Supplementary Data

Folate-conjugated stealth archaeosomes for targeted delivery of novel antitumoral peptides

Ahmad Jiblaoui,[a,c] Julie Barbeau,[a,c] Thomas Vivès,[a,c] Patrick Cormier,[b] Virginie Glippa,[b] Bertrand Cosson,[b,d] and Thierry Benvegnu*[a,c]

[a]. Ecole Nationale Supérieure de Chimie de Rennes, UMR CNRS 6226, Equipe « Chimie Organique et Supramoléculaire », 11 allée de Beaulieu, - CS 50837, 35708, Rennes Cedex 7 (France)
[b]. Sorbonne Universités, UPMC Univ Paris 06, UMR CNRS 8227, Integrative Biology of Marine Models, Translation Cell Cycle and Development, Station Biologique de Roscoff, CS 90074, 29688 Roscoff Cedex (France)
[c]. Université Bretagne et Loire (France)
[d]. Present address: Univ Paris Diderot, Sorbonne Paris Cité, Epigenetics and Cell Fate, UMR 7216, Paris F-75013 (France)
*Corresponding author: Thierry.Benvegnu@ensc-rennes.fr

Development of an analytical LC-MS method to quantify peptides A1 and A1Yala encapsulated in the formulation mixtures.

1-ESI-MS characterization of peptides A1 and A1Yala (Figure 1, Figure 2).

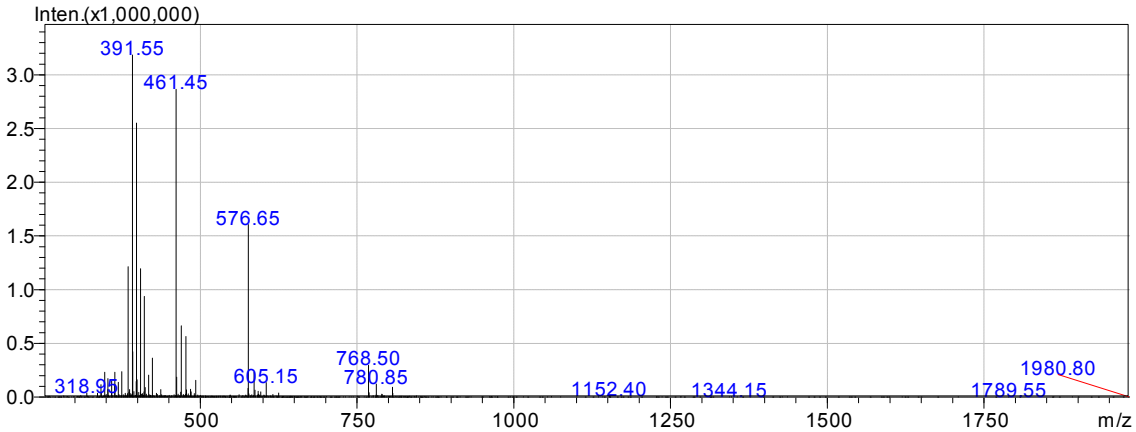


Figure 1. *ESI-MS spectrum of peptide A1.*M = 2301.4721 Da ; m/z = 384.8 : M+ 6H$^+$; m/z = 461.6 : M+ 5H$^+$; m/z = 576.8 : M+ 4H$^+$; m/z = 768.7 : M+3H$^+$; m/z = 1152.5 : M+3H$^+$.

