Characterization of different milk protein aggregates by Asymmetrical Flow Field-Flow Fractionation coupled with Multiangle Laser Light Scattering (A4F-MALLS)

Thibault Loiseleux, Agnès Rolland-Sabaté, Catherine Garnier, Thomas Croguennec, Sophie Guilois-Dubois, Marc Anton, Alain Riaublanc

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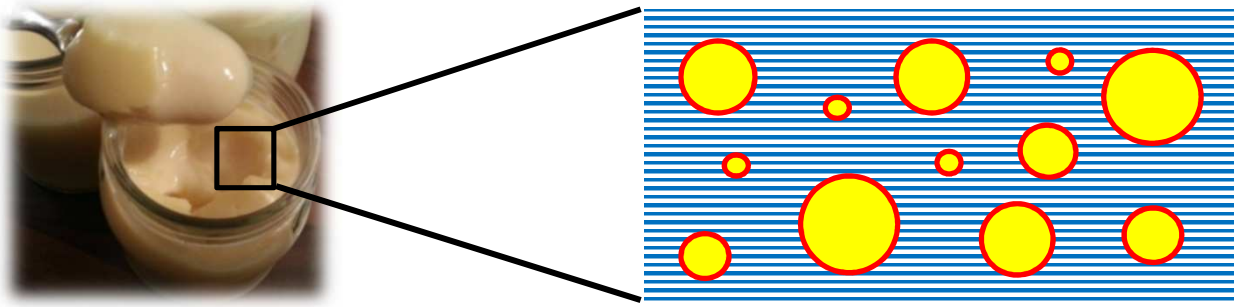
Journée Scientifique
du Groupe Francophone de Fractionnement Flux-Force
31 mars 2017

Contexte

Milk Valley PROFIL

11 Industriels

6 Scientifiques

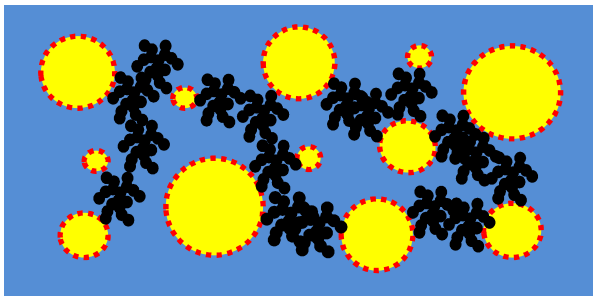


Emulsion H/E

Gélification de la phase **continue**

Objectifs

✓ Utiliser les **gouttes de matière grasse (MG)** comme des nœuds pour texturer le produit sans utiliser d'agents de texture



✓ Utiliser des **agrégats protéiques (Ag)** pour stabiliser l'interface et connecter les gouttes de MG

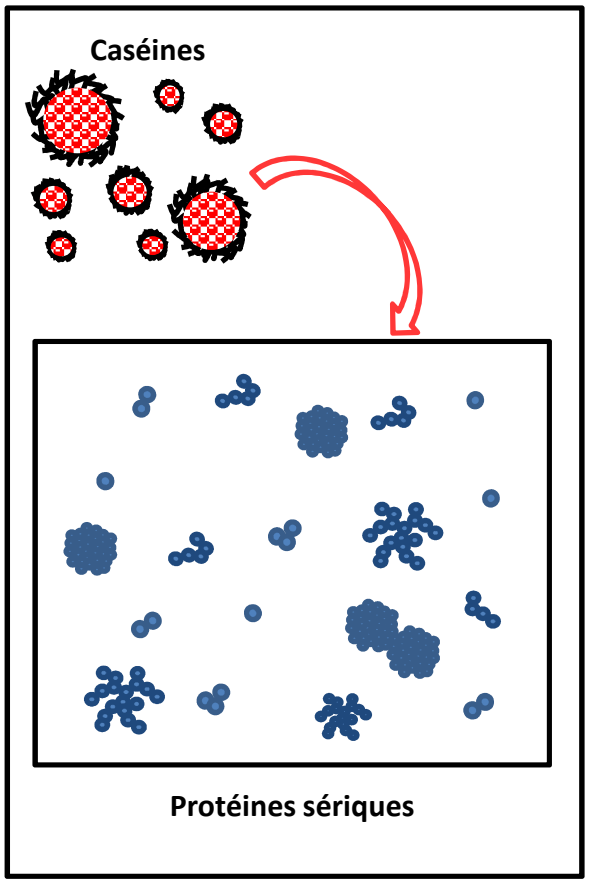
Objectifs

Eliminer les texturants de type polysaccharides

Texturer des produits à faibles taux de MG

Problématiques techniques

Agrégats de protéines laitières



Mélange monomères/agrégats

Systèmes polydispers

Différente taille/forme/densité

DLS

- ✓ Nous voyons uniquement les grosses particules
- ✓ Pas de différenciation entre les grosses particules

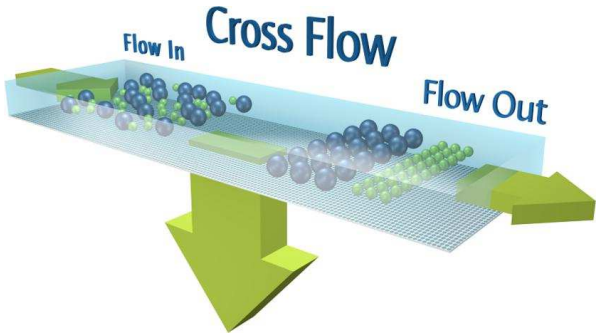
SEC

- ✓ Les caséines interagissent avec la colonne
- ✓ Nous voyons les monomères alors que les agrégats sortent dans le volume mort ou restent dans la colonne

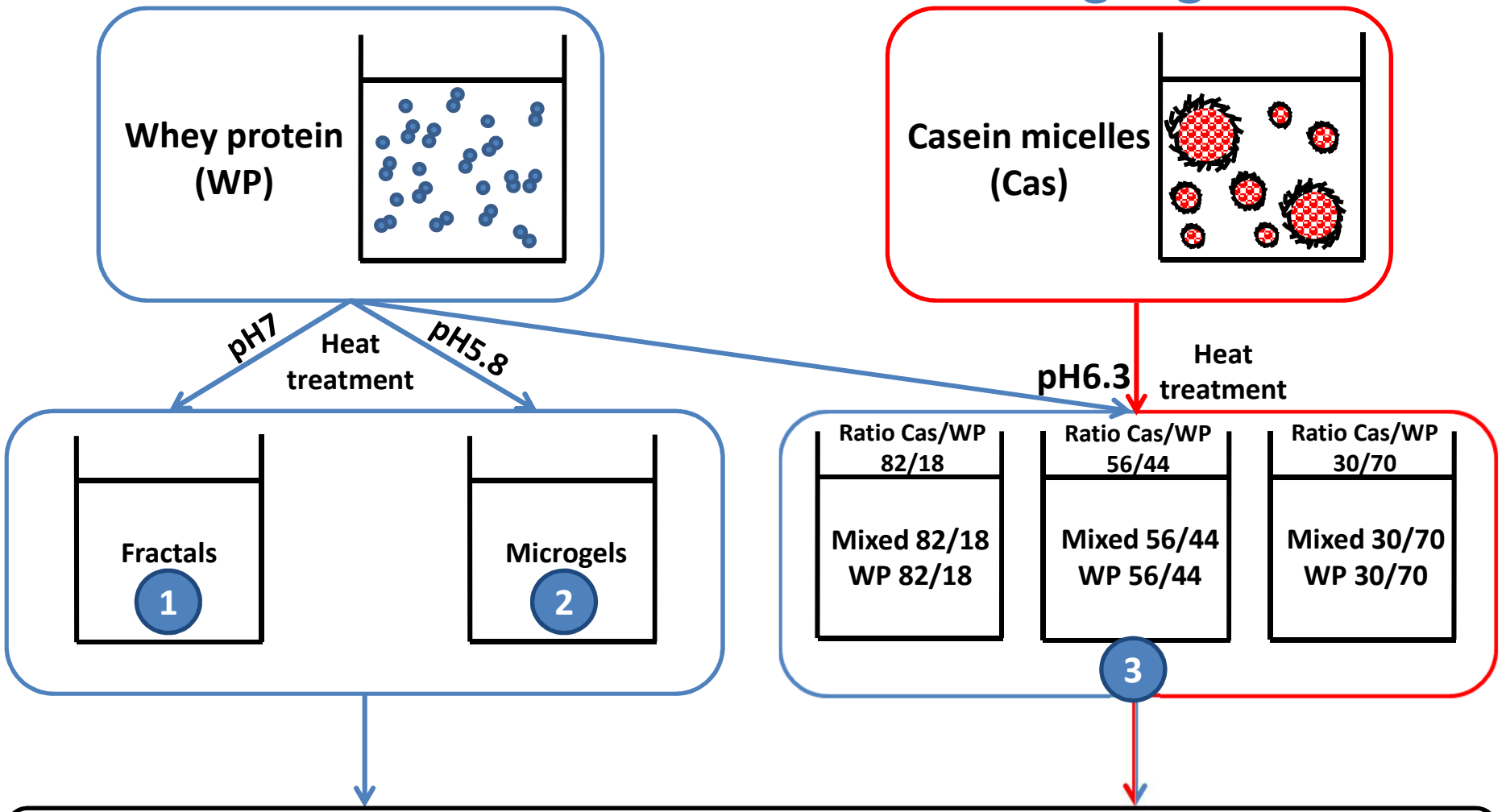
Problématiques techniques

Trouver une technique séparative capable de fractionner les différentes entités y compris les assemblages avec des caséines (contrairement à la SEC)

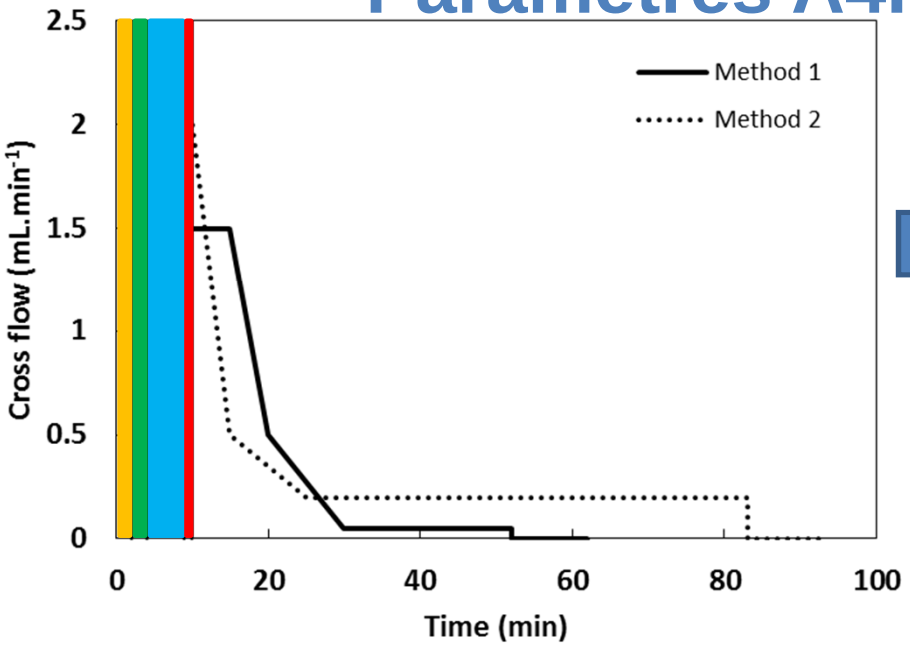
Asymmetrical Flow Field Flow Fractionation



Méthodes de fabrication des agrégats



Paramètres A4F et MET



Detectors

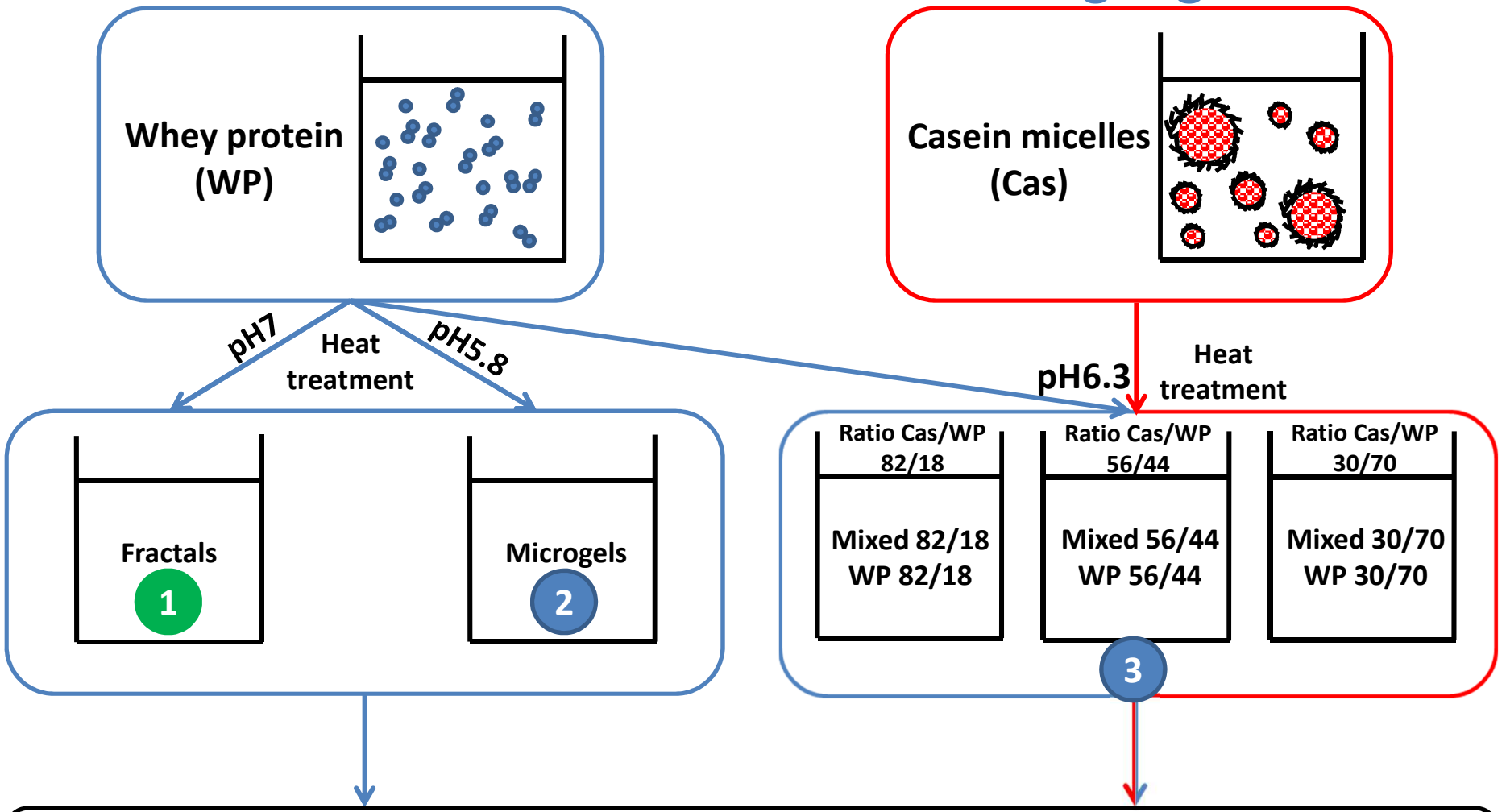
- ✓MALLS
- ✓*UV Detector*
- ✓Differential Refractometer (DRi)

