# Asymetrical Flow Field-Flow Fractionation coupled with Multi-Angle Light Scattering and Differential Refractometer (AF4-MALS-DRi): An analysis tool to characterize milk protein aggregates produced in semi-industrial way

M Hennetier, D De-Guibert, Y Gu, V Solé, Catherine Garnier, G Delaplace, Romain Jeantet, A Riaublanc

## HAL Id: hal-03370393
## https://hal.inrae.fr/hal-03370393v1

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**M.Hennetier[1]\*, D.De-Guibert[2], Y.Gu[3], V.Solé[1], C.Garnier[1], G.Delaplace[3], R.Jeantet[2], A. Riaublanc[1]**

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**[2] UMR1253 INRA, Science and Technology of Milk and Egg, Rennes, France**
**[3] UMR8207 UMET, Interface Processes and Hygiene of Materials, Lille, France**

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19TH INTERNATIONAL SYMPOSIUM ON FIELD- AND FLOW-BASED SEPARATIONS (FFF2018)
Columbia, SC | May 14-17, 2018

Milk Valley
PROFIL

INRA SCIENCE & IMPACT

bia
Biopolymères Interactions Assemblage
Nantes

STLO

UMET
Unité Matériaux Et Transformations

# Context

**Thickening or gelling agents**

**Natural product please!!!**

CLEAN LABEL
CLEAN LABEL

# Context

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Natural product please!!!

CLEAN LABEL · CLEAN LABEL
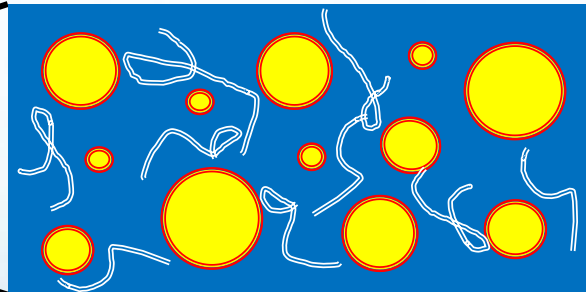
Oil/Water emulsion

Gelling agents texture the continuous phase and entrap the oil droplets

# Whey protein aggregates properties

✓ Use oil droplets to texture emulsion without gelling agents

✓ Connect oil droplets through whey protein aggregates

✓ Heating can form aggregates with different shapes and sizes (Nicolai, 2011)



**C. Surel, J. Foucquier, N. Perrot, A. Mackie, C. Garnier, A. Riaublanc, and M. Anton,** "Composition and structure of interface impacts texture of O/W emulsions," *Food Hydrocoll.*, vol. 34, pp. 3–9, 2014.
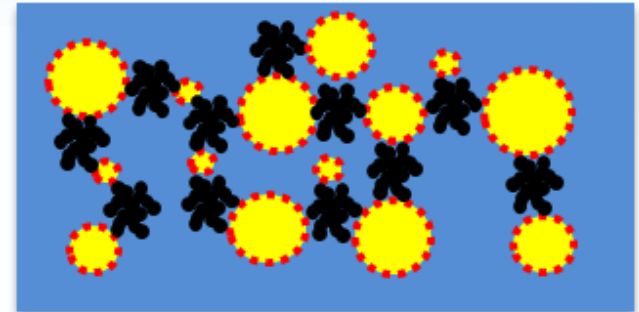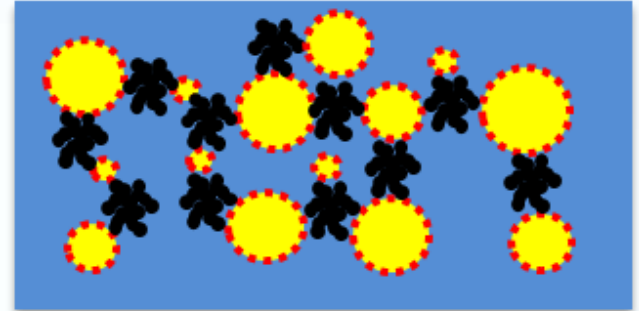
| Introduction | Methods | Results | Conclusion and perspectives |

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**Applications**

**Replace food additives like thickening or gelling agents in neutral dairy products**

**Texture emulsions even at low fat content**

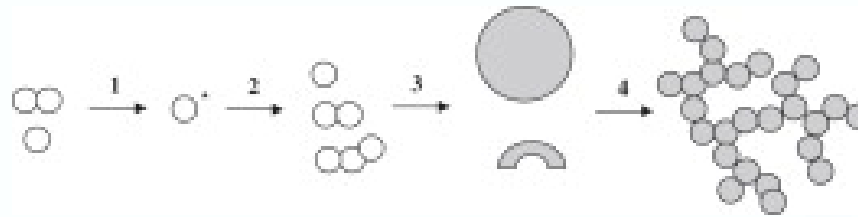**Fractal aggregates production from Whey Protein Isolate (WPI) at semi-industrial scale**

| Introduction | Methods | Results | Conclusion and perspectives |

# Fractal aggregate formation



(Nicolai, 2011)

1: Dimer/trimer of whey proteins (β-lactoglobulin) are heated at 80⁰C
  → Denaturation, unfolding and thiol/hydrophobic group exposition

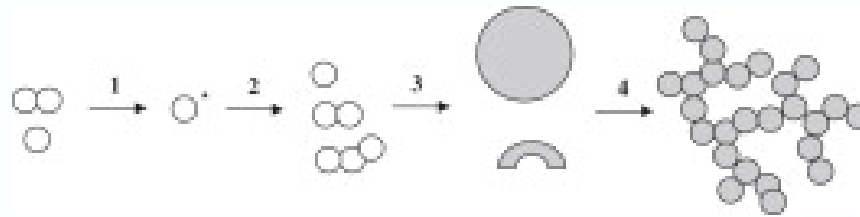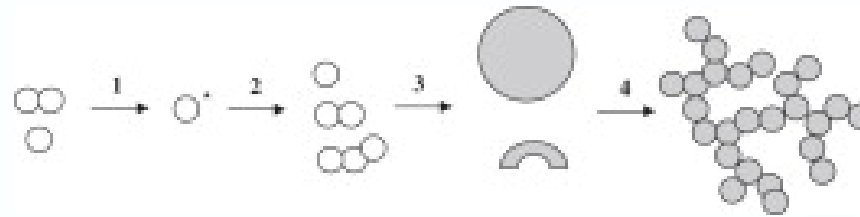# Fractal aggregate formation



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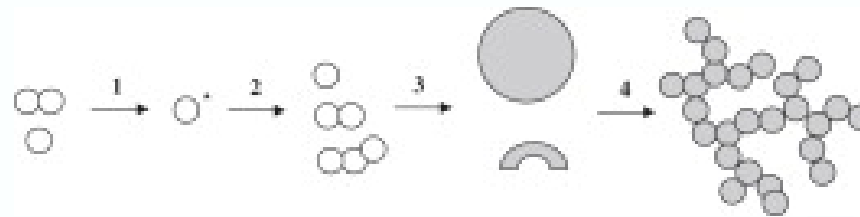2: Denatured proteins form oligomers (irreversible)

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4: When Salt concentration is sufficient: Branched aggregate formation (disulfides bonds, hydrogen and hydrophobic interactions)

| **Introduction** | Methods | Results | Conclusion and perspectives |
|---|---|---|---|

