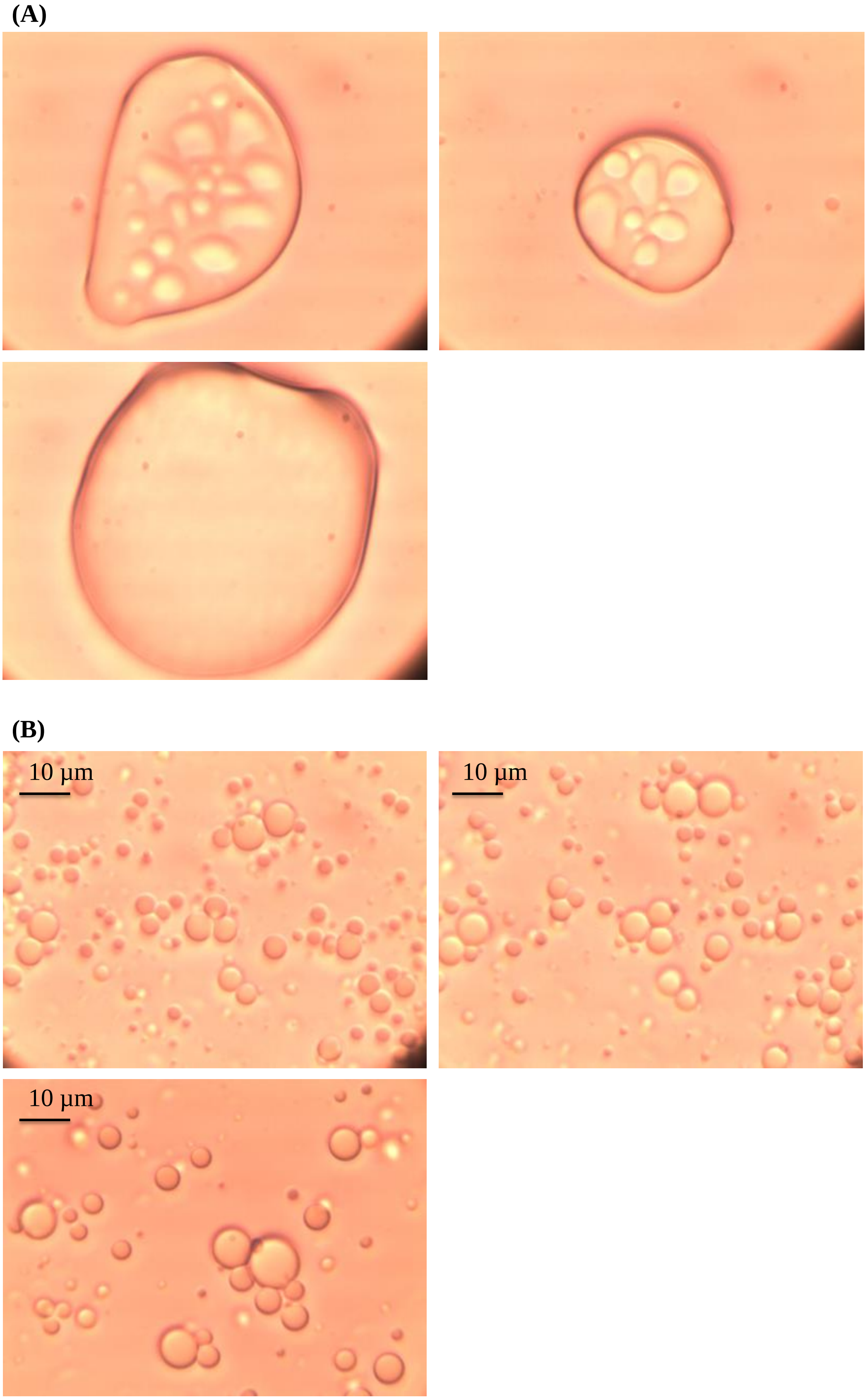

Figure S8. Optical microscopy images of the emulsion formed by GAP **1** after one, two, three, four, five, six, seven and eight weeks.