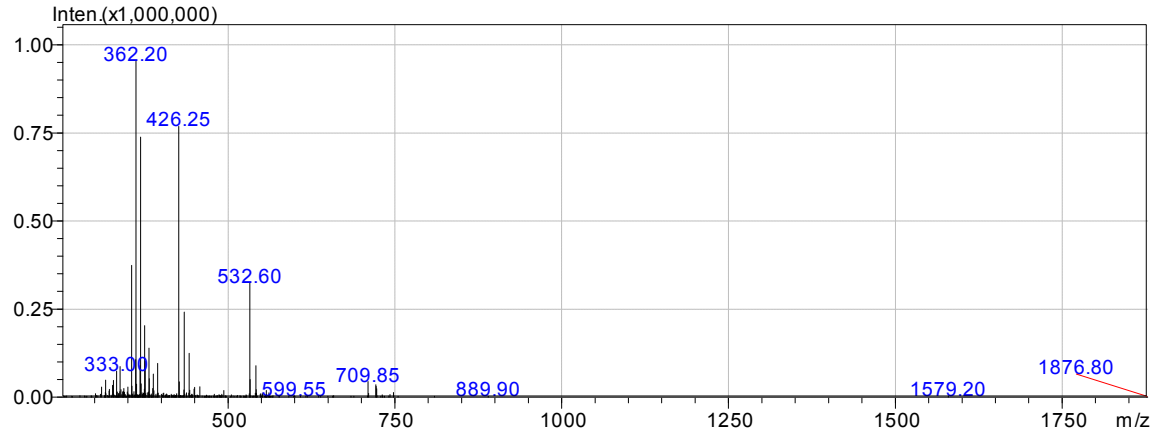


Figure 2. *ESI-MS spectrum of peptide A1Yala.* M = 2125.1821 Da ; m/z = 359.2 : M+5H$^+$+ Na$^+$; m/z = 362.8 : M+ 4H$^+$+ 2Na$^+$; m/z = 426.3 : M+ 5H$^+$; m/z = 532.6 : M+ 4H$^+$; m/z = 362.8 : M+ 3H$^+$.

2-Development of HPLC conditions for quantitative peptide analysis.

The separation conditions described below were used to isolate peptides A1 and A1Yala from lipids (Egg PC, **8**, **9**) present in the mixture after the formulation step.

HPLC-materials:

HPLC Prominence with LCMS2020 from Shimadzu

Acclaim column 120 C18 5µm 120A 2.1x100 mm

Separation method:

Route A: H_2O + 0.1% HCOOH

Route B: ACN + 0.1% HCOOH

Flow: 0.4 mL/min

Gradient mode:

Time (min)	% route B
0	5
4	5
20	60
25	100
35	100
40	5
45	5

Acquisition time: 45 min

Detection: ESI -LCMS

3 events: 1-SCAN (+) 250-2000

2- SIM (+) 760.6 / 461.45 /426.3

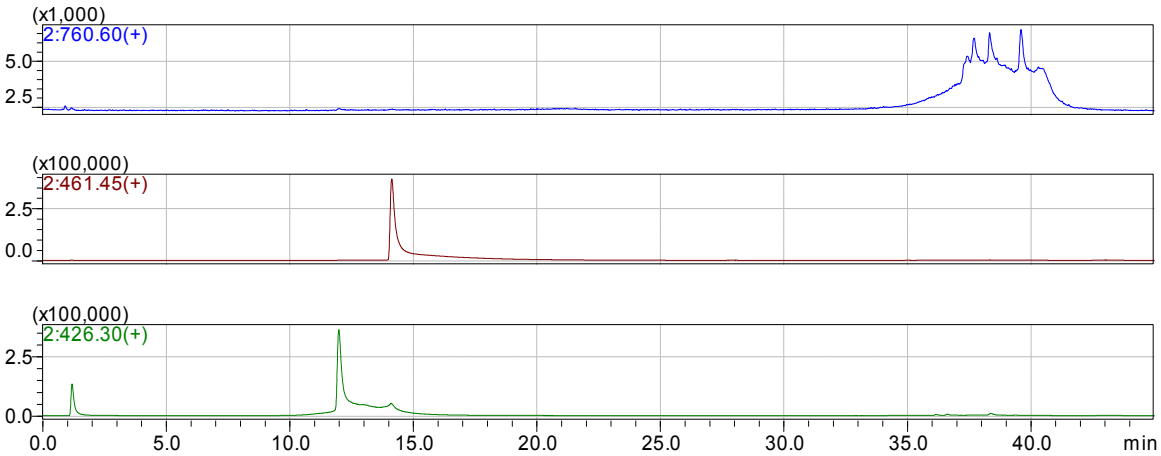


Figure 3. HPLC Chromatograms of Egg PC (blue), A1 (brown) and A1Yala (green).

3- Development of the calibration curve.

In order to quantify the amount of peptide encapsulated in archaeosomes, a calibration LC-MS curve was setting up by plotting the chromatogram area of peptide detected by LC-MS in function of the real concentration. The concentrations of each peptide was ranged from 5 to 20 µM (Figure 4, Figure 5).

ID# : 1
m/z : 461.45
Name Compound : peptide A1-WT
Quantitative Method : External Standard

Function :
f(x) = 4.15462e+006*x-1.24053e+007
Rr1 = 0.9995659
Rr2 = 0.9991319

FitType : Linear
ZeroThrough : Not Through
Weighted Regression : None

#	Data File Name	Conc std	Area
3	TVS99-A1-5_006.lcd	5 µM	8165360
4	TVS99-A1-10_008.lcd	10 µM	29949109
5	TVS99-A1-15_010.lcd	15 µM	48905156
6	TVS99-A1-20_012.lcd	20 µM	71090345