Elution : 2 min
Focus : 2 min
Focus + inject : 5 min
Relaxation : 1 min

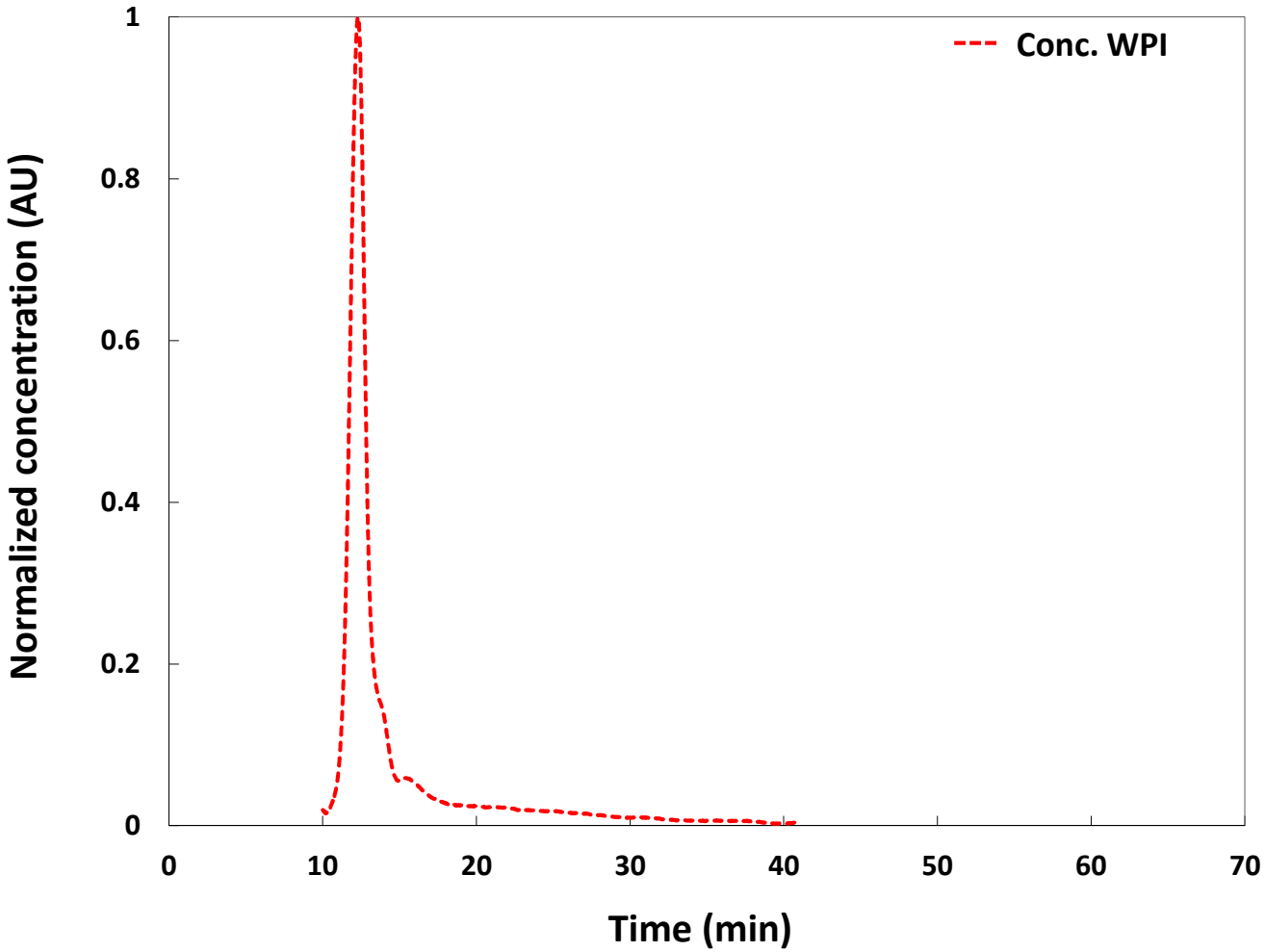
	Fractal aggregates	Microgel aggregates	Mixed aggregates
Eluent (pH)	Millipore water containing 0.02 w/v% NaN_3 and 45 mM NaCl (pH 7)	Millipore water containing 0.02 w/v% NaN_3 and 45 mM NaCl (pH 7)	Millipore water containing 0.02 w/v% NaN_3, 45 mM NaCl and 10mM $CaCl_2$ (pH 6.3 or 7)
A4F method	2	1	2
Membrane	Regenerated cellulose		
Spacer (µm)	350		
Temperature (°C)	25		

MET: Coloration négative

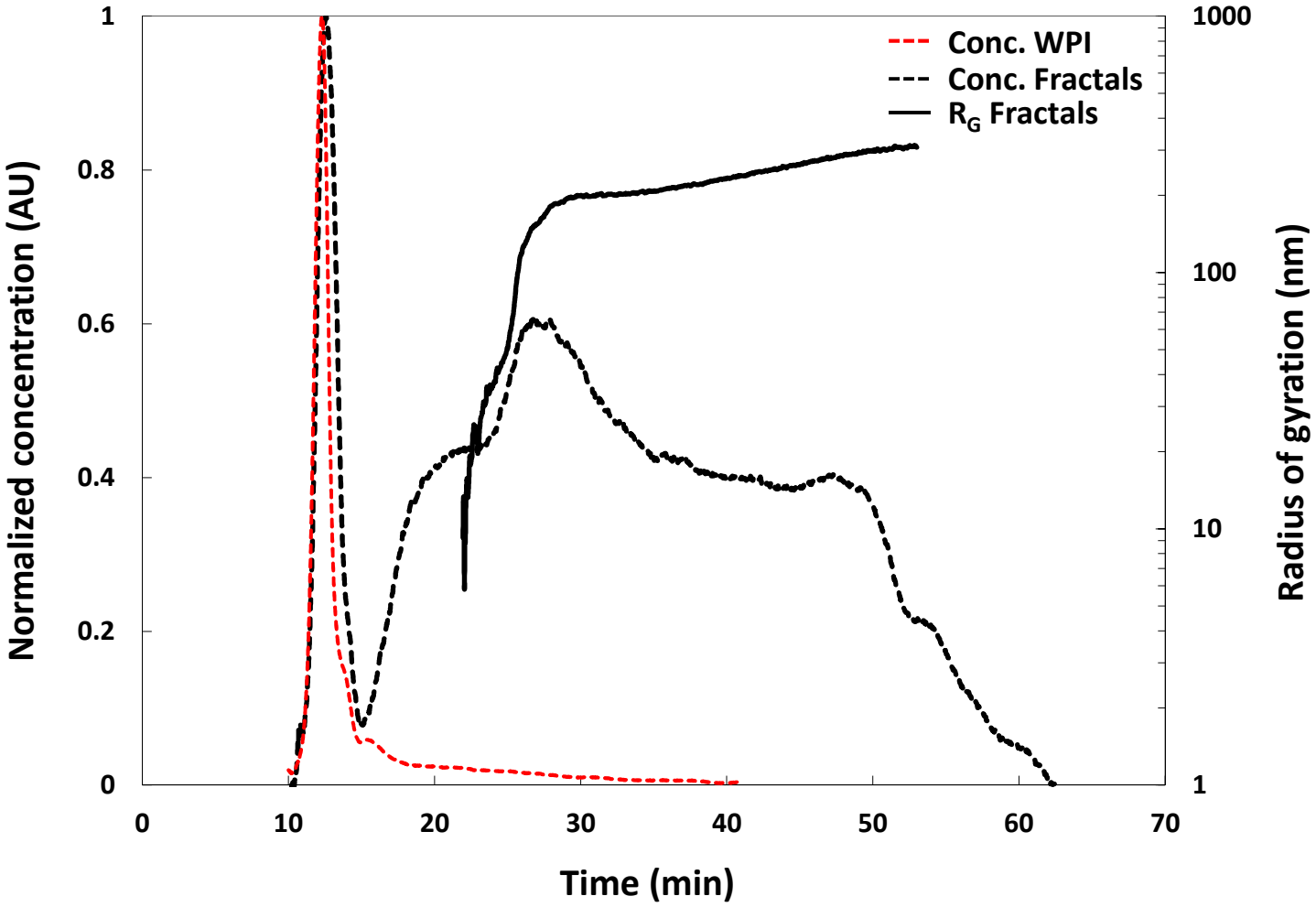
Méthodes de fabrication des agrégats



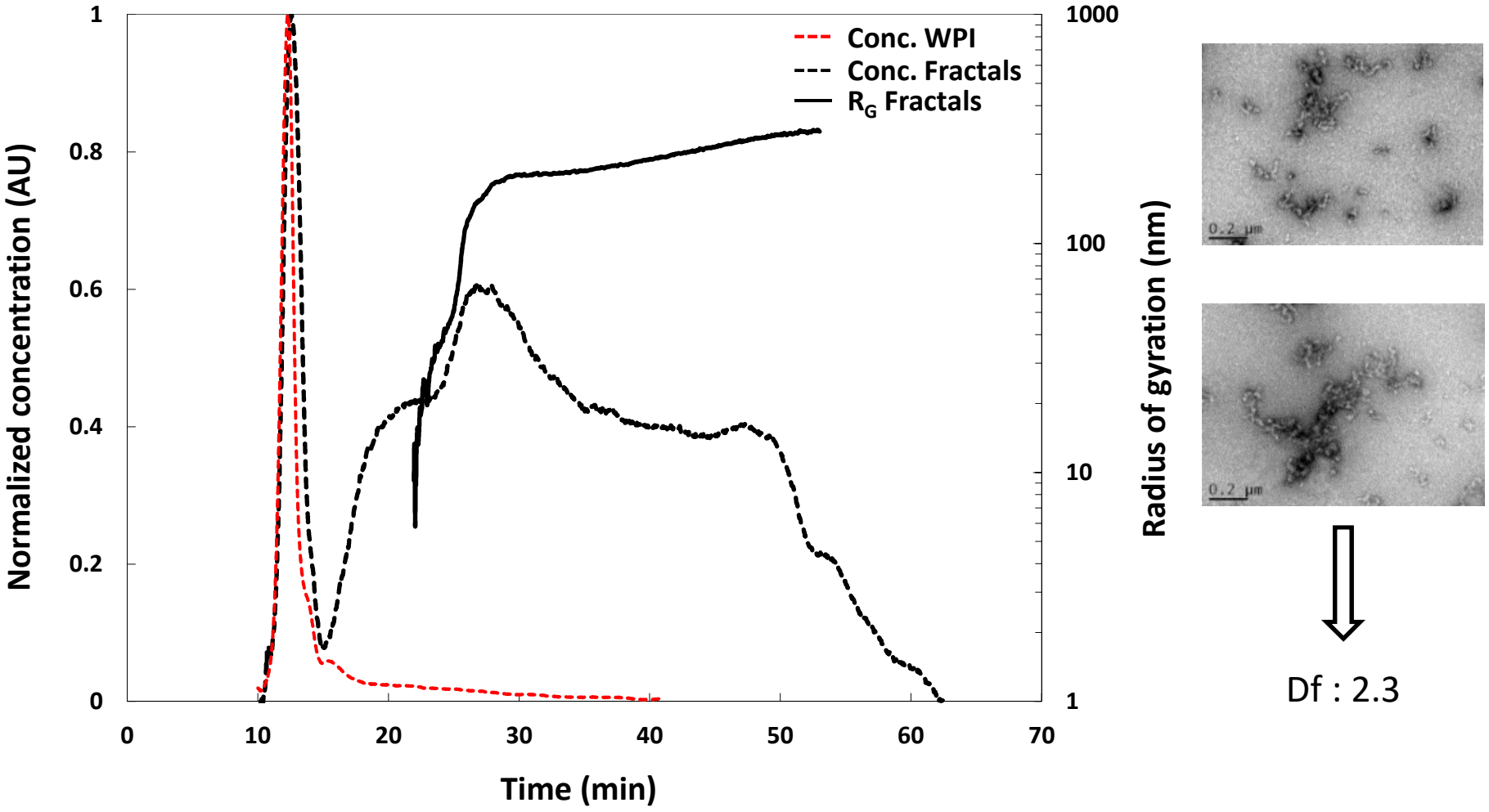
Fractals


Conc. WPI
Normalized concentration (AU)
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
70
Time (min)

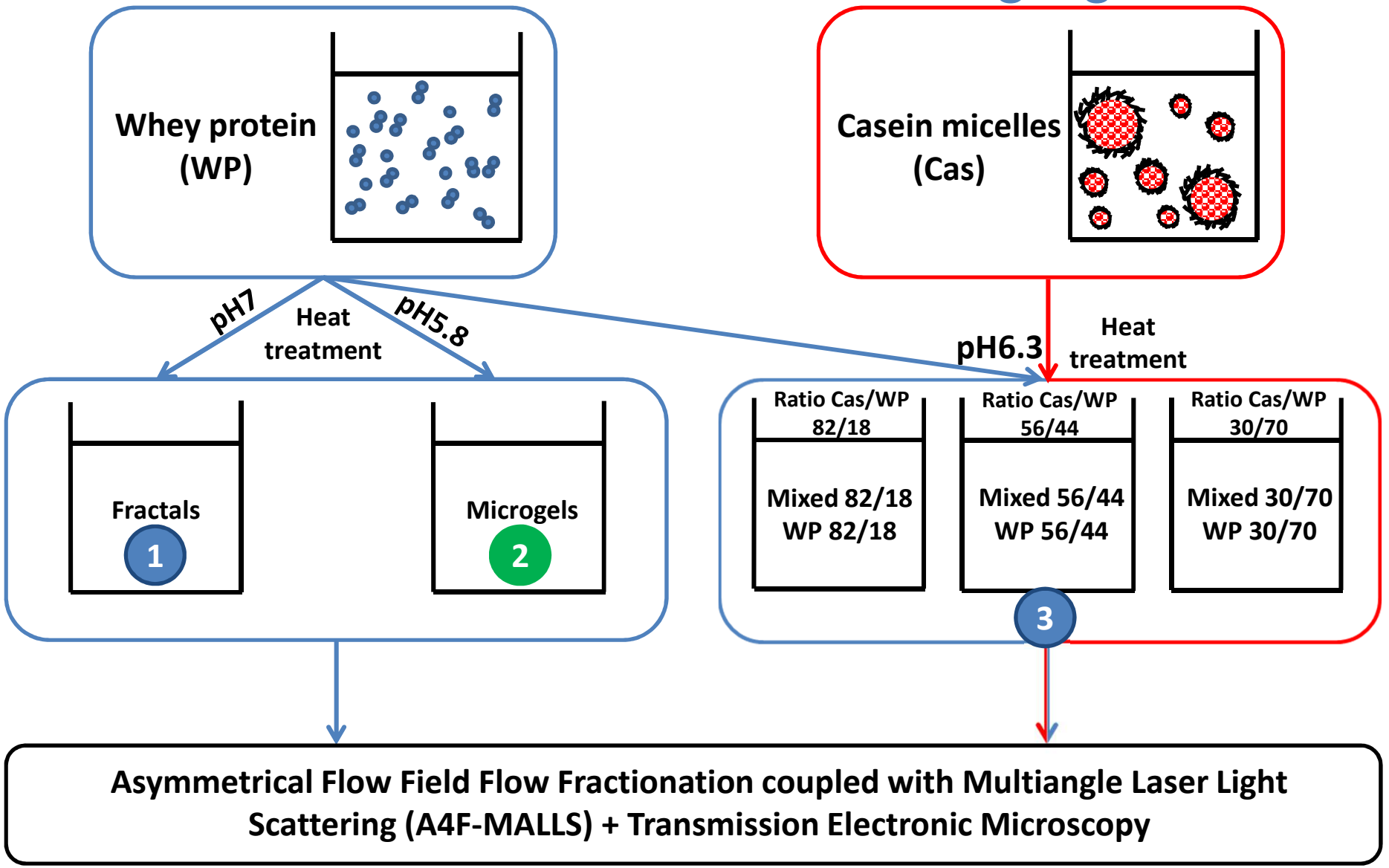
Fractals


Conc. WPI
Conc. Fractals
R_G Fractals
Normalized concentration (AU)
Radius of gyration (nm)
Time (min)
0
0.2
0.4
0.6
0.8
1
1
10
100
1000
0
10
20
30
40
50
60
70