# Objective:
## Characterization of aggregates produced at semi-industrial scale

**Dynamic light scattering (DLS) in batch**

- Large objects are exacerbated
- No differentiation in large particle populations

**Size exclusion chromatography (SEC)**

- Aggregates obstruct the column

# Objective:
## Characterization of aggregates produced at semi-industrial scale

**Dynamic light scattering (DLS) in batch**

**As-FlFFF**

- Large objects are exacerbated
- No differentiation in large particle populations

- Injection of entire product without filtration

- Fractionation of large particles
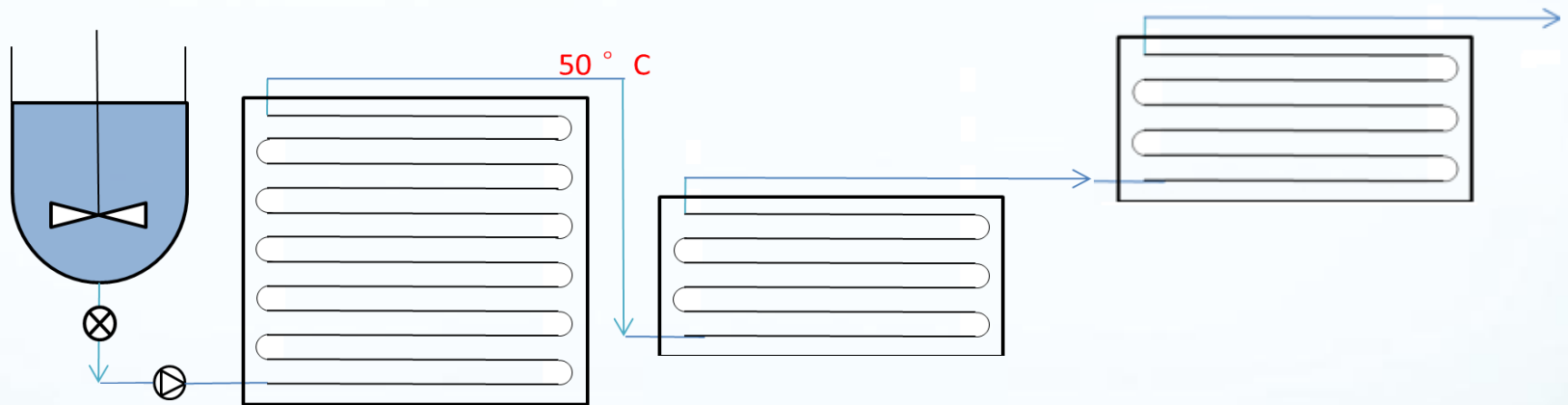
**Size exclusion chromatography (SEC)**

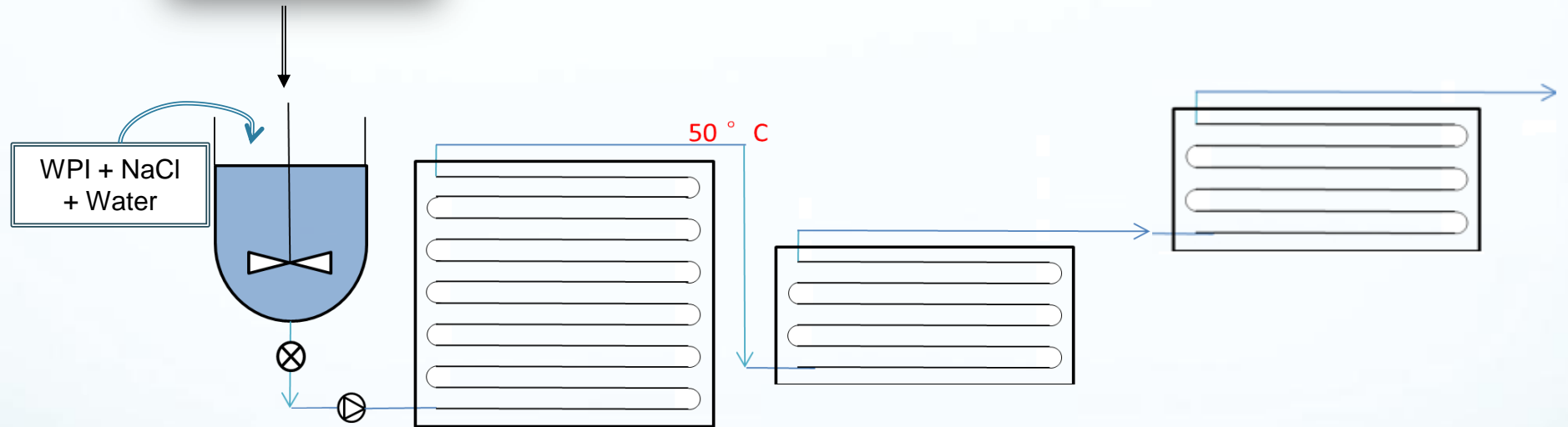**Transmission Electronic Microscopy (TEM)**

- Aggregates obstruct the column

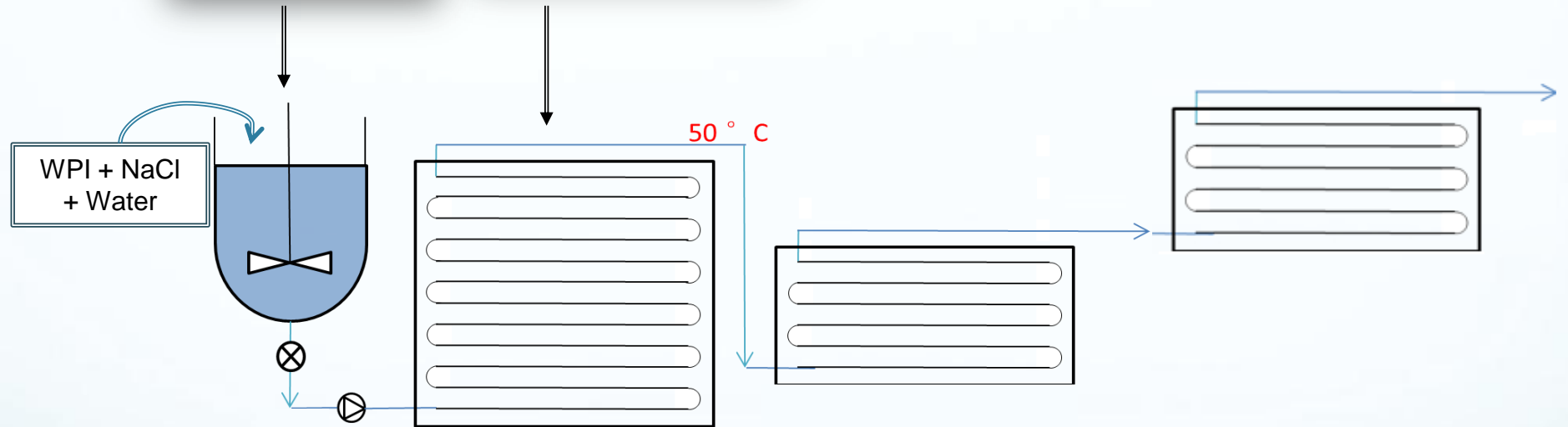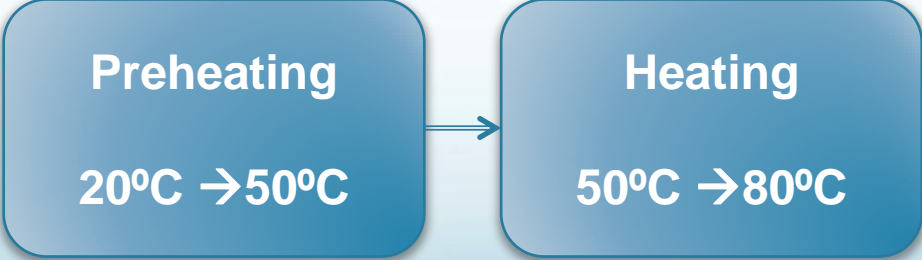- Correlation between size/form factor (As-FlFFF) and microscopic visual