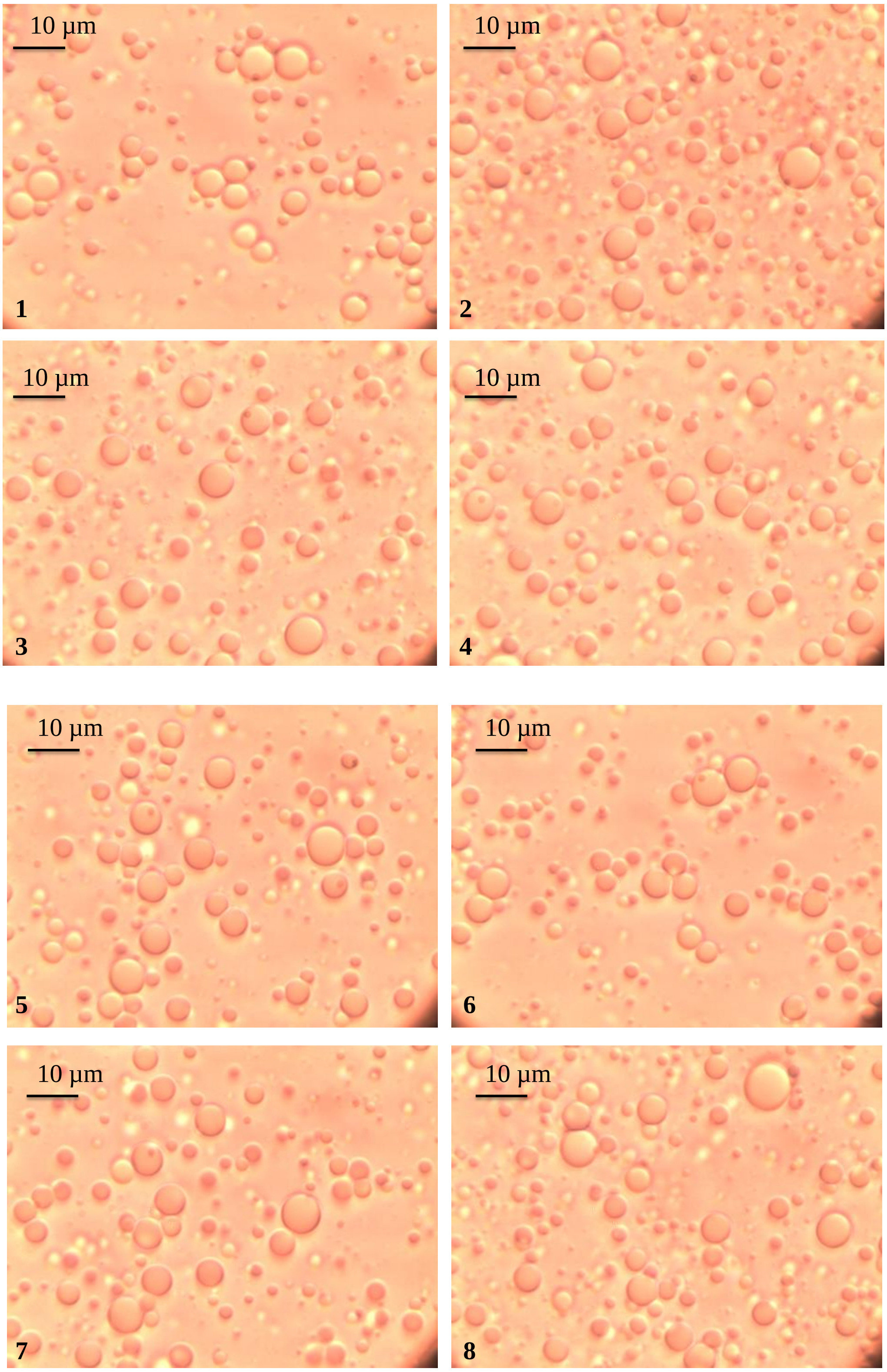

Figure S9. Optical microscopy of o/w emulsion formed by GAP **1** after centrifugation at 3000 rpm for 30 minutes; mechanical stability test (A) 5 min, (B) 10 min, (C) 15 min, (D) 20 min, (E) 25 min and (F) 30 min.