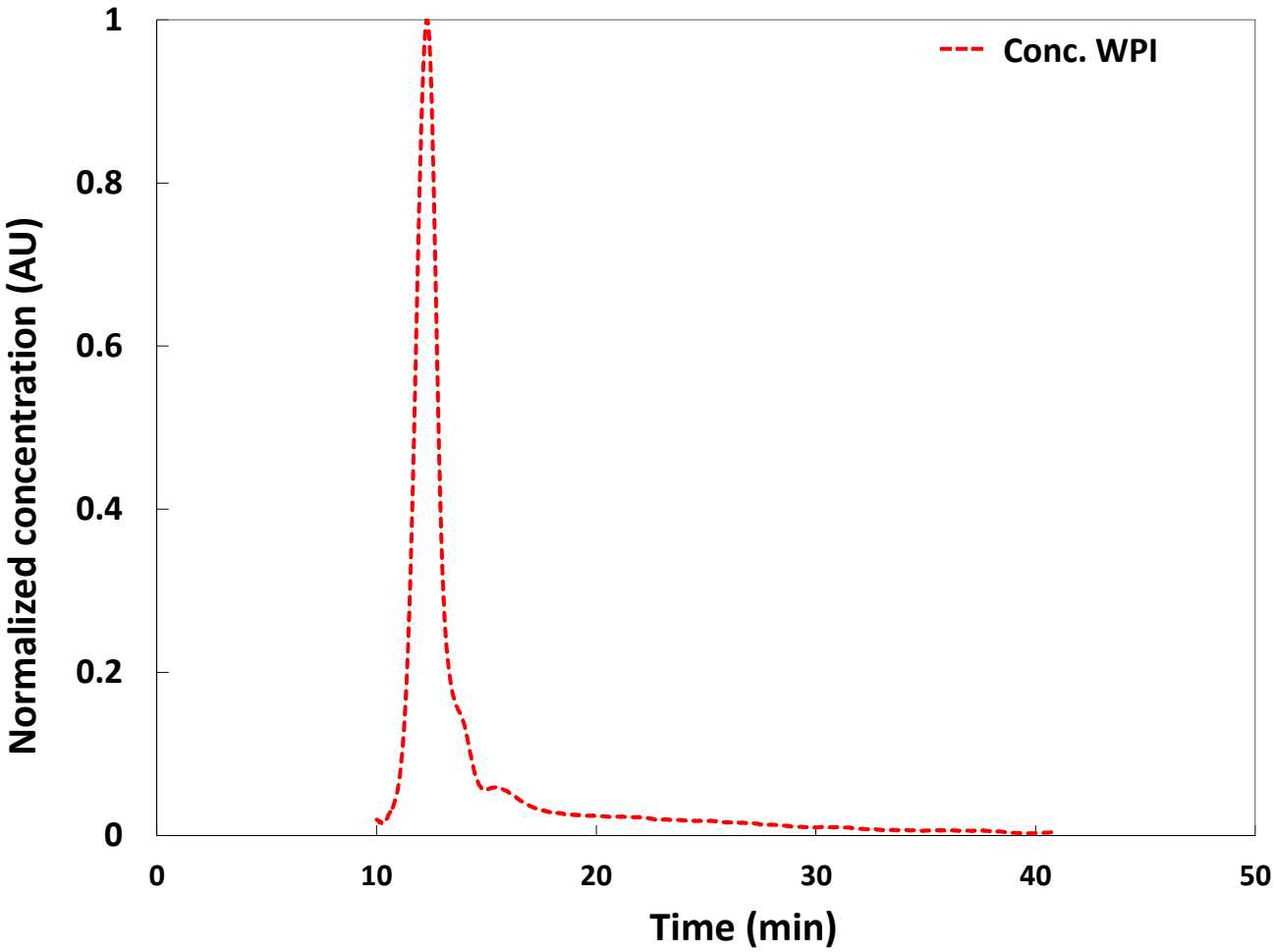
Fractals


Conc. WPI
Conc. Fractals
R_G Fractals
Normalized concentration (AU)
Radius of gyration (nm)
Time (min)
0
0.2
0.4
0.6
0.8
1
1
10
100
1000
0
10
20
30
40
50
60
70
0.2 µm
0.2 µm
Df : 2.3

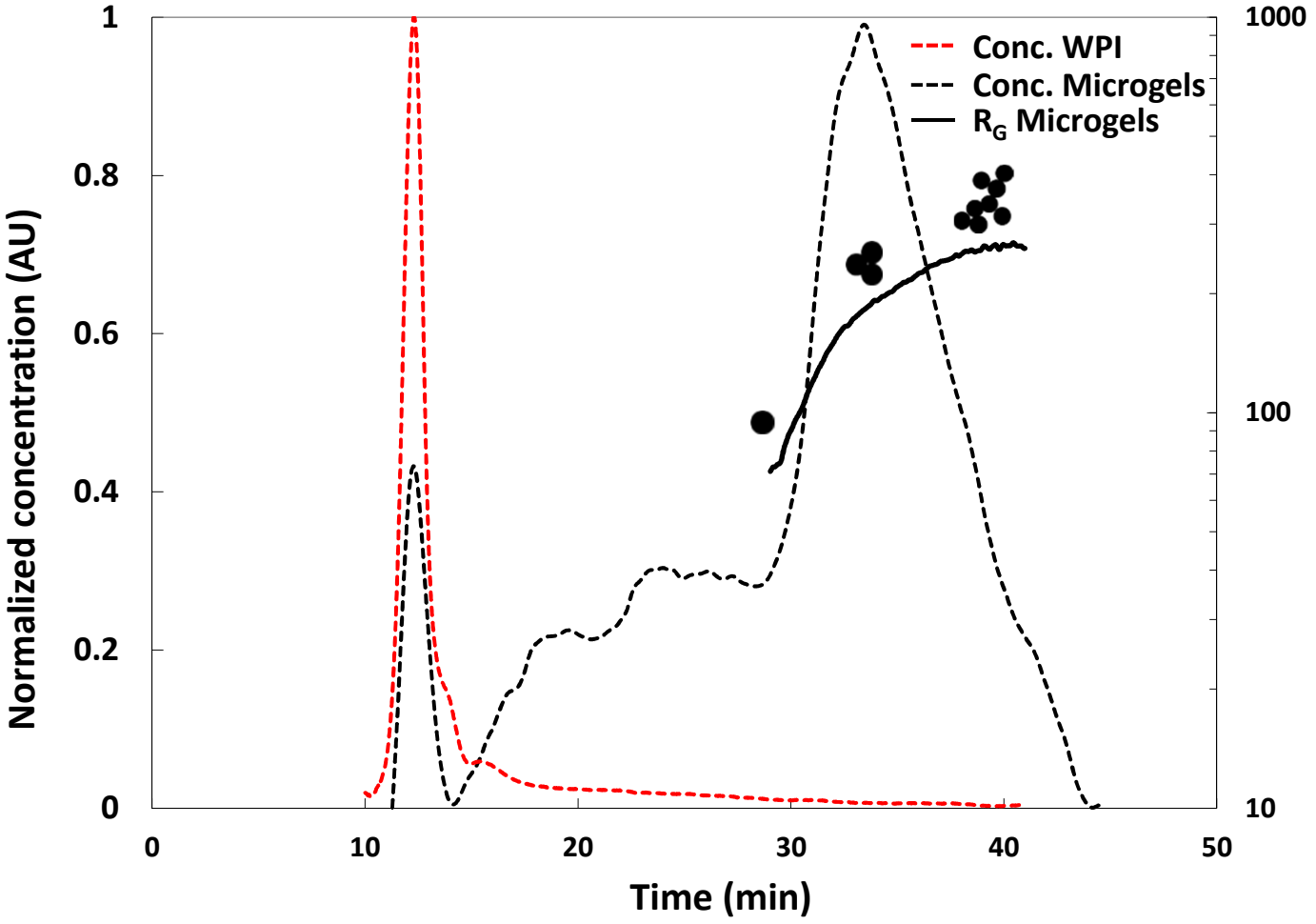
Méthodes de fabrication des agrégats

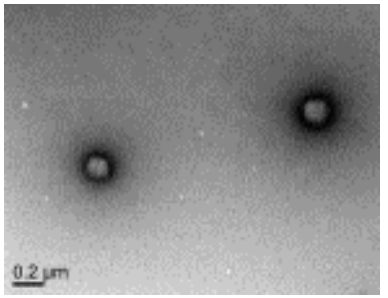


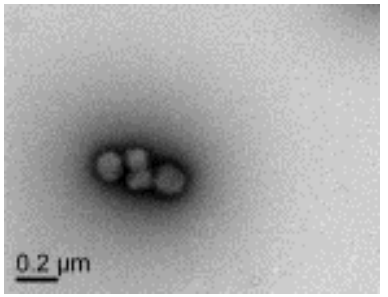
Microgels


Conc. WPI
Normalized concentration (AU)
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
Time (min)