# Aggregates production by semi-industrial way

50 ° C

# Aggregates production by semi-industrial way

WPI + NaCl + Water

50 ° C

# Aggregates production by semi-industrial way



WPI + NaCl + Water

50 ° C

**Preheating**

**20ºC →50ºC**

# Aggregates production by semi-industrial way



WPI + NaCl + Water

50 ˚C

80ºC
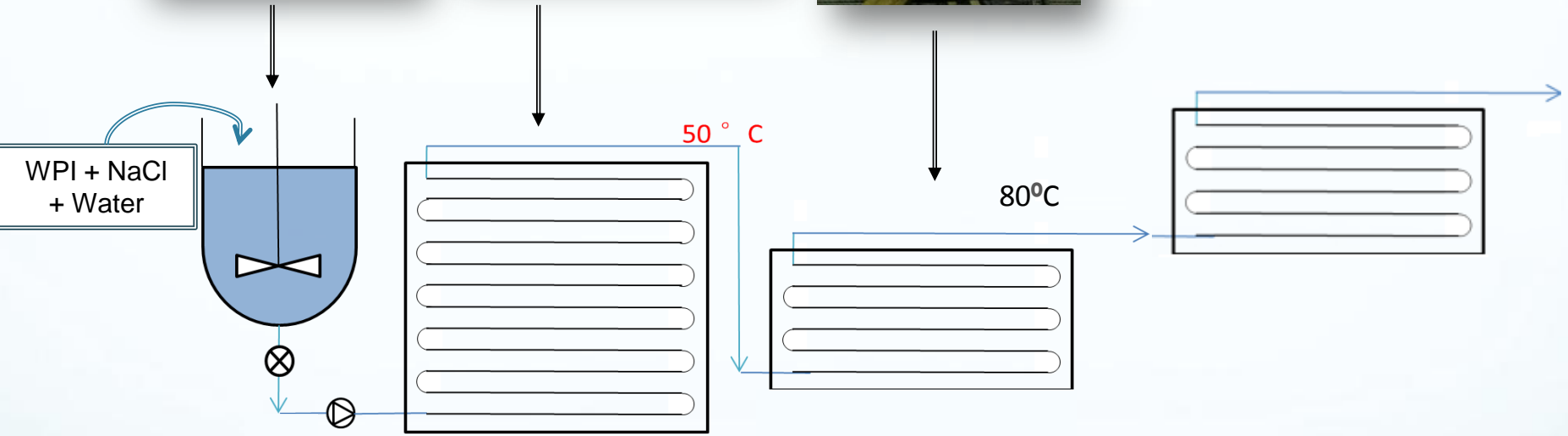
**Preheating**

20ºC → 50ºC

**Heating**

50ºC → 80ºC

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# Aggregates production by semi-industrial way



WPI + NaCl + Water

50 ° C

80⁰C

80⁰C

**Preheating**

**20⁰C →50⁰C**

**Heating**

**50⁰C →80⁰C**

**Static or dynamic holding 80⁰C ; up to 1h**

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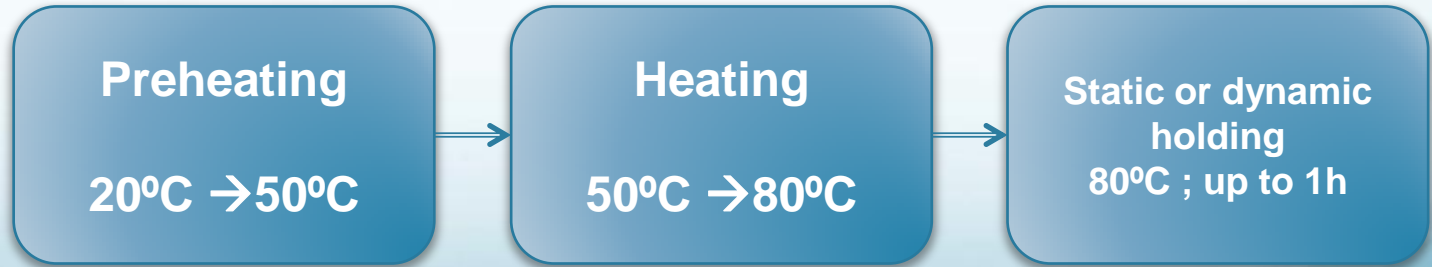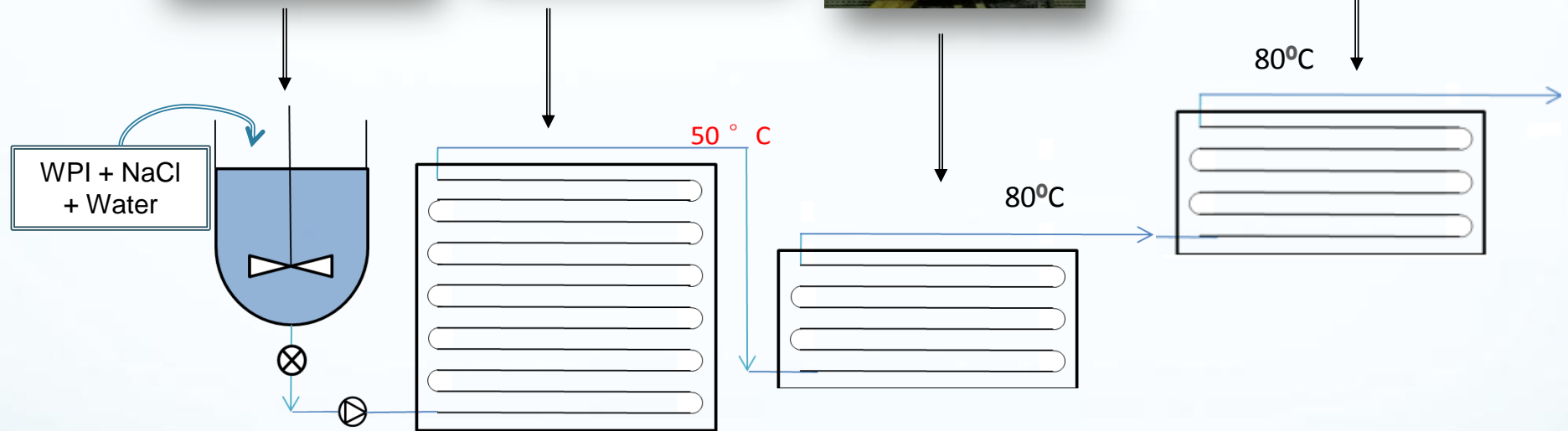
# As-FIFFF method
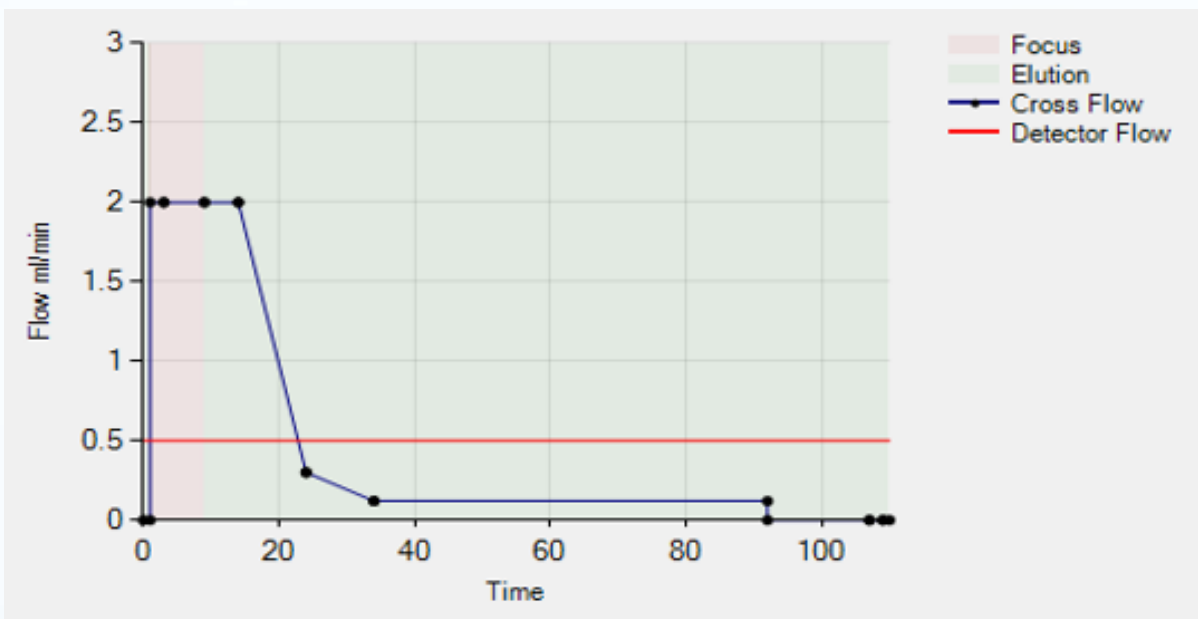