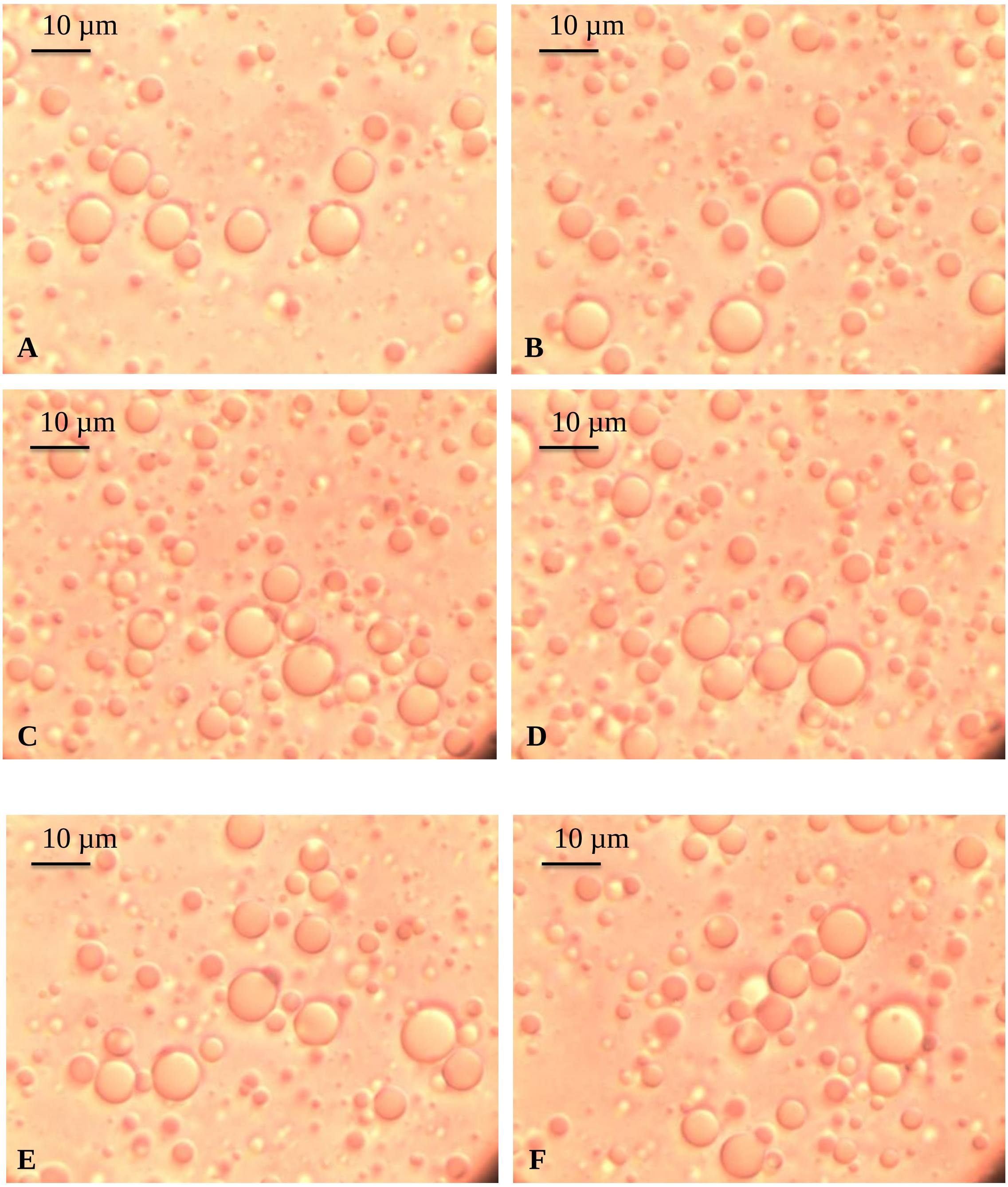

Figure S10. Optical microscopy of o/w emulsion formed by GAP **1** after four months heating at 45ºC.