Microgels





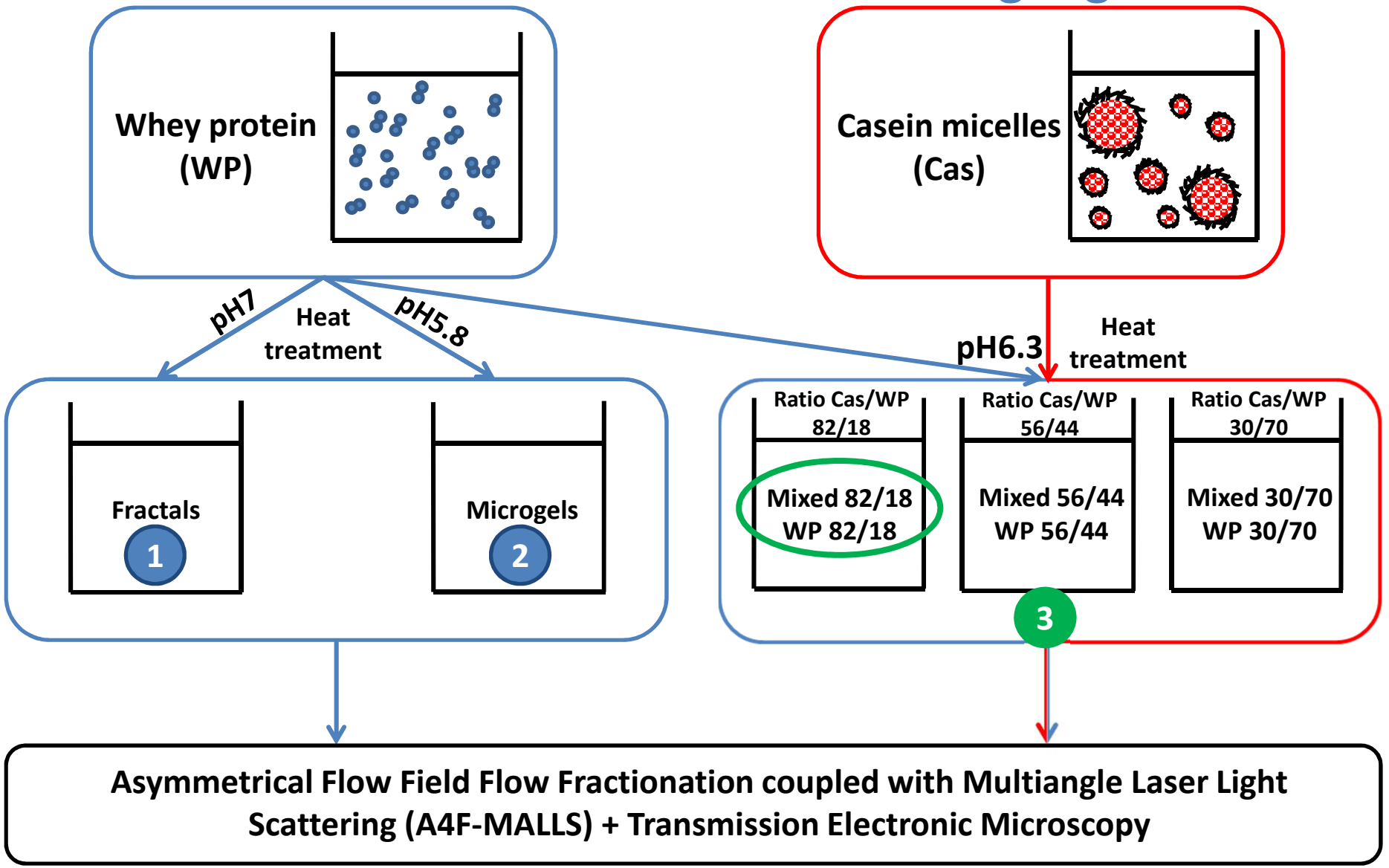


R_G = 100nm

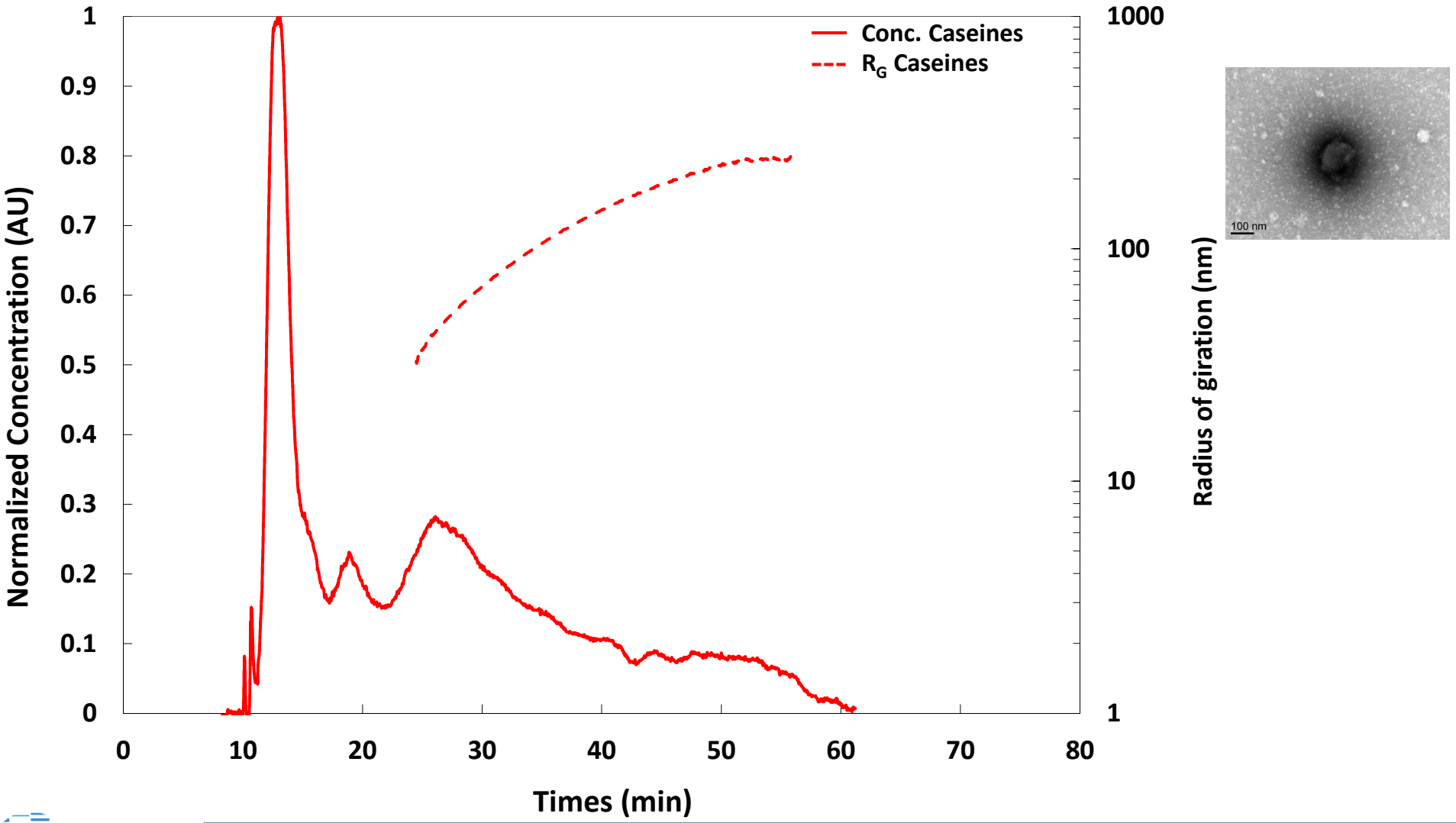
$M_W = 8.4.10^7$ g.mol^{-1}

$M_W = 5.5.10^8$ g.mol^{-1}

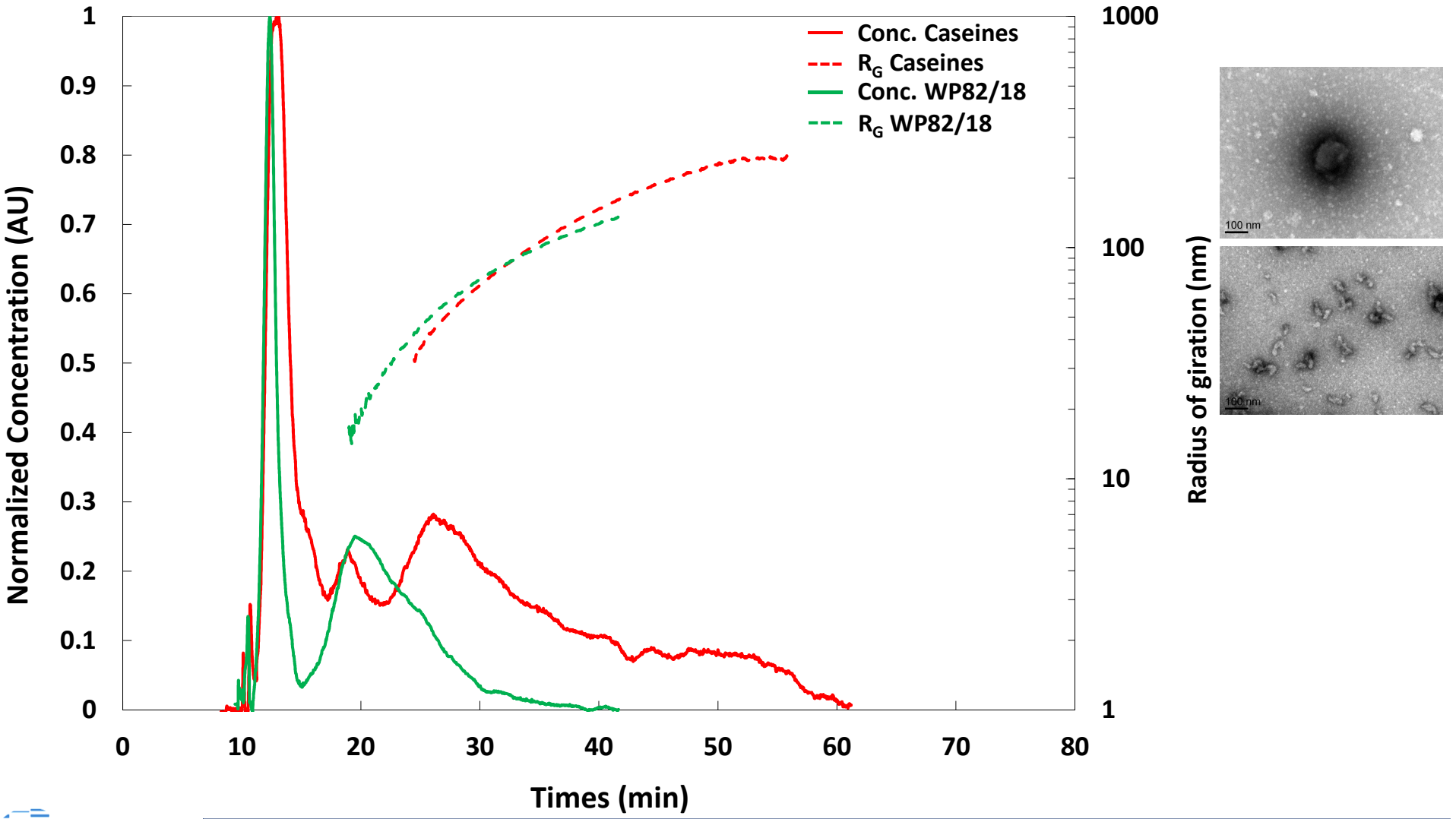
Méthodes de fabrication des agrégats



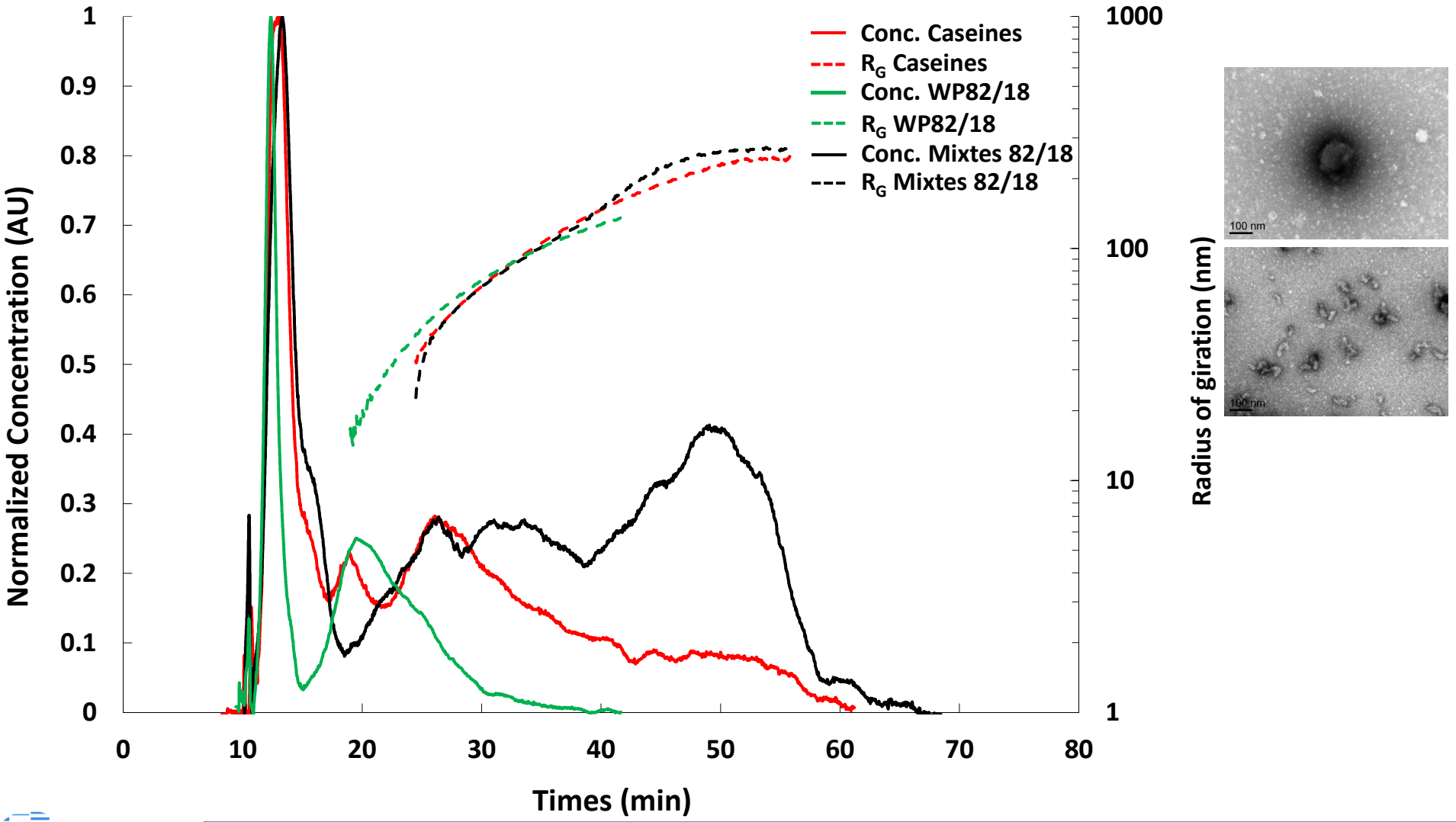
Mixtes 82/18


Conc. Caseines
R_G Caseines
Normalized Concentration (AU)
Radius of giration (nm)
Times (min)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1
10
100
1000
0
10
20
30
40
50
60
70
80
100 nm

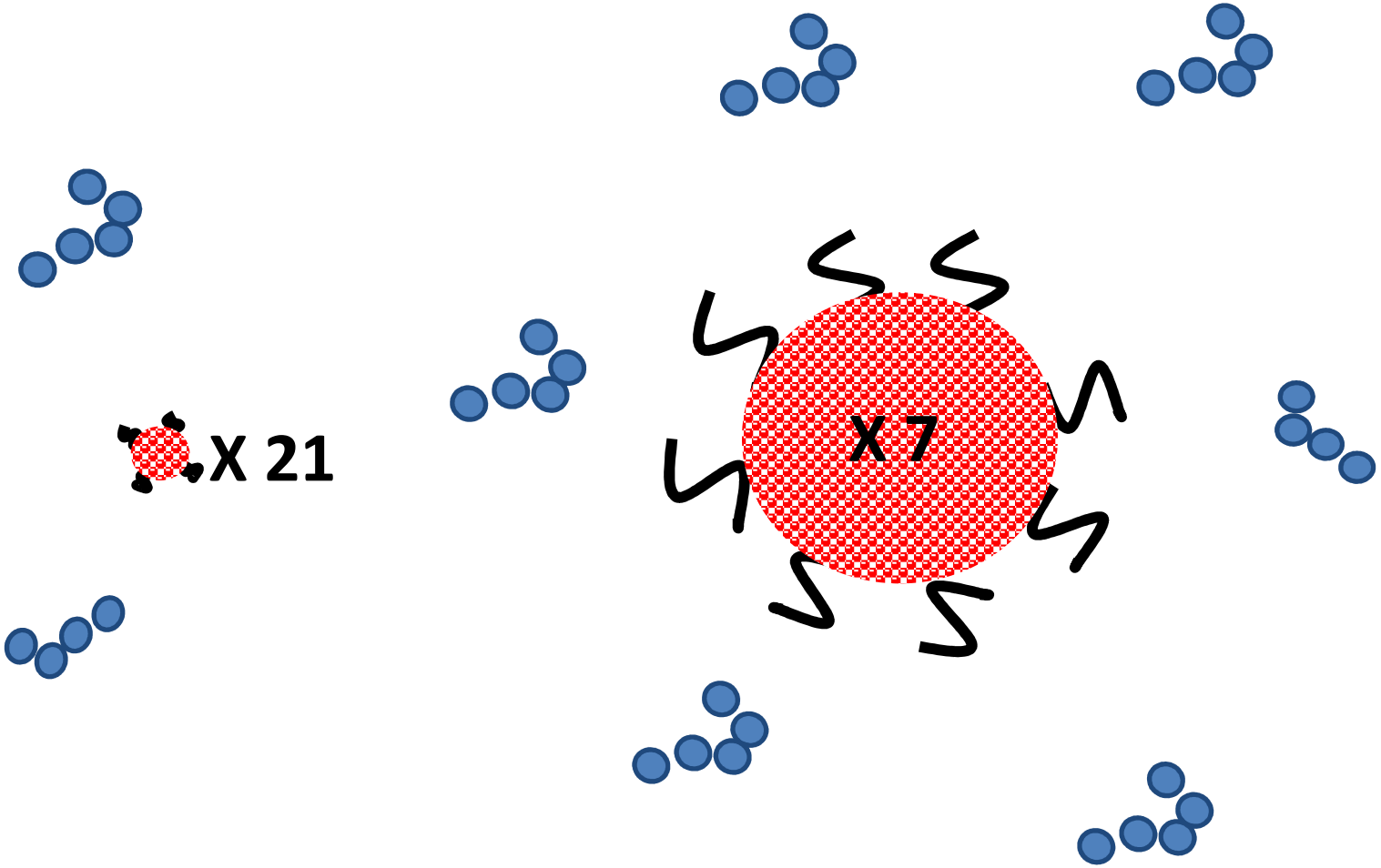
Mixtes 82/18


Normalized Concentration (AU)
Radius of giration (nm)
Times (min)
Conc. Caseines
R_G Caseines
Conc. WP82/18
R_G WP82/18
100 nm
100 nm

Mixtes 82/18


Normalized Concentration (AU)
Radius of giration (nm)
Times (min)
Conc. Caseines
R_G Caseines
Conc. WP82/18
R_G WP82/18
Conc. Mixtes 82/18
R_G Mixtes 82/18
100 nm
100 nm

Mixtes 82/18

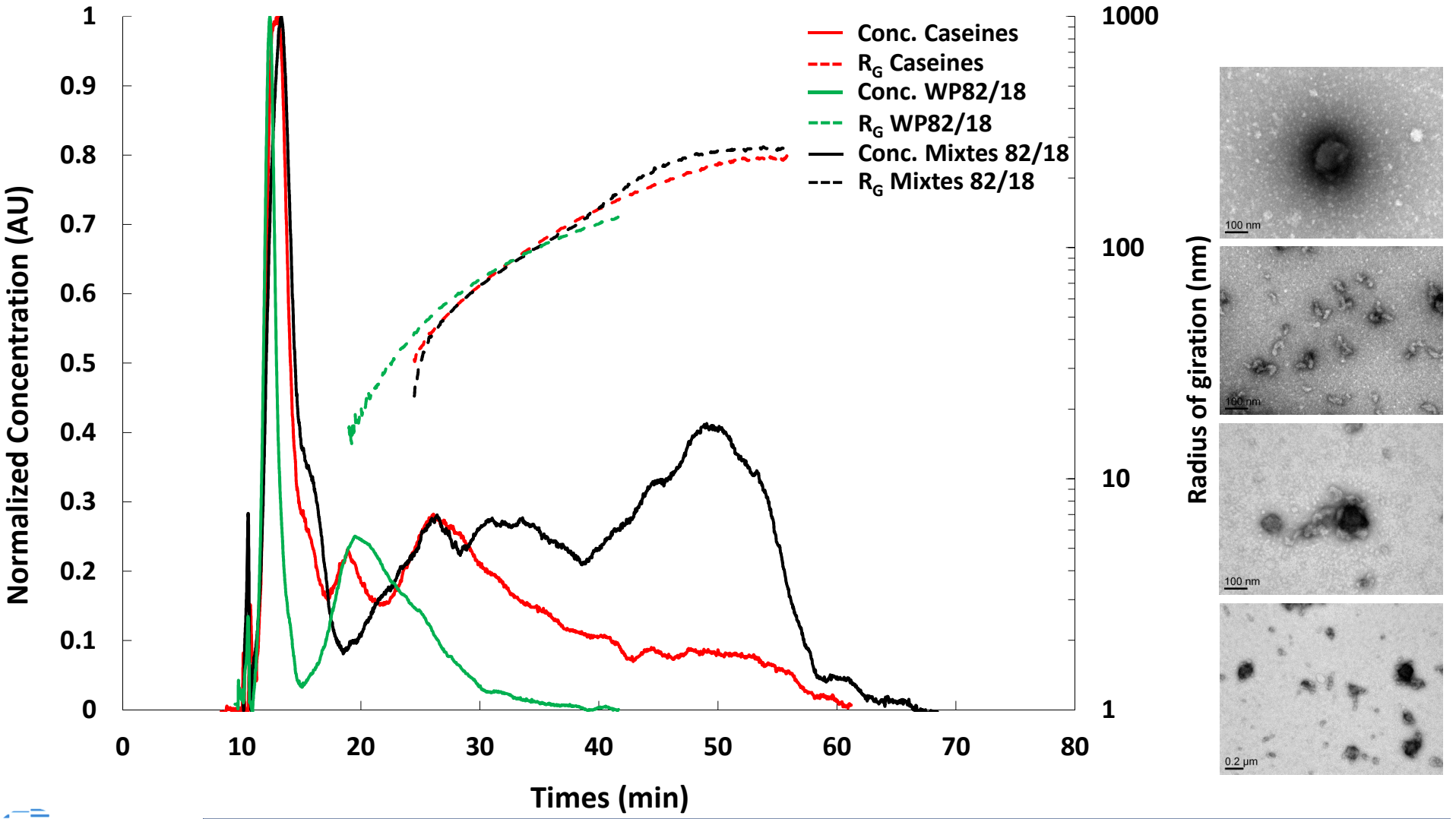


Pourquoi uniquement les micelles de grosses taille sont concernées?