✓**Wyatt Dualtec System**

✓**MALS**

✓**Differential Refractometer**

**Elution : 1 min**
**Focus : 2 min**
**Focus + inject : 6 min**

| Eluent | Ultrapure water + 0.02% NaN$_3$ + 45mM NaCl |
|---|---|
| Membrane | Regenerated cellulose, cut-off 10kDa |
| Spacer (µm) | 350 |
| Canal | Short Canal |
| Injected mass (µg) | 20 |

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# Interest parameters

- Radius of gyration (Rg) distribution

- Molar mass (M) distribution

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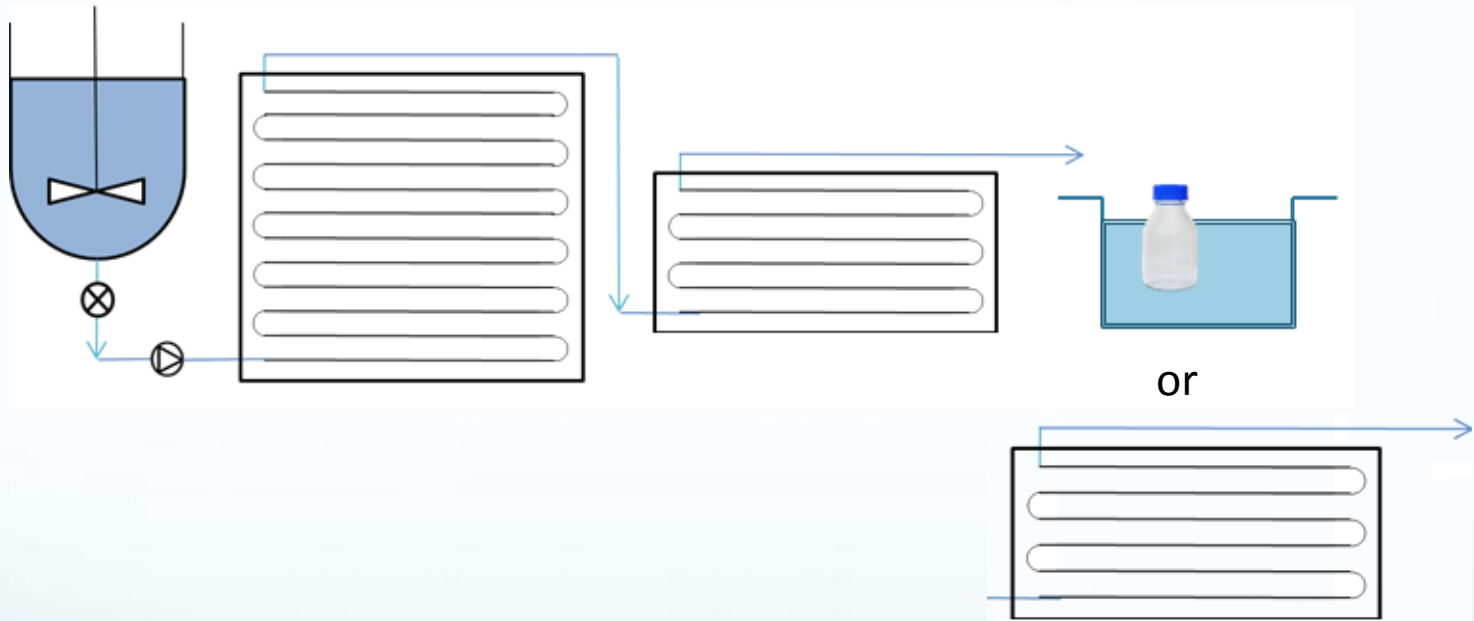- Fractal dimension (Df): $\log(\text{Rg}).\,Df = f(\log(M))$
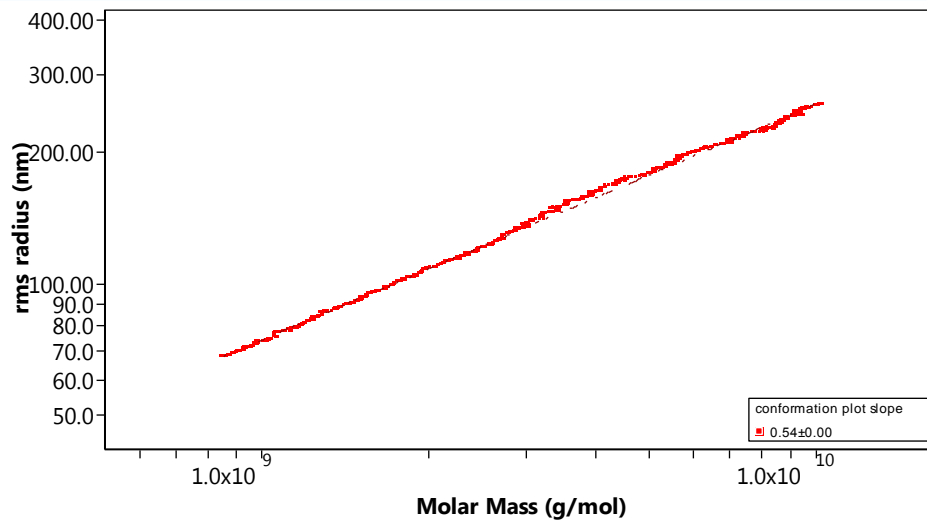