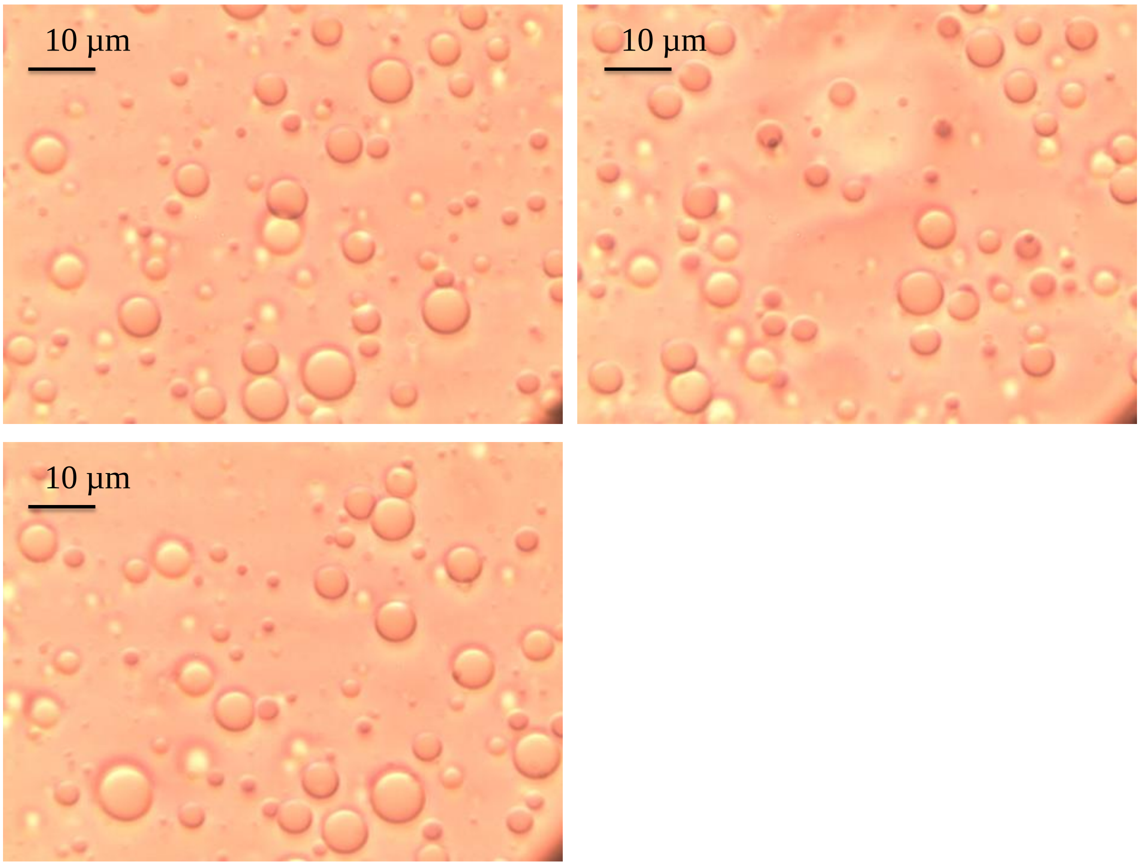

Figure S11. Optical microscopy of o/w emulsion formed by GAP **1** in acidic medium: pH 4 (A),pH 3 (B), pH 2 (C), pH 1 (D) and pH 0 (E&F)