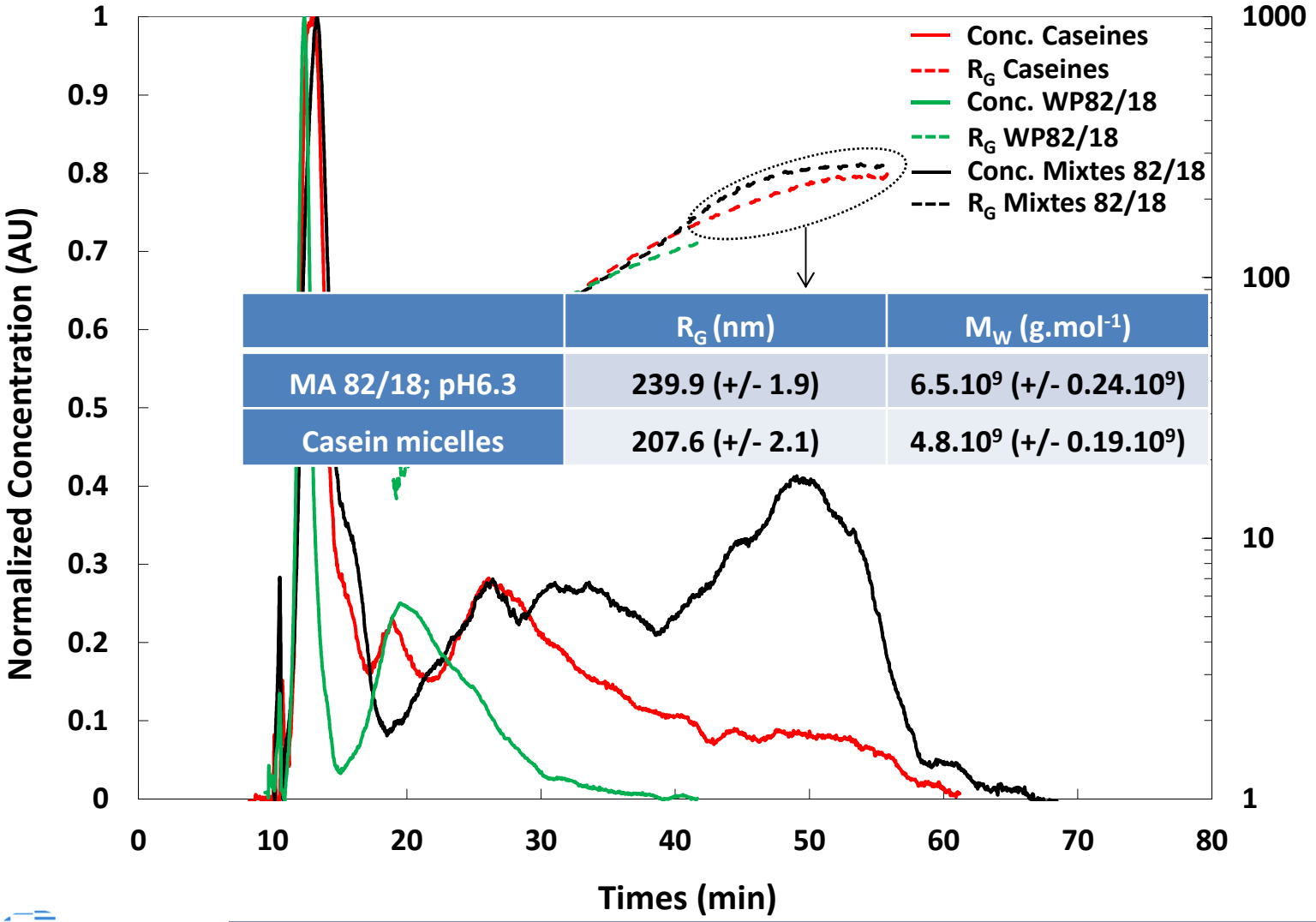
✓ Plus grande quantité en nombre de caséines κ

✓ Encombrement stérique sur les petites micelles

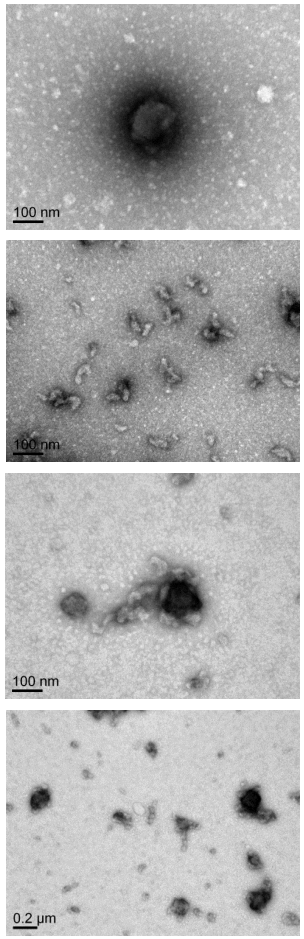
Mixtes 82/18


Normalized Concentration (AU)
Radius of giration (nm)
Times (min)
Conc. Caseines
R_G Caseines
Conc. WP82/18
R_G WP82/18
Conc. Mixtes 82/18
R_G Mixtes 82/18
100 nm
100 nm
100 nm
0.2 µm

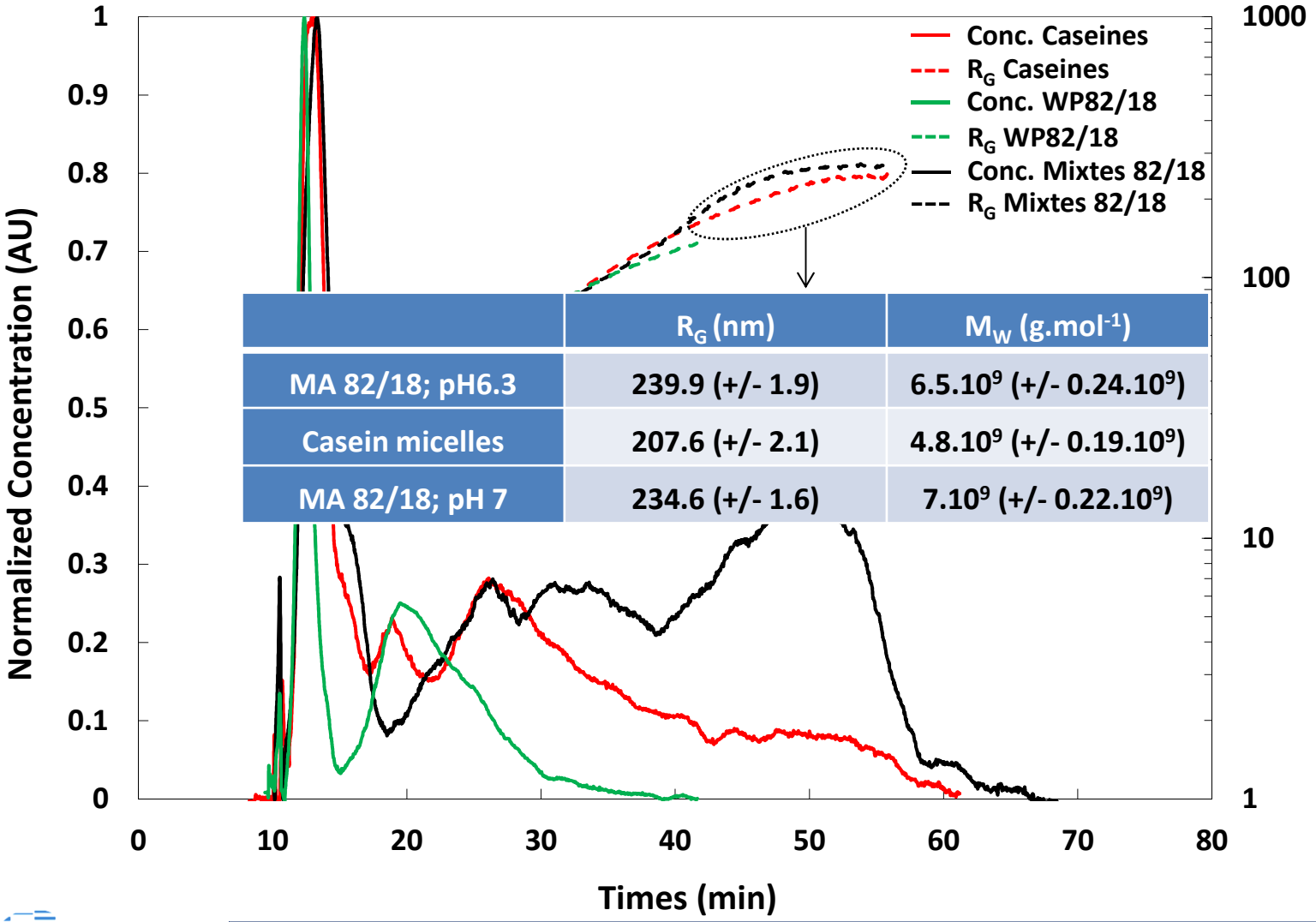
Mixtes 82/18



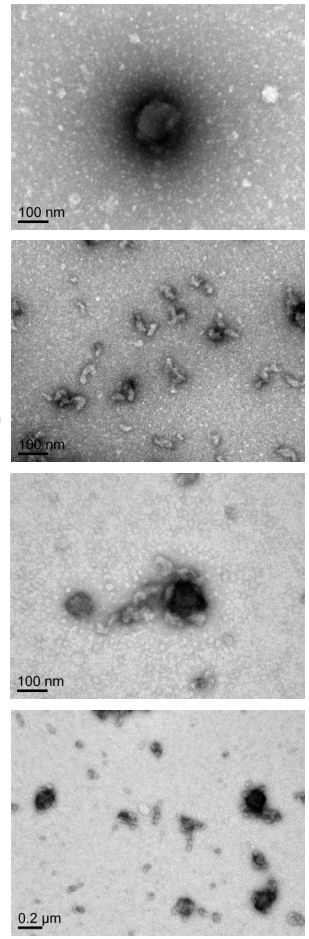
	R_G (nm)	M_W (g.mol^{-1})
MA 82/18; pH6.3	239.9 (+/- 1.9)	6.5.10^9 (+/- 0.24.10^9)
Casein micelles	207.6 (+/- 2.1)	4.8.10^9 (+/- 0.19.10^9)