Molecular conformation model :

Df = 1 : Rod shape
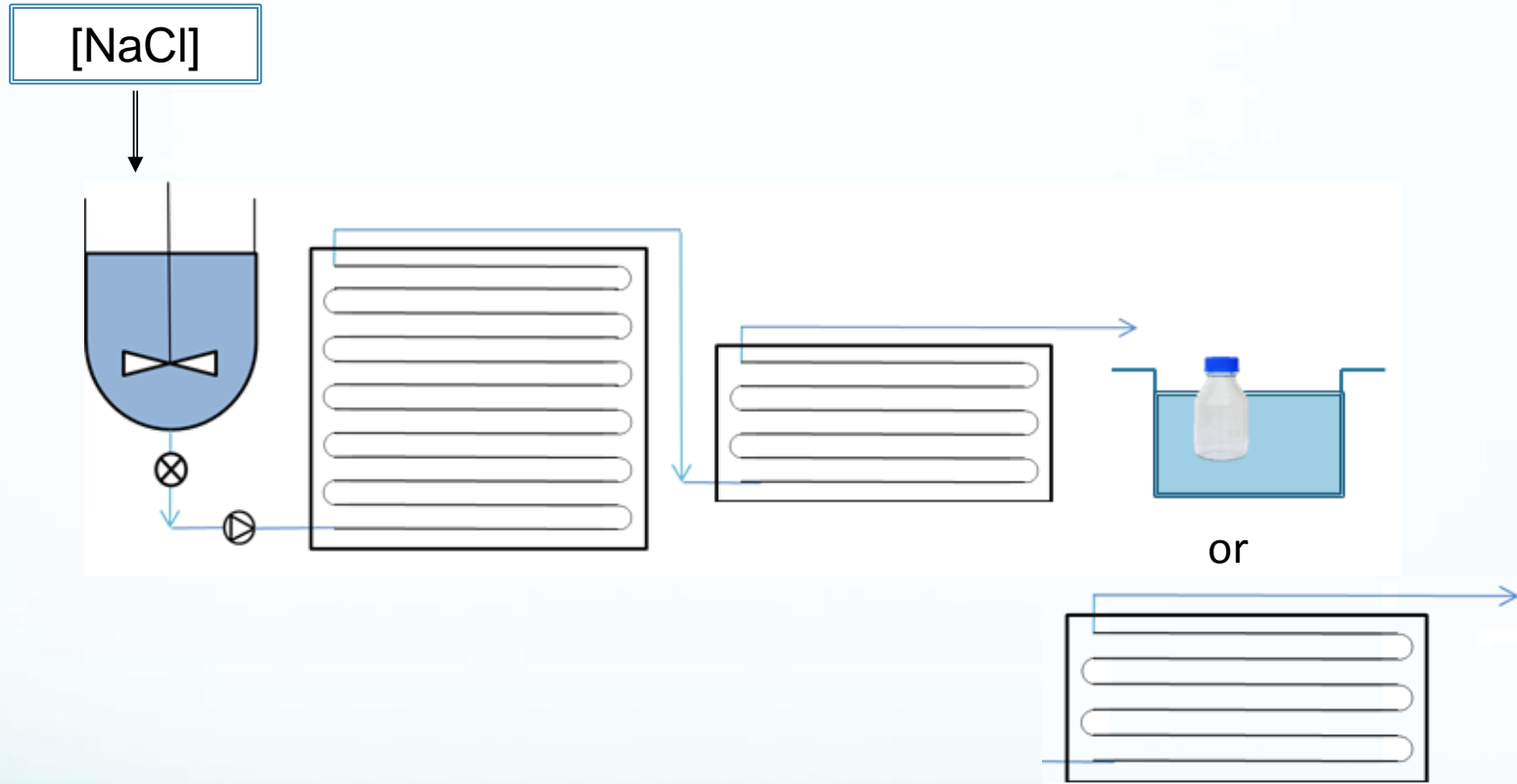Df ~ 2 : Coil
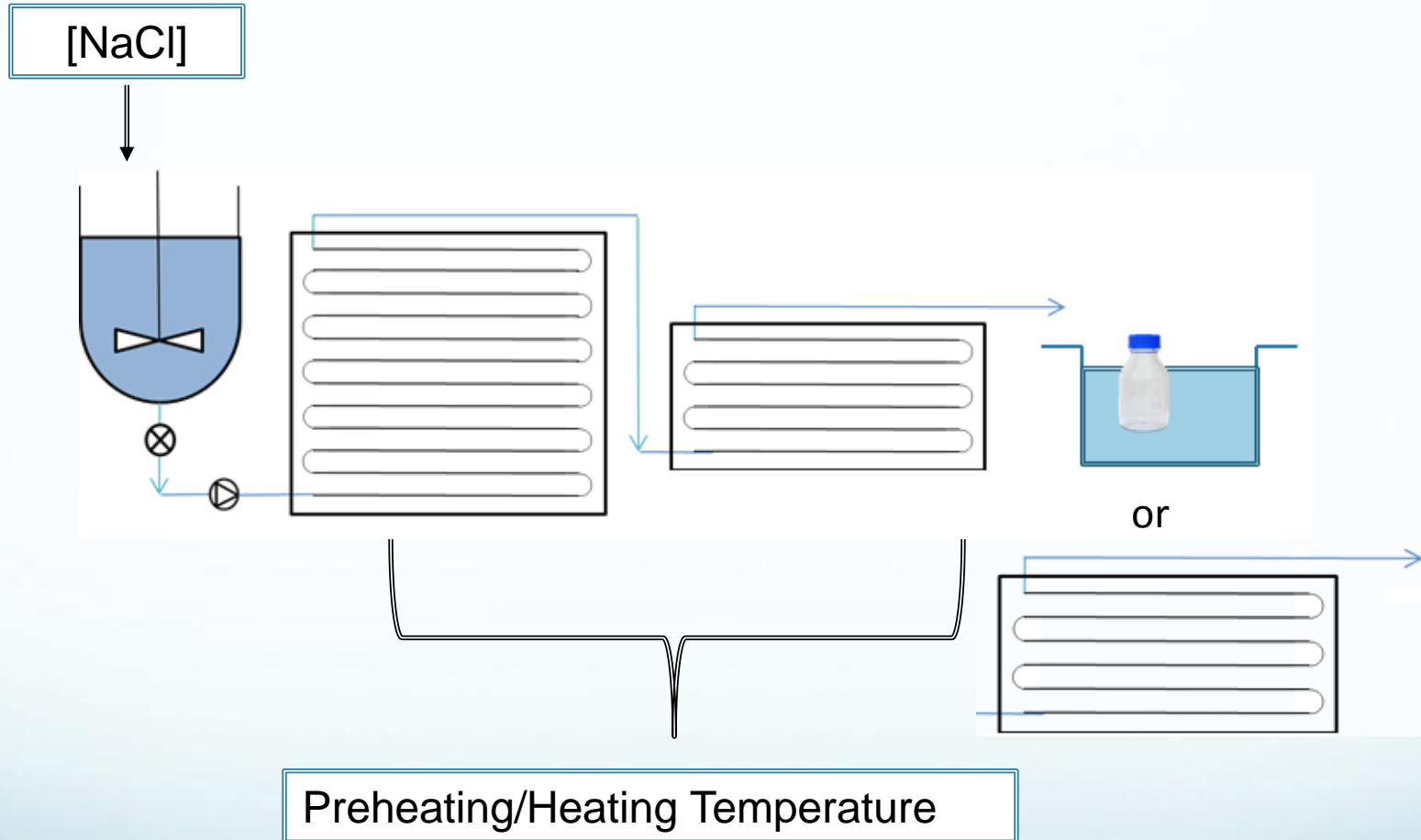Df = 3 : Spherical shape