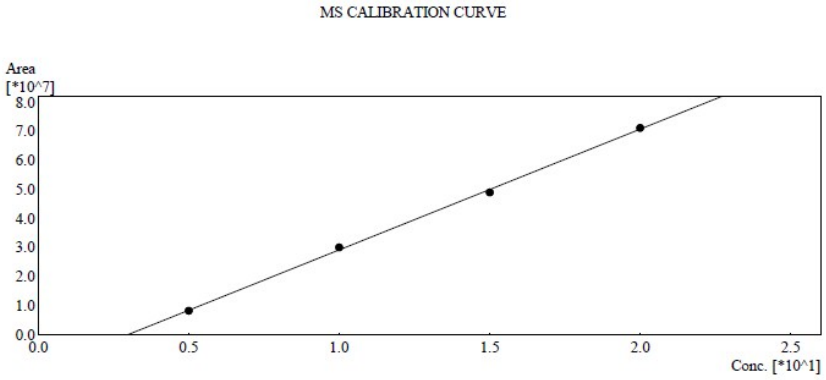


Figure 4. LC-MS calibration curve of peptide A1 ranging from 5 to 20 µM. [*10^1] and [*10^7] correspond to [x 10^1] and [x 10^7] respectively.

ID# : 1
m/z : 426.30
Name Compound : A1-YALA
Quantitative Method : External Standard

Function :
f(x) = 3.26457e+006*x-8.39092e+006
Rr1 = 0.9998789
Rr2 = 0.9997578

FitType : Linear
ZeroThrough : Not Through
Weighted Regression : None

#	Data File Name	Conc std	Area
4	TVS99-A1-YALA-5_008.lcd	5 µM	7799935
5	TVS99-A1-YALA-10_009.lcd	10 µM	24220651
6	TVS99-A1-YALA-15_010.lcd	15 µM	41041663
7	TVS99-A1-YALA-20_011.lcd	20 µM	56602355

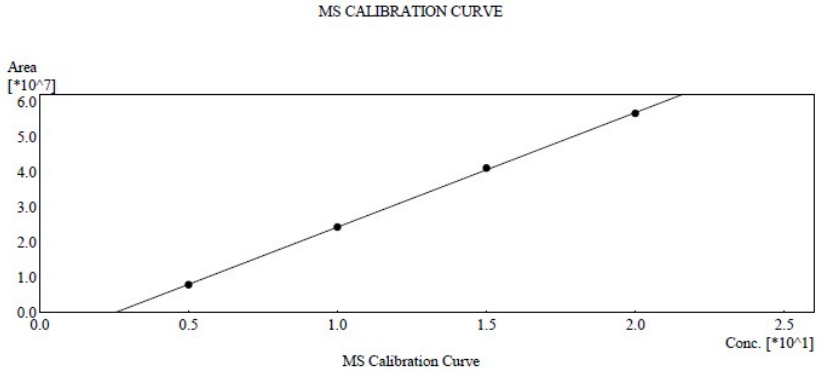
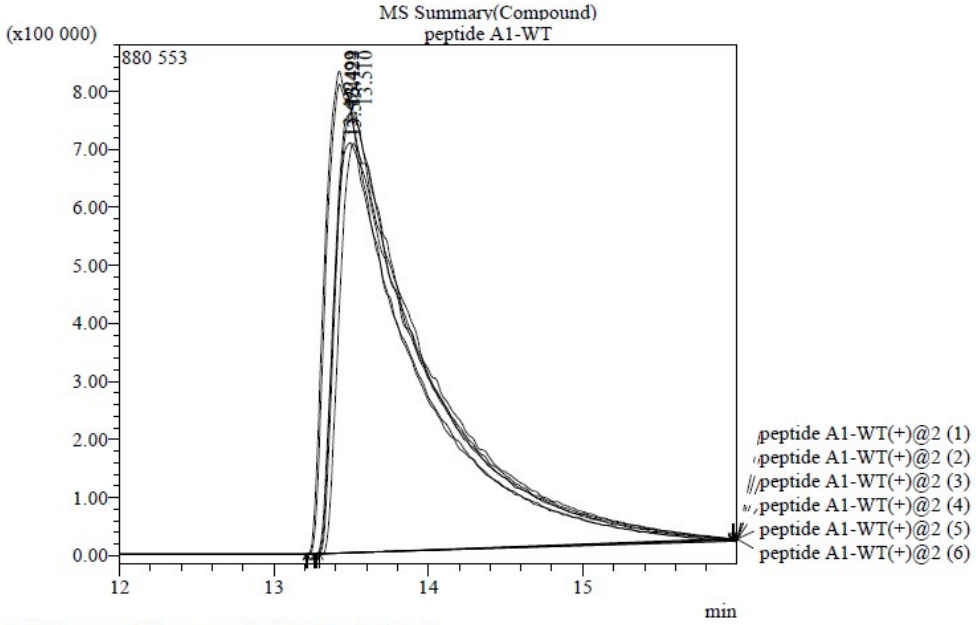


Figure 5. LC-MS calibration curve of peptide A1Yala ranging from 5 to 20 µM. [*10^1] and [*10^7] correspond to [x 10^1] and [x 10^7] respectively.