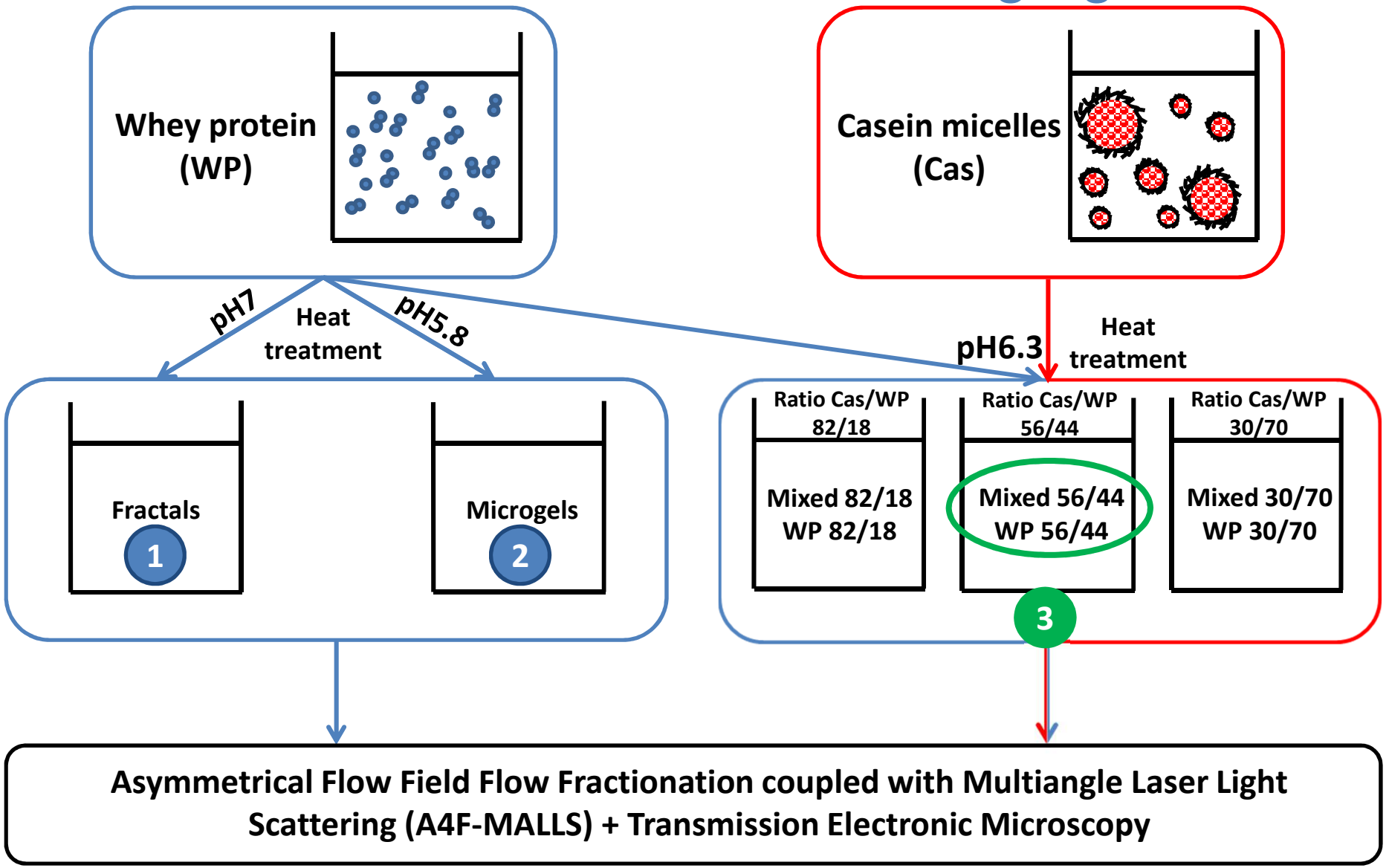
Mixtes 82/18



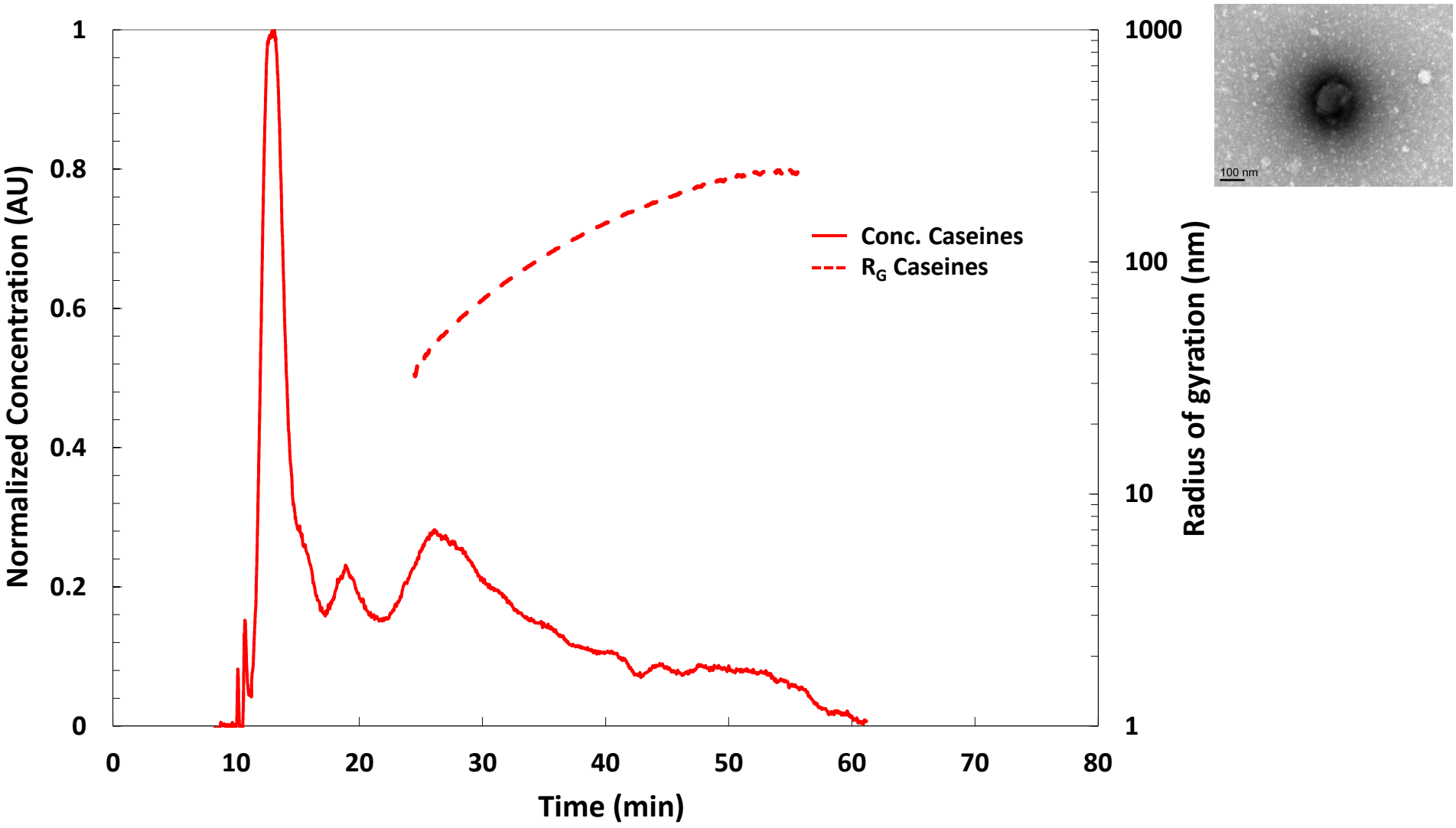
	R_G (nm)	M_W (g.mol^{-1})
MA 82/18; pH6.3	239.9 (+/- 1.9)	$6.5.10^9$ (+/- $0.24.10^9$)
Casein micelles	207.6 (+/- 2.1)	$4.8.10^9$ (+/- $0.19.10^9$)
MA 82/18; pH 7	234.6 (+/- 1.6)	7.10^9 (+/- $0.22.10^9$)



Méthodes de fabrication des agrégats

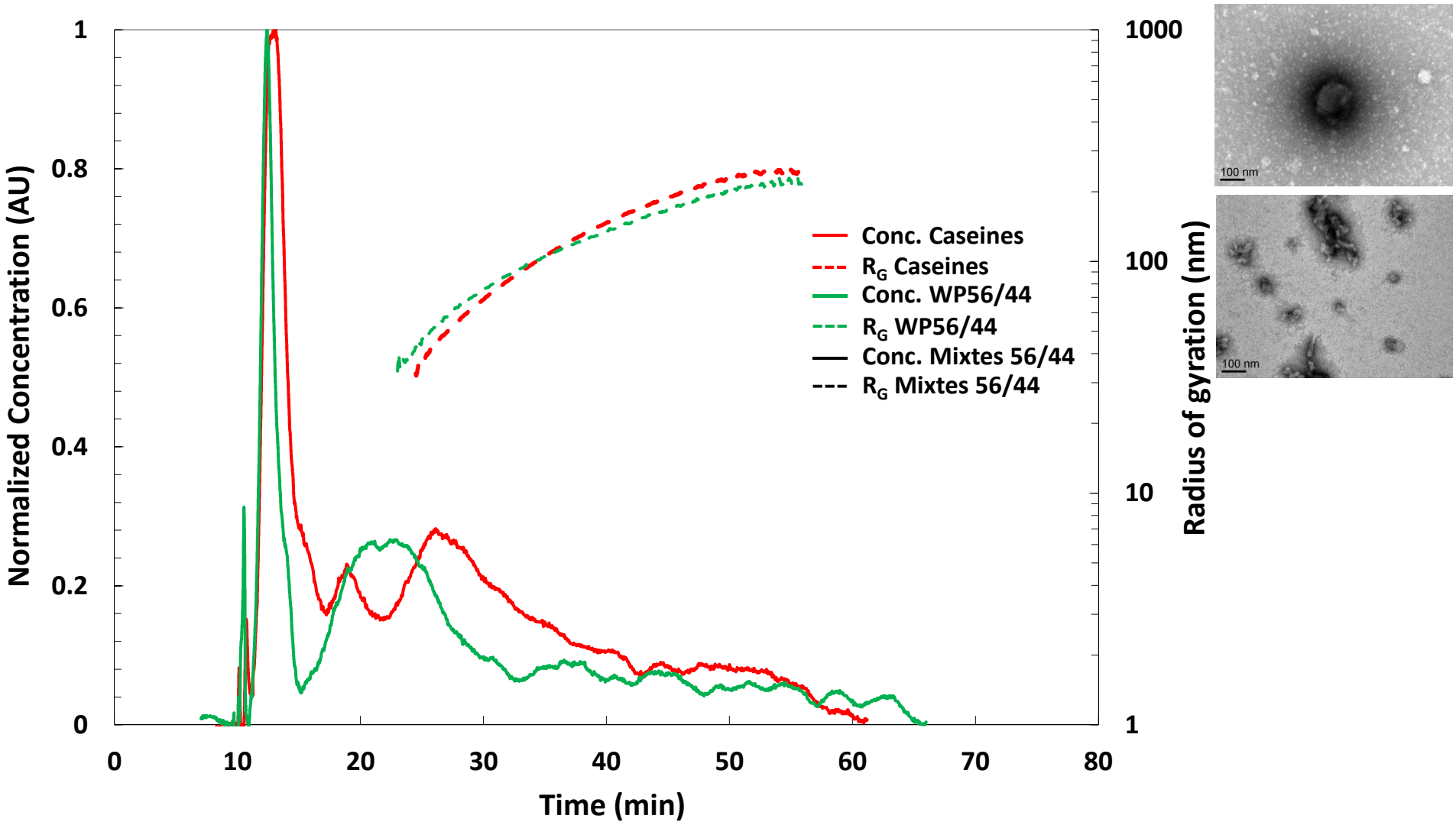


Mixtes 56/44

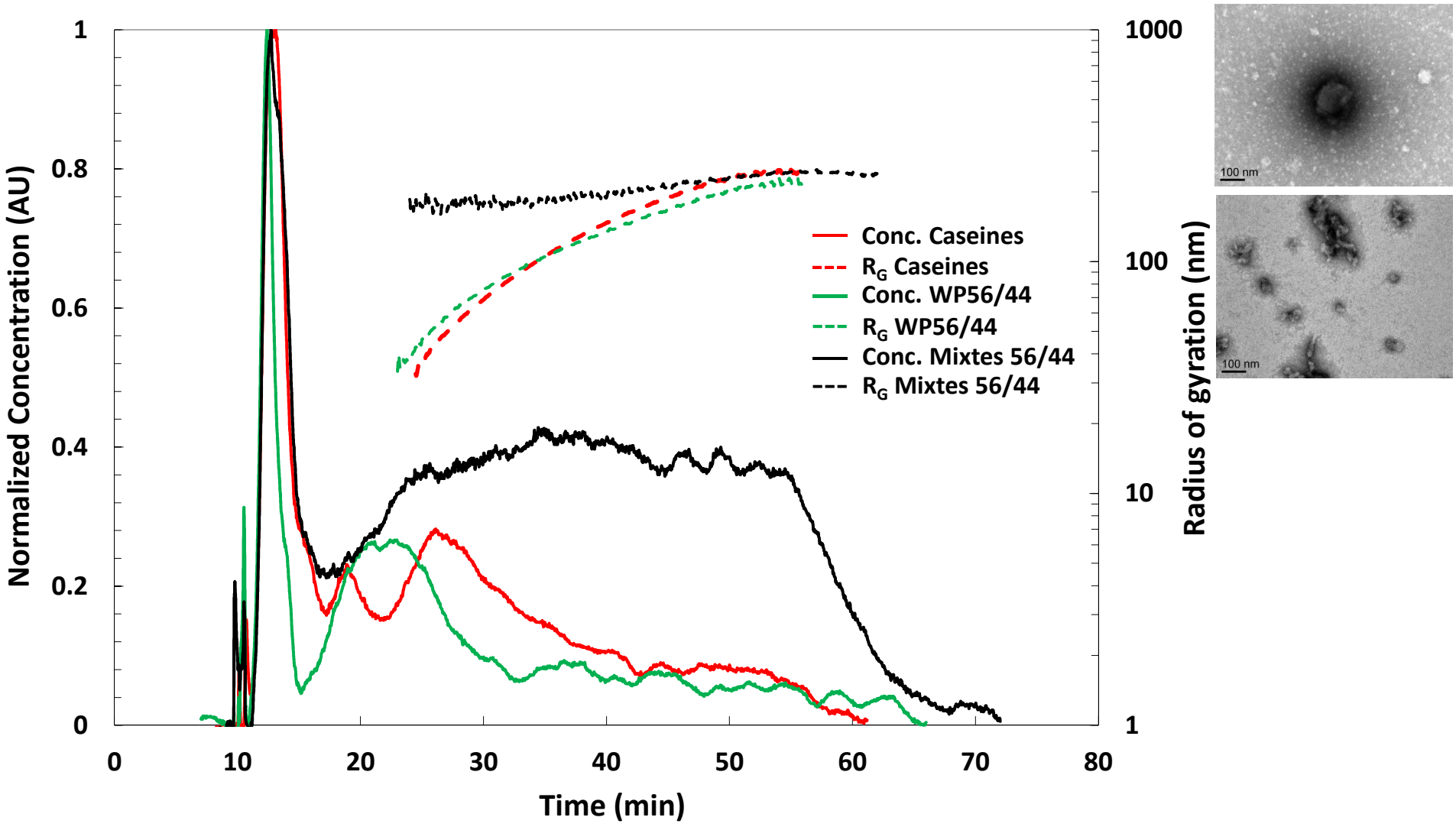

Normalized Concentration (AU)
Radius of gyration (nm)
Time (min)
Conc. Caseines
R_G Caseines
0
0.2
0.4
0.6
0.8
1
1
10
100
1000
0
10
20
30
40
50
60
70
80

100 nm

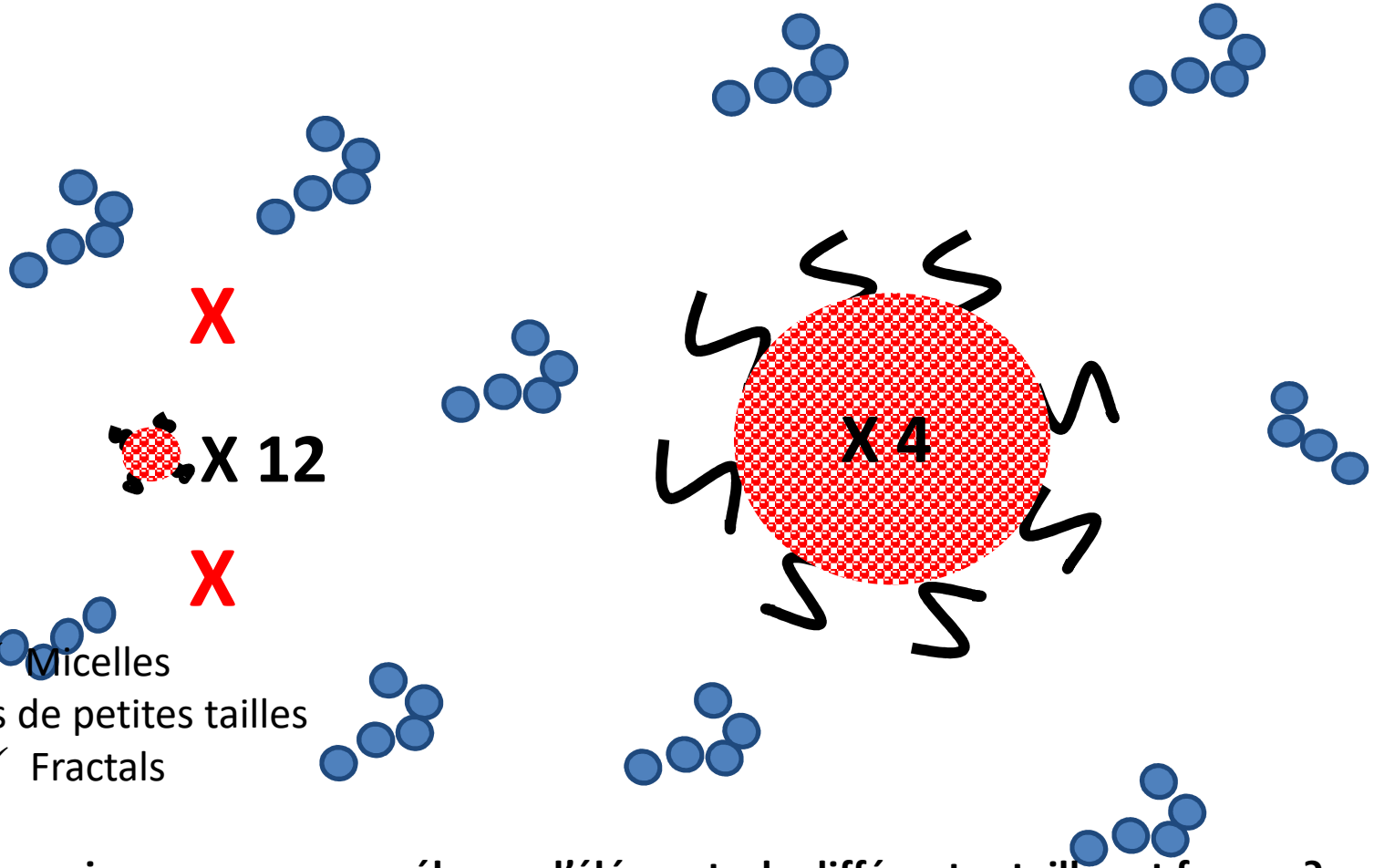
Mixtes 56/44


Normalized Concentration (AU)
Radius of gyration (nm)
Time (min)
0
0.2
0.4
0.6
0.8
1
1
10
100
1000
0
10
20
30
40
50
60
70
80
Conc. Caseines
R_G Caseines
Conc. WP56/44
R_G WP56/44
Conc. Mixtes 56/44
R_G Mixtes 56/44
100 nm
100 nm