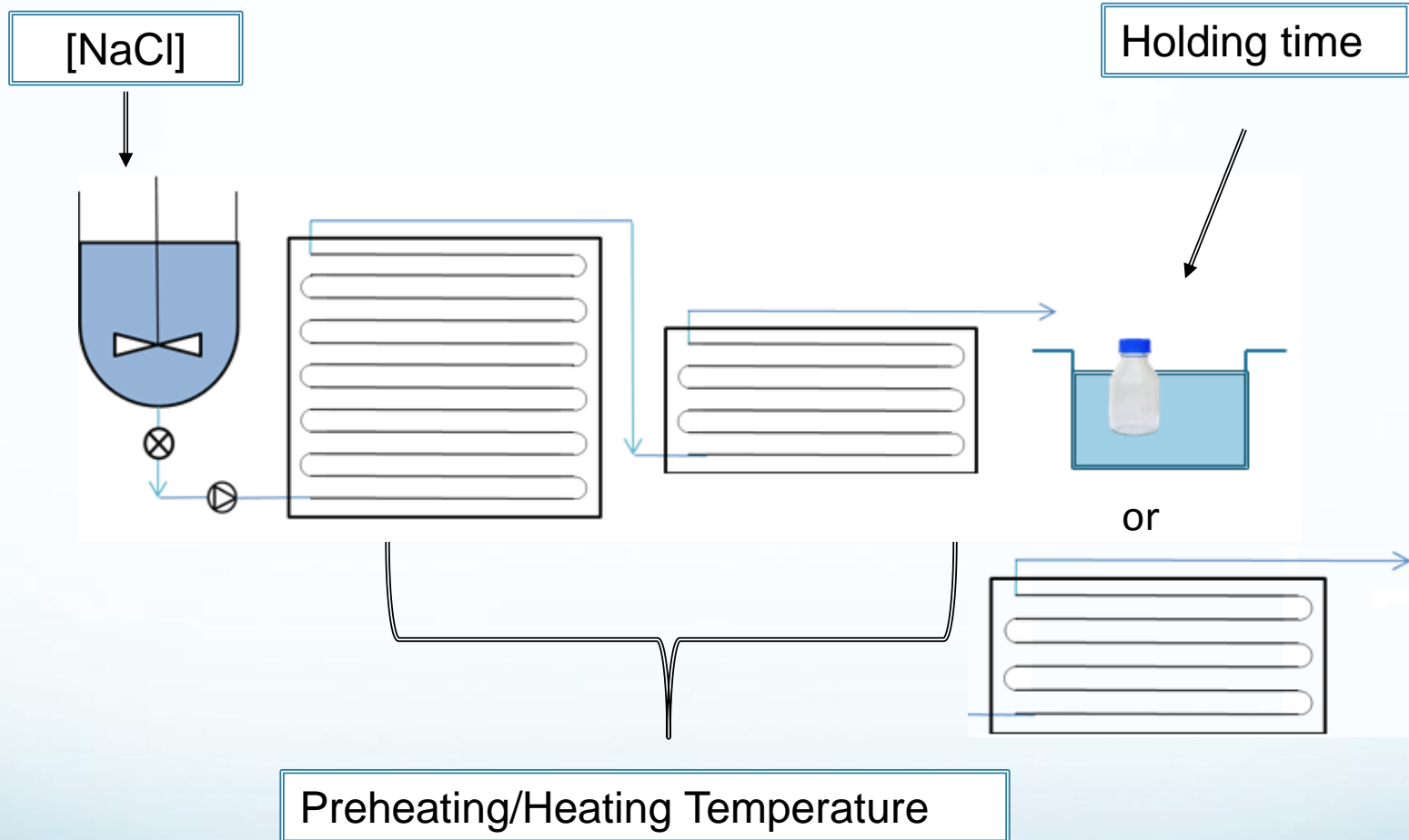# Size/shape aggregate control : process parameter variations



or

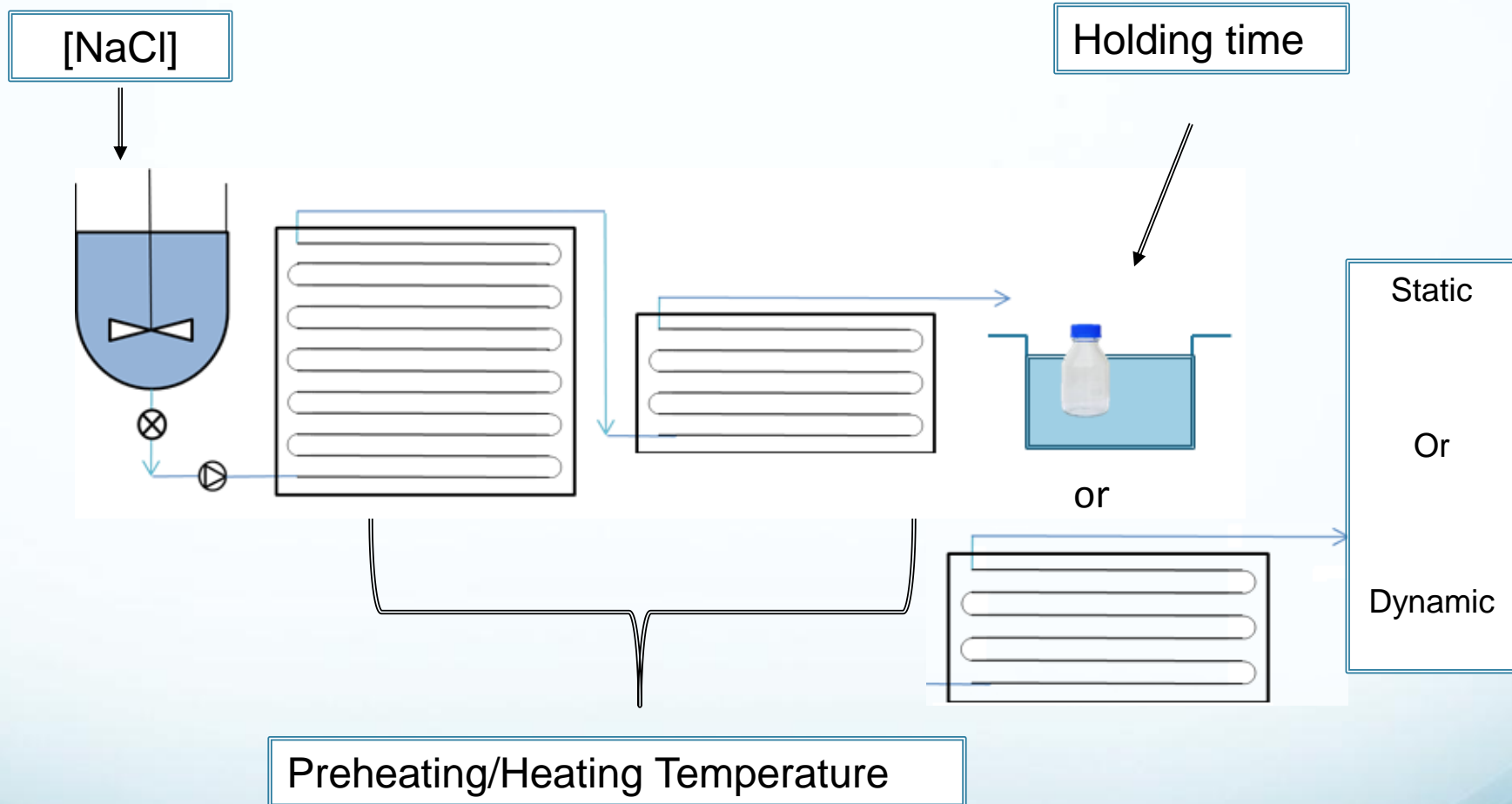# Size/shape aggregate control : process parameter variations