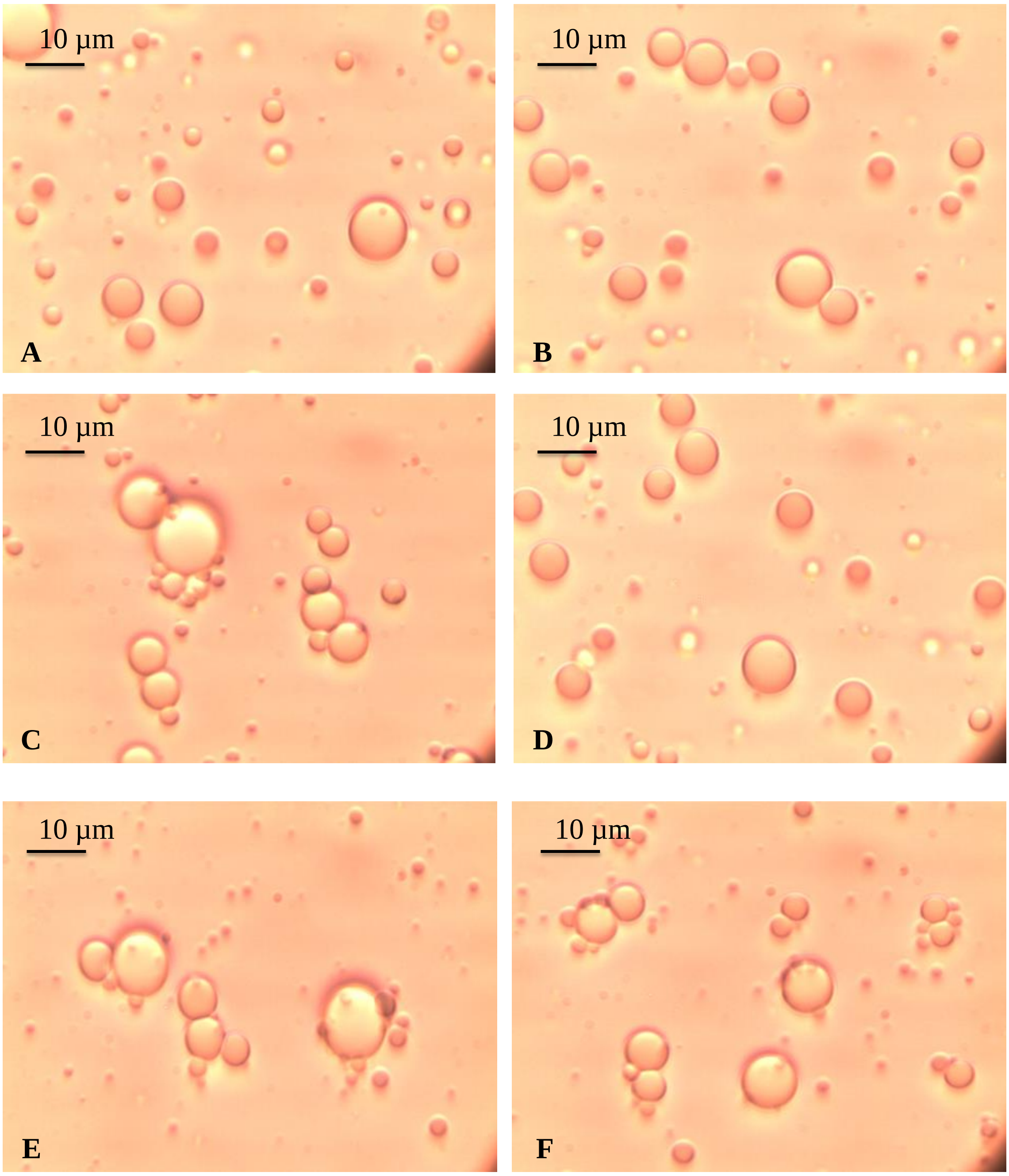

Figure S12. Optical microscopy of o/w emulsion formed by GAP **1** in basic medium i.e. pH 10