Mixtes 56/44


Normalized Concentration (AU)
Radius of gyration (nm)
Time (min)
Conc. Caseines
R_G Caseines
Conc. WP56/44
R_G WP56/44
Conc. Mixtes 56/44
R_G Mixtes 56/44
100 nm
100 nm

Mixtes 56/44

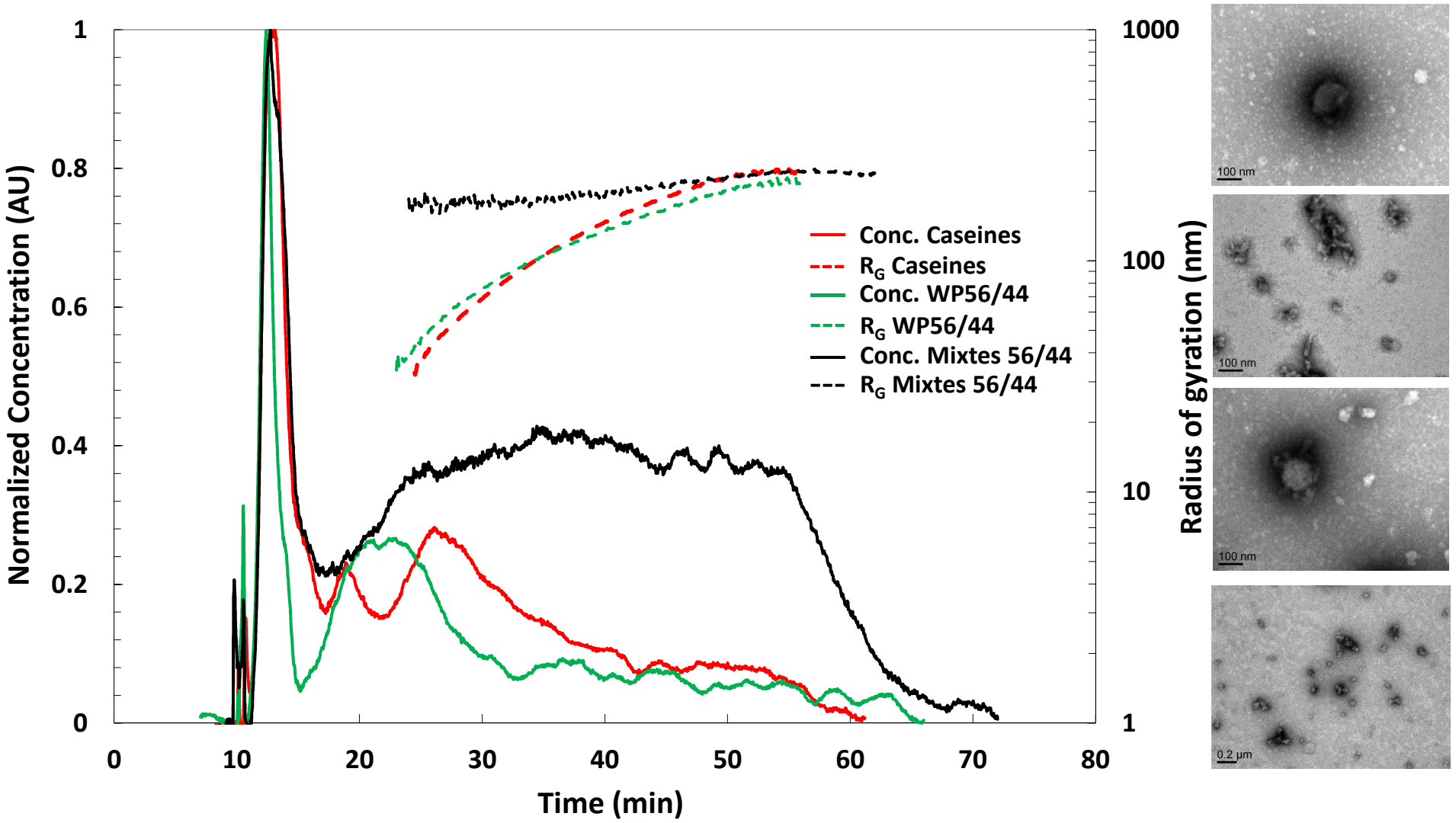


- ✓ Micelles
- ✓ Mixtes de petites tailles
- ✓ Fractals

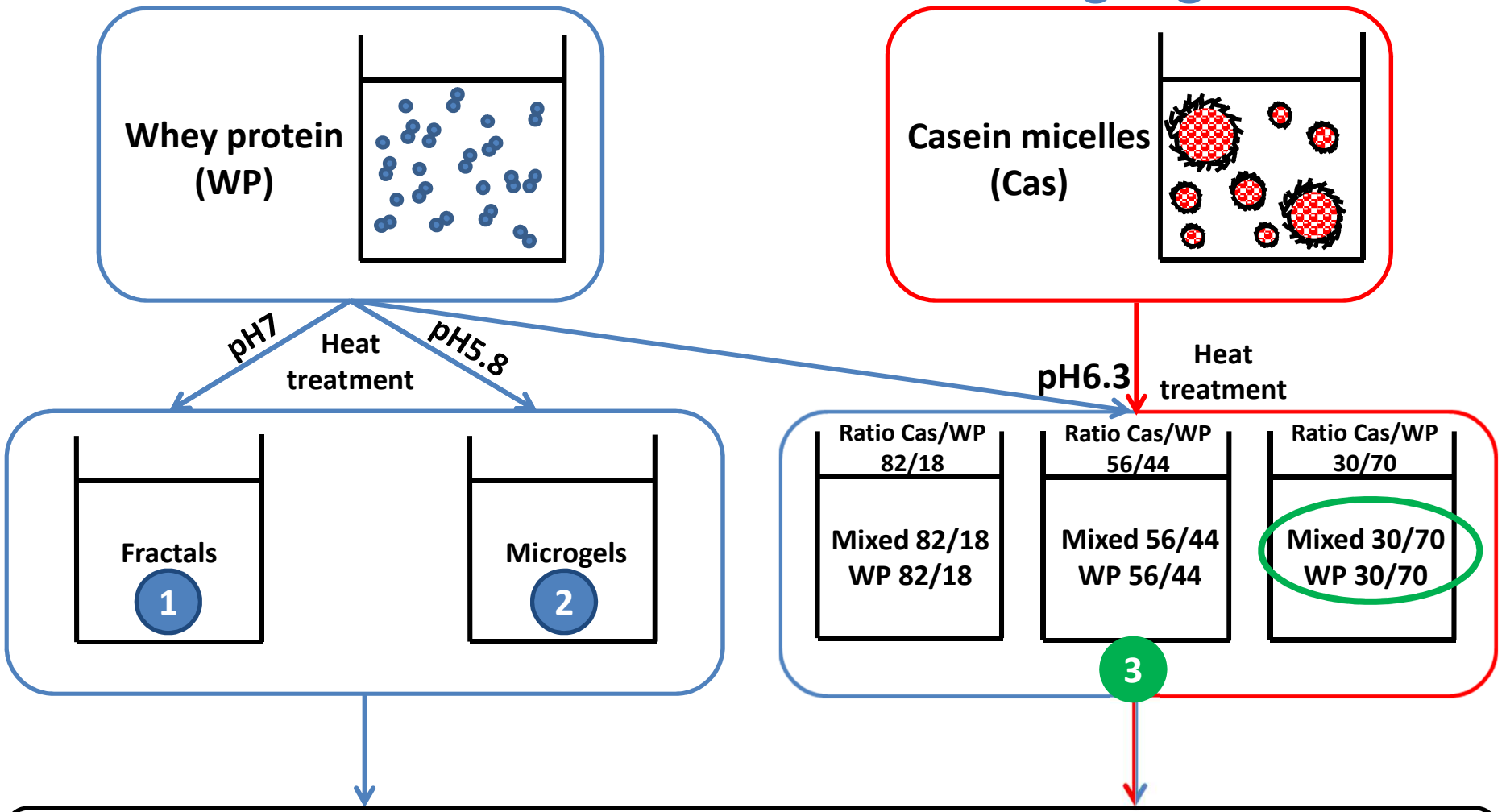
Pourquoi nous avons un mélange d'éléments de différentes tailles et formes?

- ✓ Formation essentiellement de mixtes de petites tailles car peu de grosses micelles
- ✓ Présence d'agrégats fractals en solution car problème d'encombrement stérique sur les petites micelles (cinétique d'adsorption plus lente)

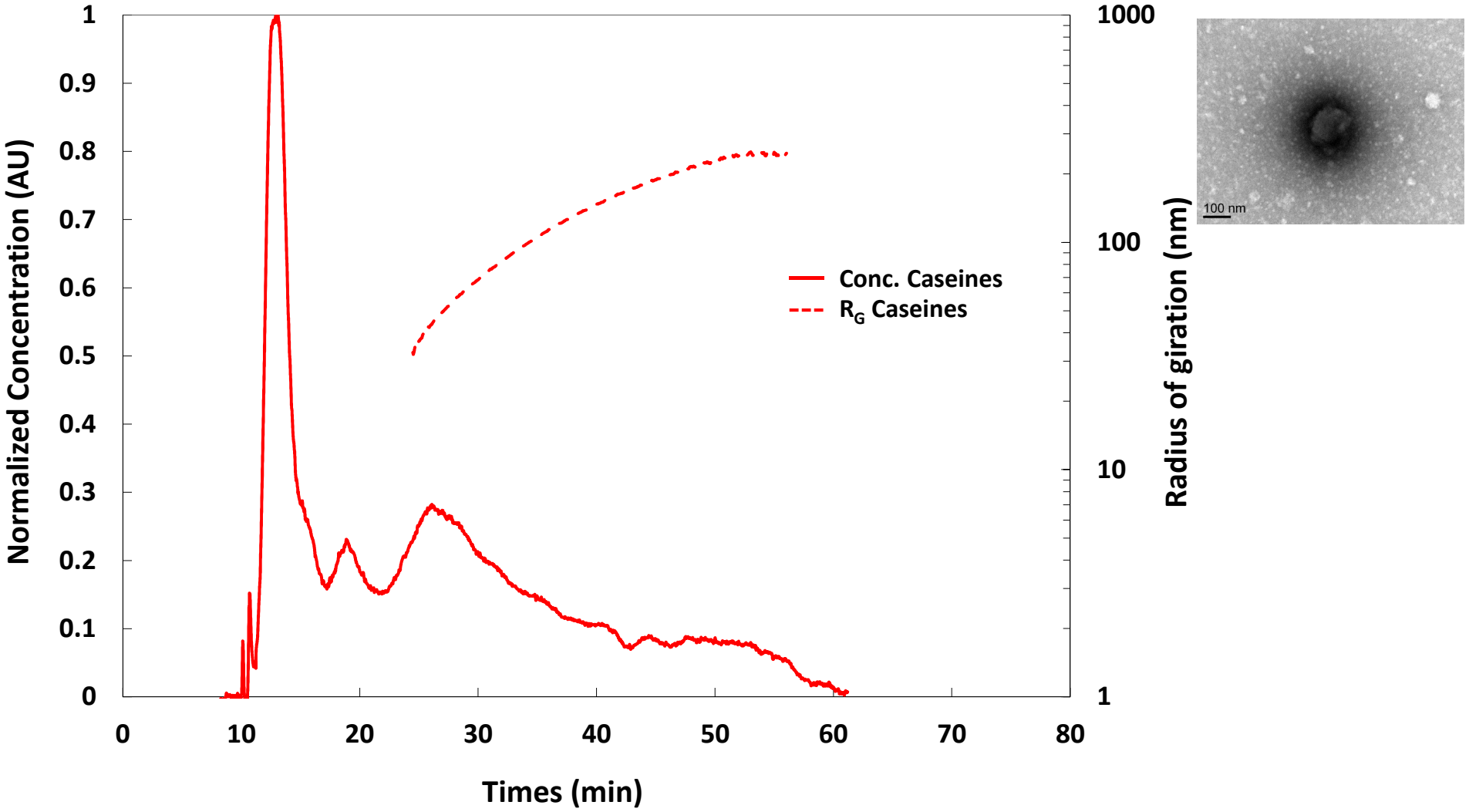
Mixtes 56/44


Normalized Concentration (AU)
Radius of gyration (nm)
Time (min)
Conc. Caseines
R_G Caseines
Conc. WP56/44
R_G WP56/44
Conc. Mixtes 56/44
R_G Mixtes 56/44
100 nm
100 nm
100 nm
0.2 µm