[NaCl]

or

# Size/shape aggregate control : process parameter variations



[NaCl]

or

Preheating/Heating Temperature

# Size/shape aggregate control : process parameter variations



[NaCl]

Holding time

or

Preheating/Heating Temperature

# Size/shape aggregate control : process parameter variations



[NaCl]

Holding time

Static

Or

Dynamic

or

Preheating/Heating Temperature

# First production results

**5%** proteins ; **10mM** NaCl ; **Static** holding ; **15min** of holding time ; **50/80°C** Preheating/Heating
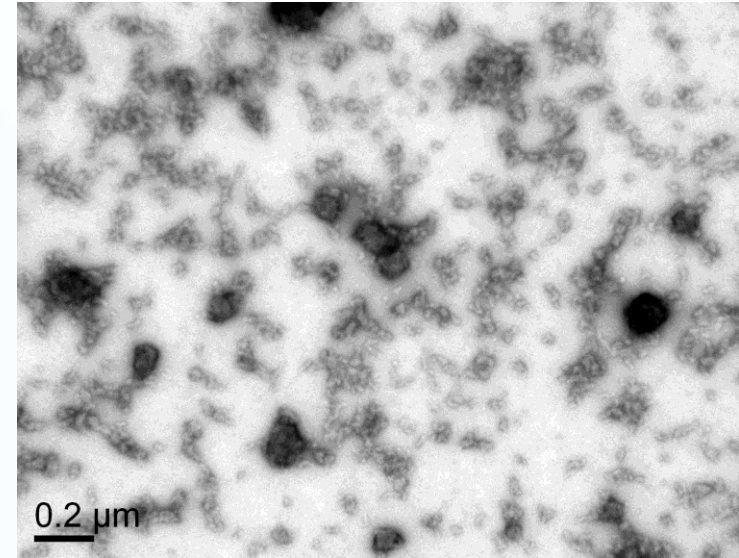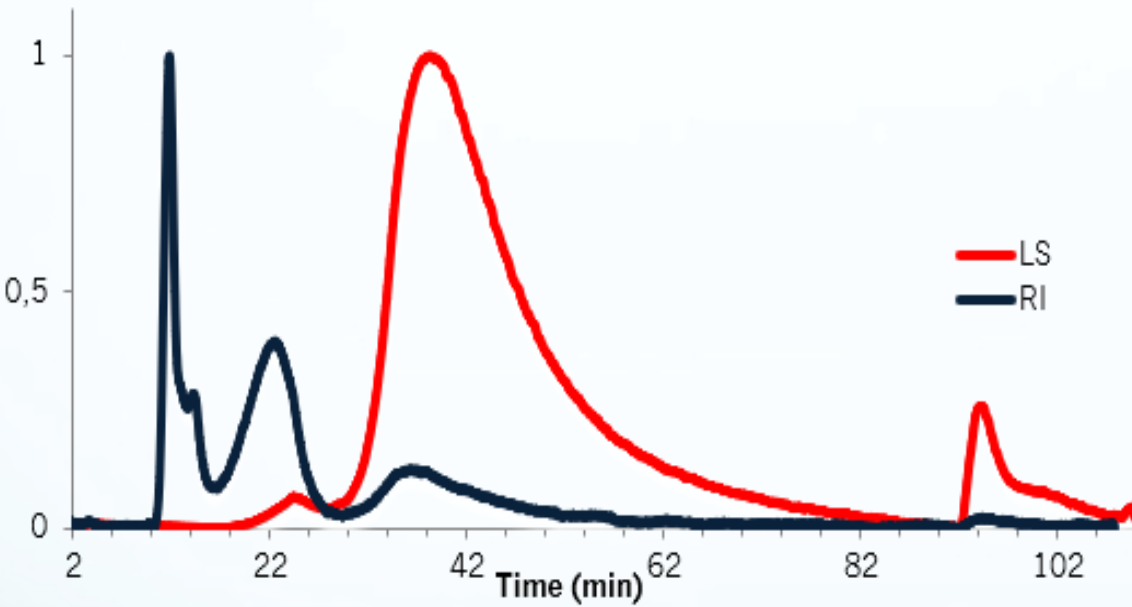
| Introduction | Methods | **Results** | Conclusion and perspectives |

# First production results

**5%** proteins ; **10mM** NaCl ; **Static** holding ; **15min** of holding time ; **50/80°C** Preheating/Heating

**Relative Scale**





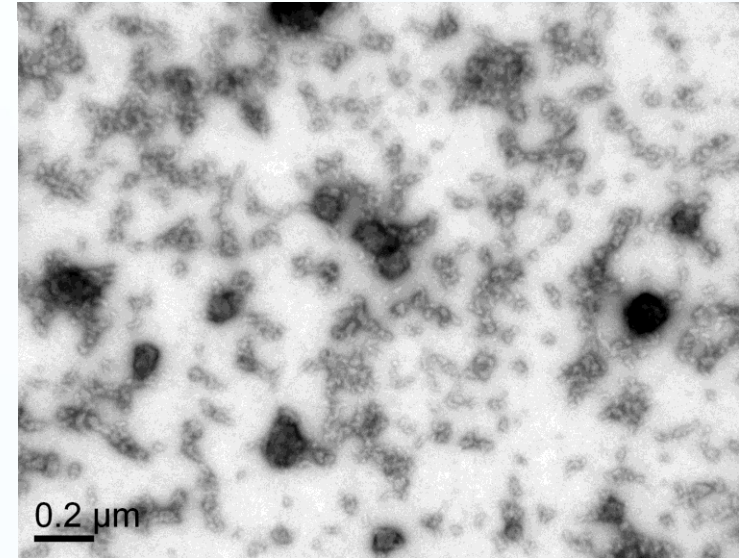Transmission Electronic microscopy
(negative coloration)