4- Reproducibility and accuracy of results.

In order to ensure that the developed LC-MS analytical method is reproducible and accurate, we injected the same sample of each peptide 6 times to control the exactitude of the measured area of the chromatogram (Figure 6, Figure 7). Also, we opted to realize the calibration curve experiments and to quantify the desired peptide on the same day.

Accuracy of the injection

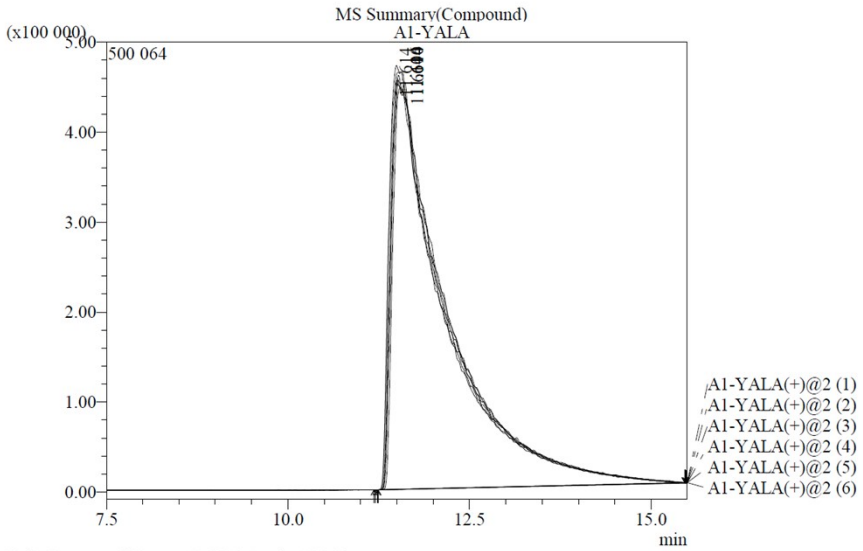


ID#1 Compound Name: peptide A1-WT m/z: 461.45

Title	Sample Name	Ret. Time	Area	Height	Conc.
TVS99-A1-10_014.lcd	TVS99-A1-10	13.422	30835773	829970	10.408
TVS99-A1-10_016.lcd	TVS99-A1-10	13.424	30638657	807757	10.360
TVS99-A1-10_018.lcd	TVS99-A1-10	13.510	30009899	760056	10.209
TVS99-A1-10_020.lcd	TVS99-A1-10	13.499	29903693	758267	10.184
TVS99-A1-10_022.lcd	TVS99-A1-10	13.492	29610520	706151	10.113
TVS99-A1-10_024.lcd	TVS99-A1-10	13.514	29385397	705617	10.059
Average		13.477	30063990	761303	10.222
%RSD		0.314	1.894	6.705	1.341
Maximum		13.514	30835773	829970	10.408
Minimum		13.422	29385397	705617	10.059

Figure 6. Control of the accuracy of the measured area values by injecting the same sample of A1 6 times.

Accuracy of the injection



ID#1 Compound Name: A1-YALA m/z: 426.30

Title	Sample Name	Ret. Time	Area	Height	Conc.
TVS99-A1-YALA-10_01	TVS99-A1-YALA-1(	11.614	24210961	439995	9.987
TVS99-A1-YALA-10_01	TVS99-A1-YALA-1(	11.614	24180945	426911	9.977
TVS99-A1-YALA-10_01	TVS99-A1-YALA-1(	11.606	24242803	440621	9.996
TVS99-A1-YALA-10_01	TVS99-A1-YALA-1(	11.614	24091062	431897	9.950
TVS99-A1-YALA-10_01	TVS99-A1-YALA-1(	11.610	24310467	451865	10.017
TVS99-A1-YALA-10_01	TVS99-A1-YALA-1(	11.614	23890763	434356	9.889
Average		11.612	24154500	437607	9.969
%RSD		0.027	0.613	1.981	0.455
Maximum		11.614	24310467	451865	10.017
Minimum		11.606	23890763	426911	9.889

Figure 7. Control of the accuracy of the measured area values by injecting the same sample of A1Yala 6 times.