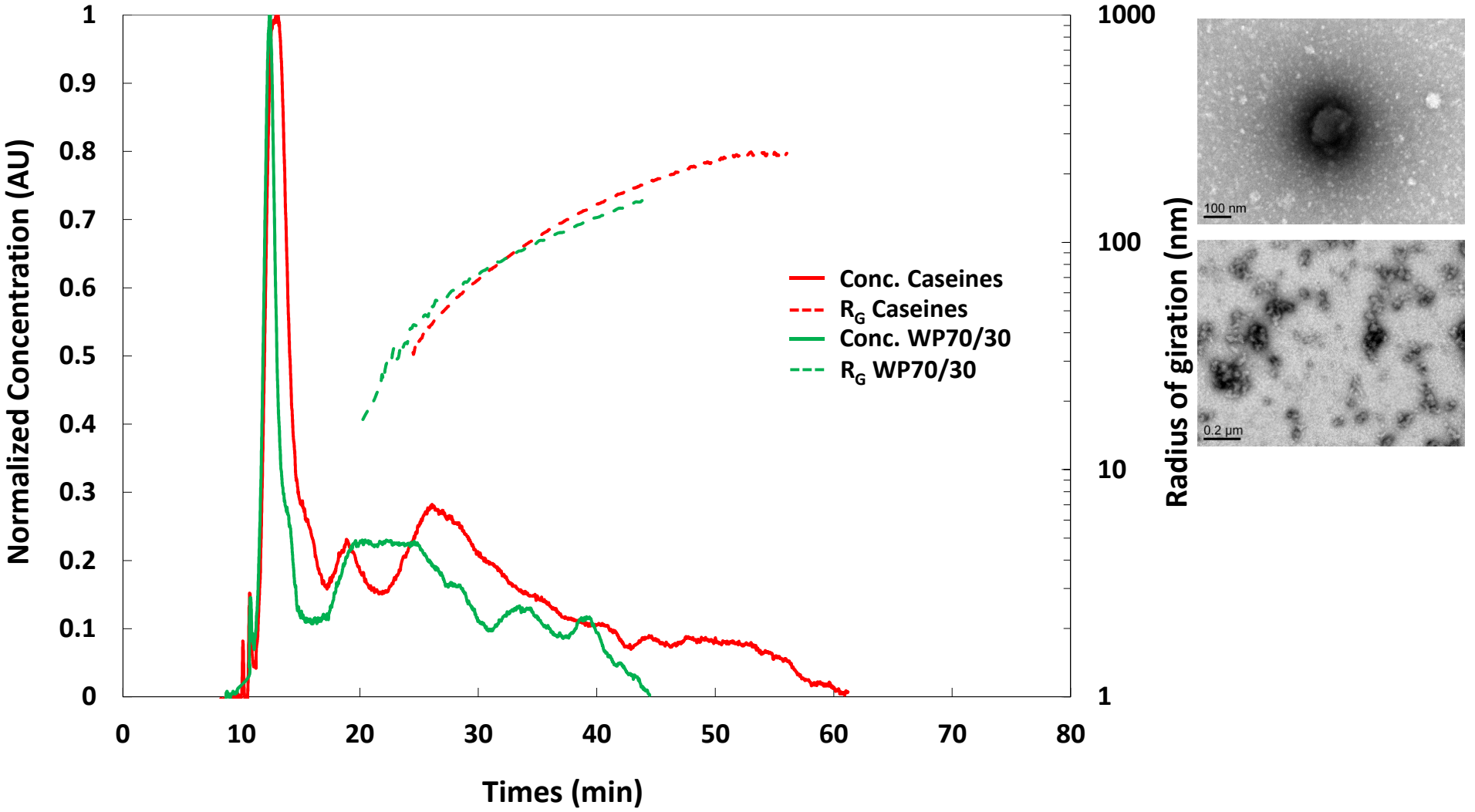
Méthodes de fabrication des agrégats



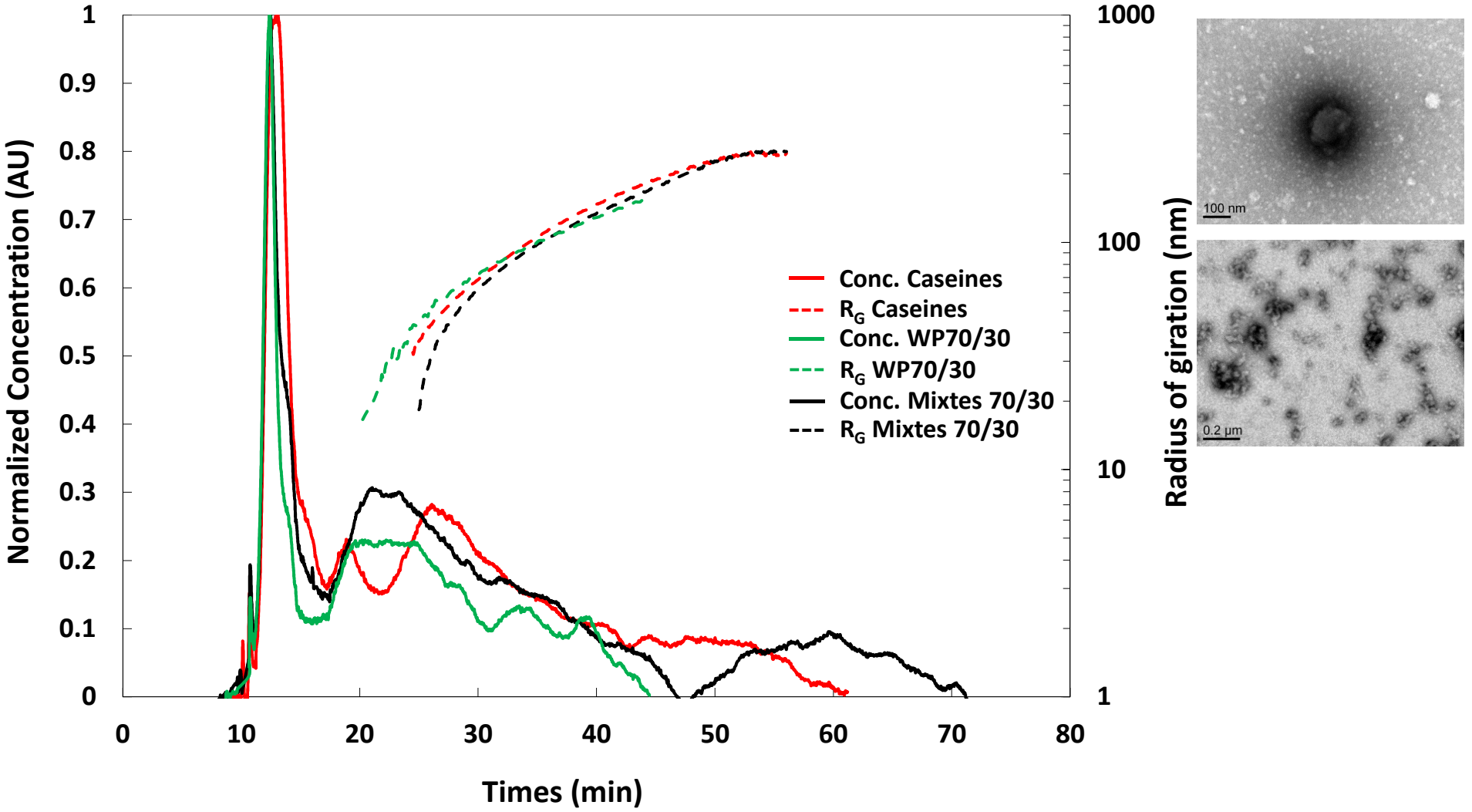
Mixtes 30/70


Normalized Concentration (AU)
Radius of giration (nm)
Times (min)
Conc. Caseines
R_G Caseines
100 nm

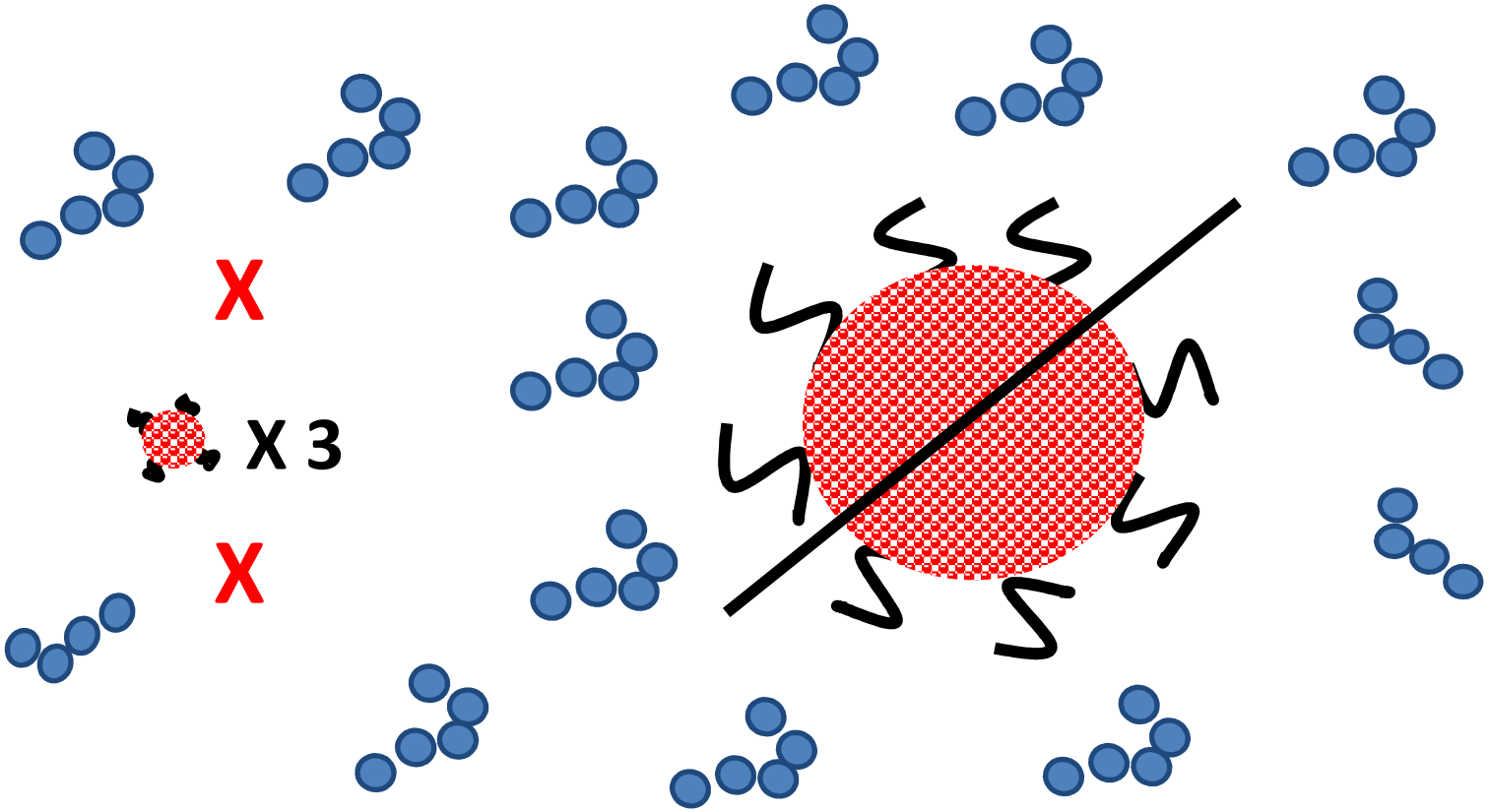
Mixtes 30/70


Normalized Concentration (AU)
Radius of giration (nm)
Times (min)
Conc. Caseines
R_G Caseines
Conc. WP70/30
R_G WP70/30
100 nm
0.2 µm

Mixtes 30/70


Normalized Concentration (AU)
Radius of giration (nm)
Times (min)
Conc. Caseines
R_G Caseines
Conc. WP70/30
R_G WP70/30
Conc. Mixtes 70/30
R_G Mixtes 70/30
100 nm
0.2 µm

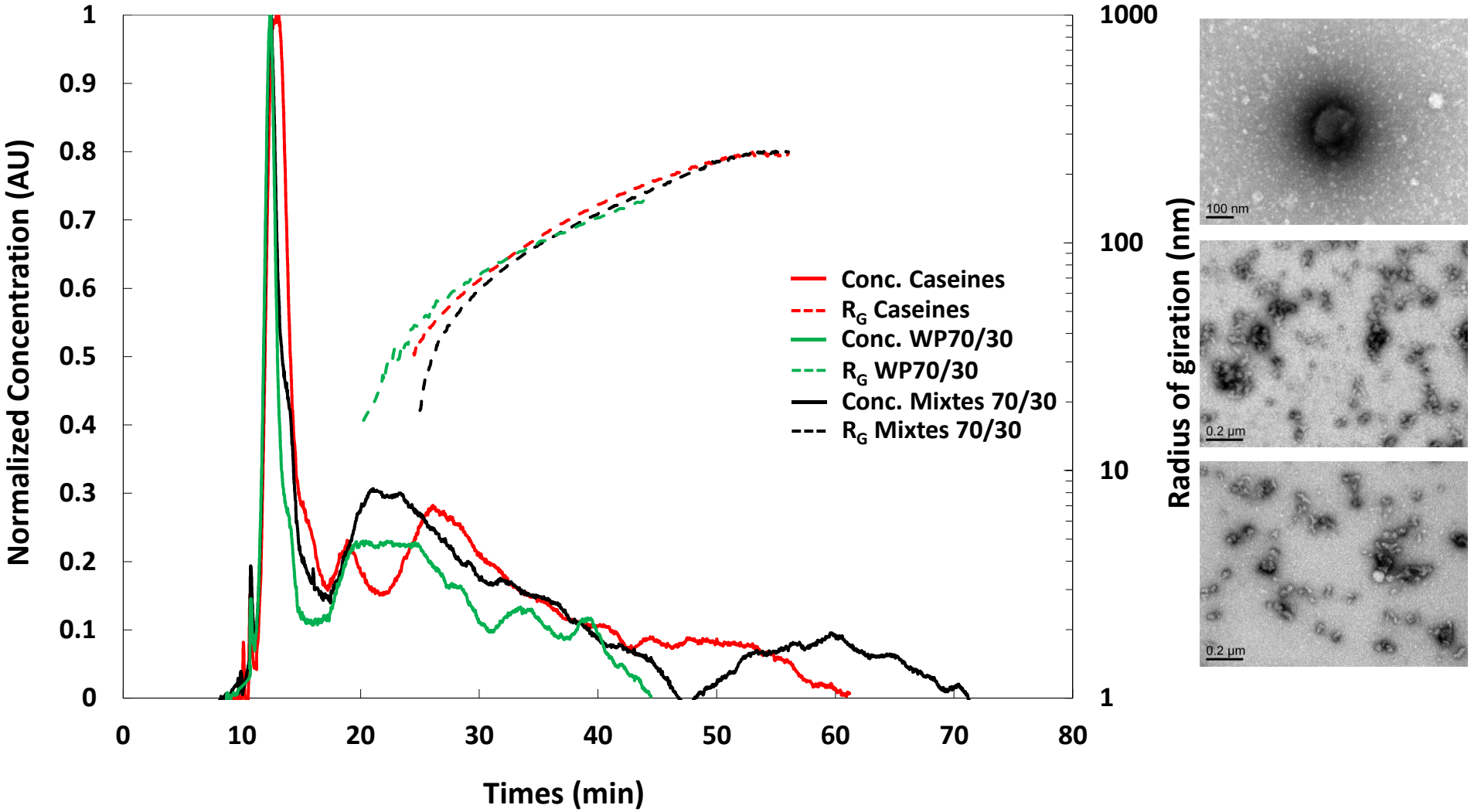
Mixtes 30/70



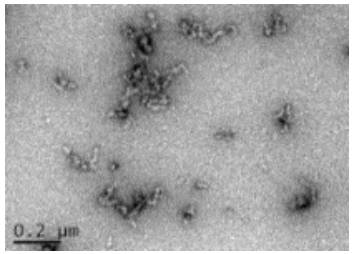
Pourquoi le profil des mixtes 30/70 et des fractals sont similaires?

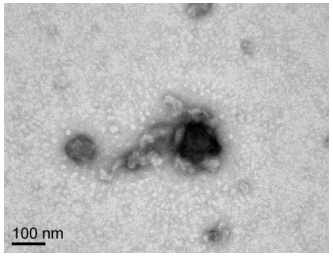
✓ Très peu de petites caséines présentent dans la solution et quasiment aucune grosse micelle

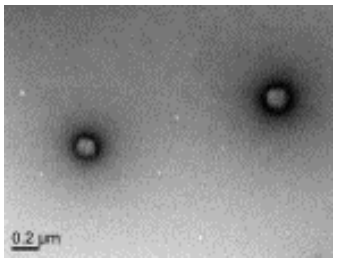
Mixtes 30/70


Normalized Concentration (AU)
Radius of giration (nm)
Times (min)
Conc. Caseines
R_G Caseines
Conc. WP70/30
R_G WP70/30
Conc. Mixtes 70/30
R_G Mixtes 70/30
100 nm
0.2 µm
0.2 µm

Conclusion

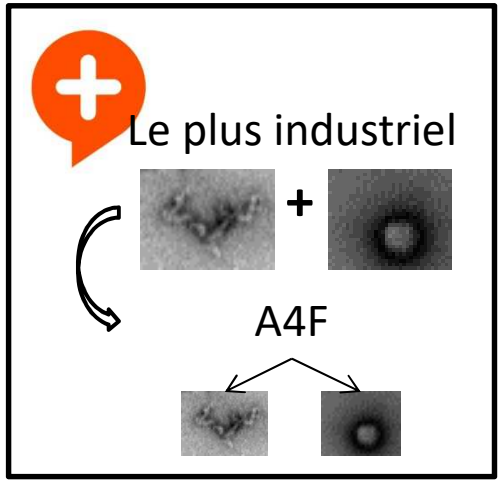






- ✓ Polydisperses
- ✓ Mélange monomères/agrégats
- ✓ Différente taille et forme
- ✓ Présence de caséines

Asymmetrical Flow Field Flow Fractionation	Fractionner les monomère et les agrégats
	Fractionner les agrégats
	Travailler avec des agrégats contenant des micelles
	Idée des mécanismes de formation des agrégats mixtes



Merci de votre attention

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