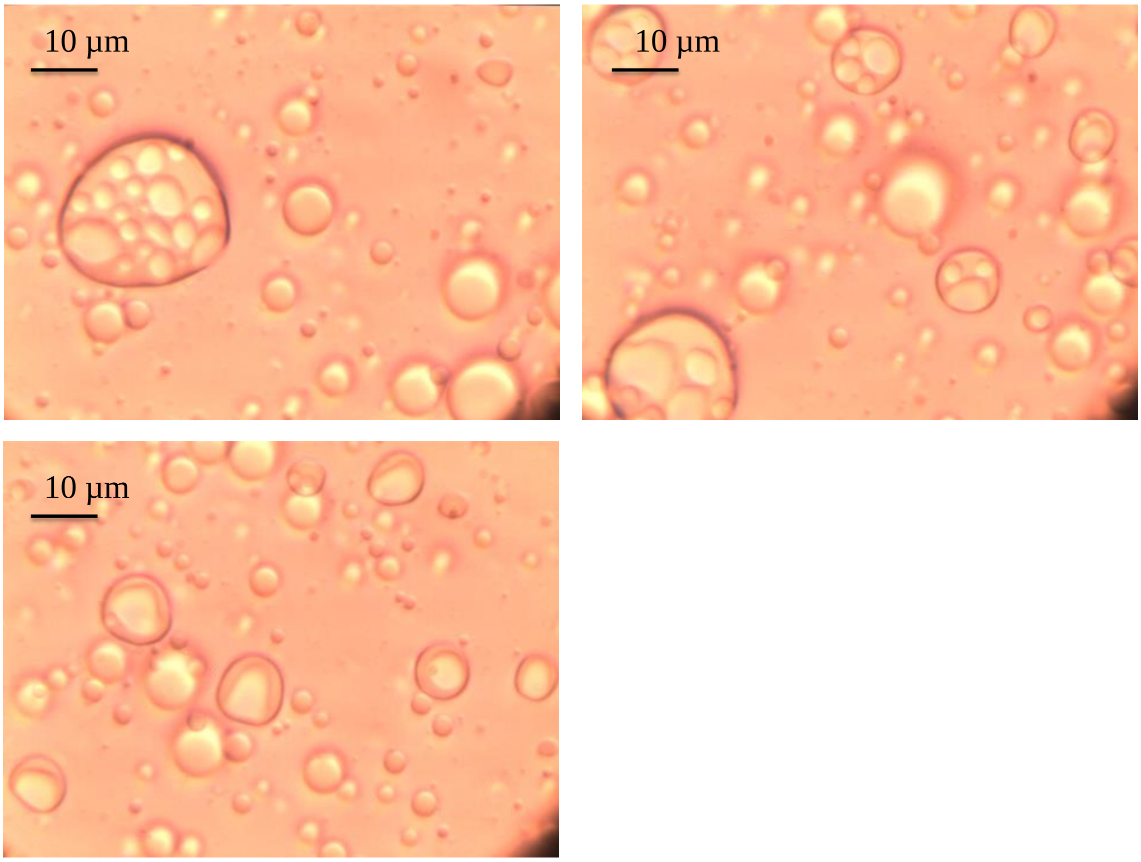

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Figure S13. Optical microscopy of o/w emulsion formed by GAP **1** after incubation with thermolysin (1.5 mg/mL).

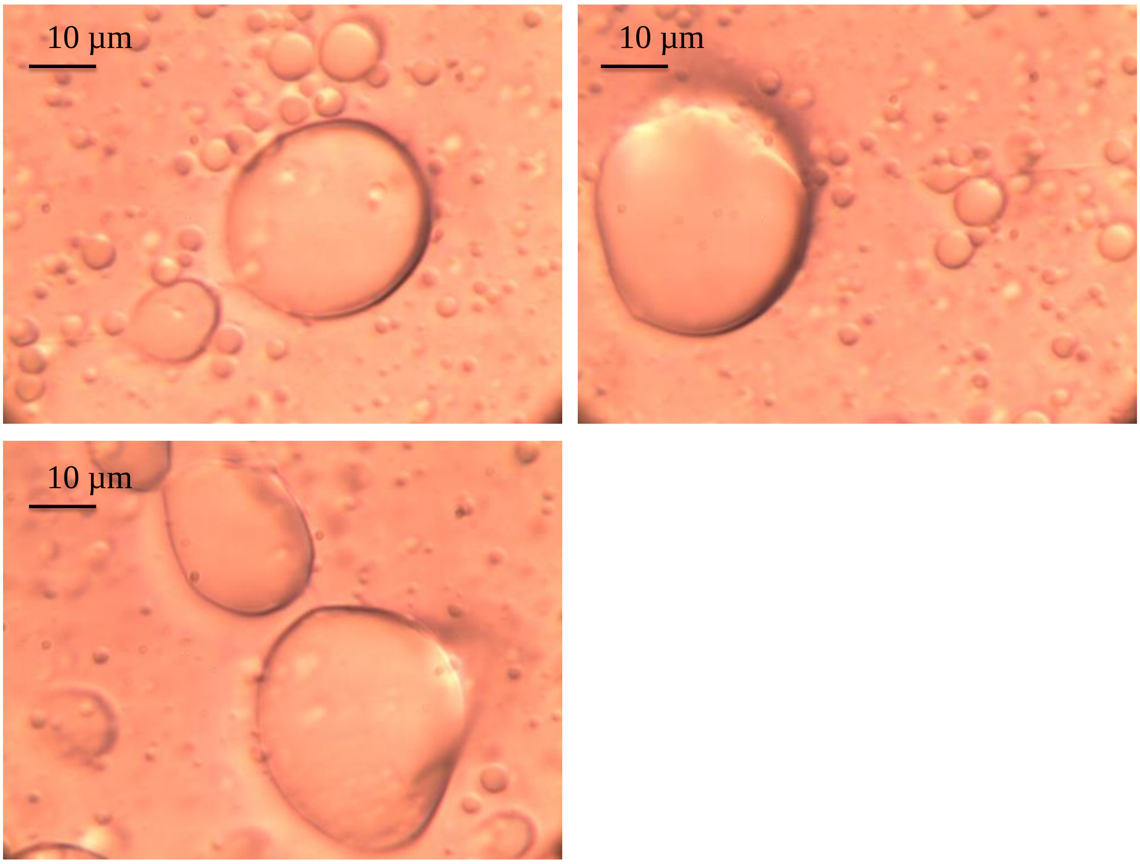

Figure S14. Size distribution diagram of GAPs vesicles in o/w emulsion