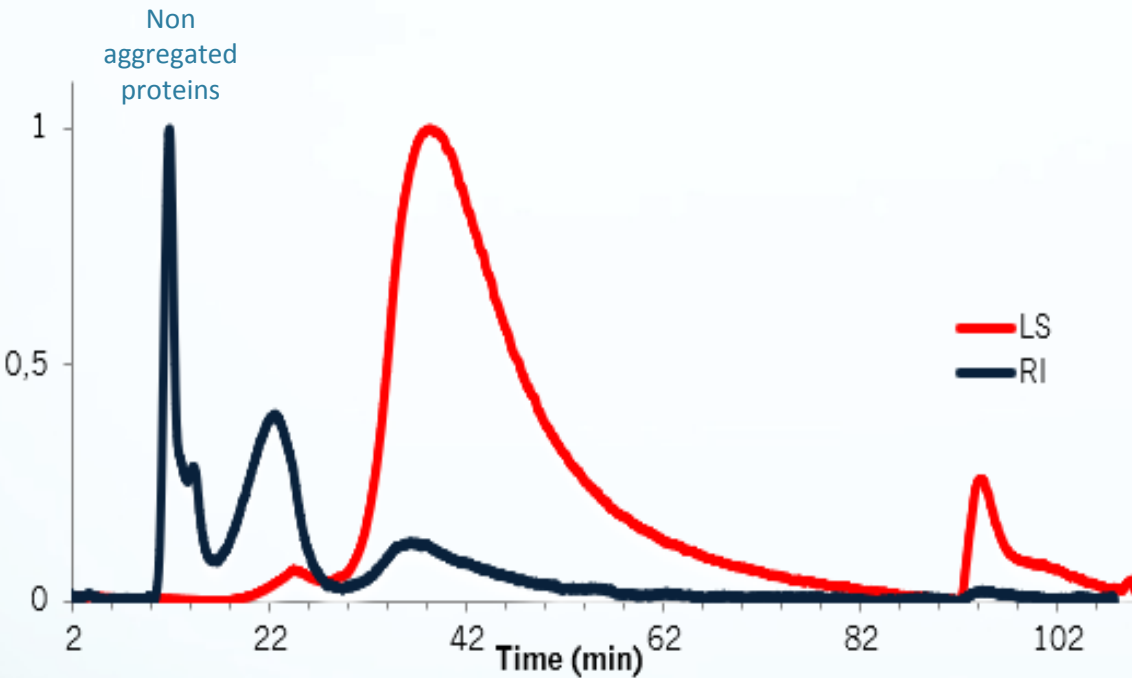# First production results
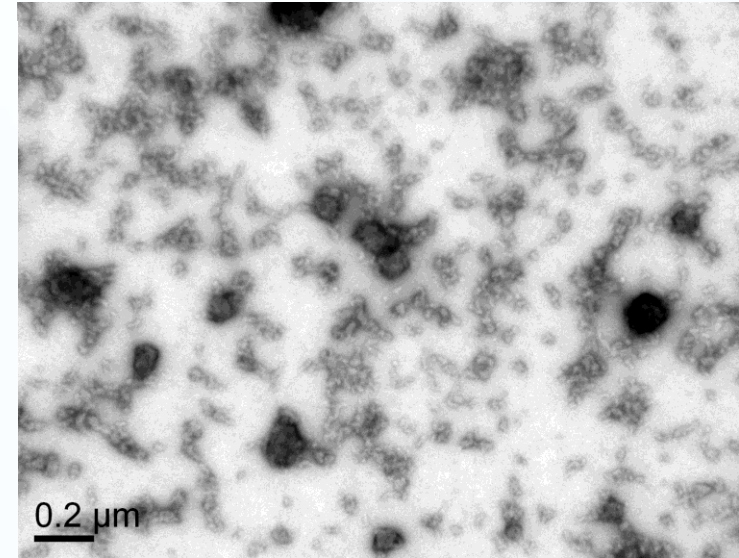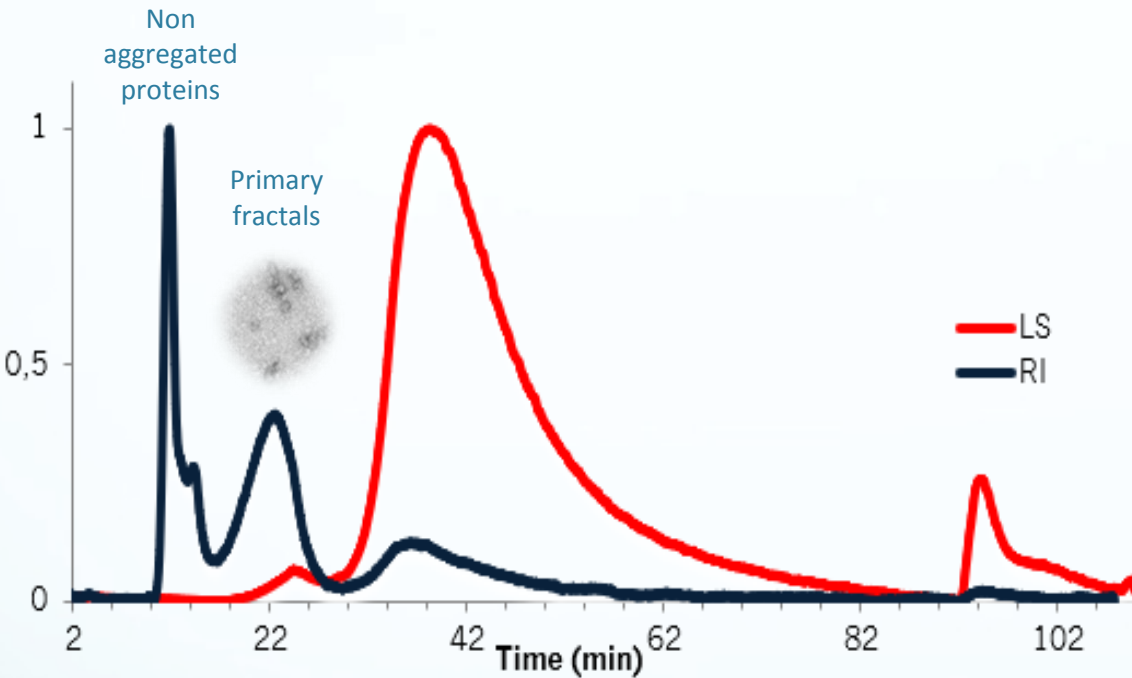
**Relative Scale**



Non aggregated proteins

LS
RI



0.2 µm

Transmission Electronic microscopy
(negative coloration)

- 1$^{rst}$ population (10-15min): non aggregated whey proteins

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# First production results

**5%** proteins ; **10mM** NaCl ; **Static** holding ; **15min** of holding time ; **50/80°** C Preheating/Heating
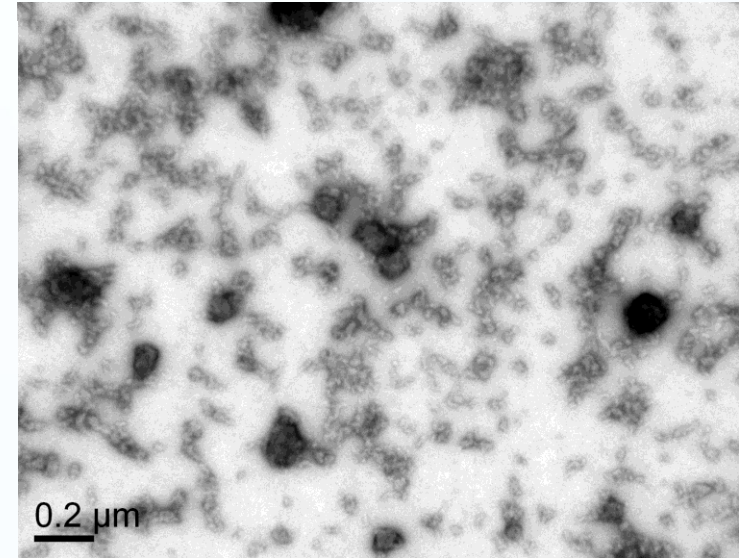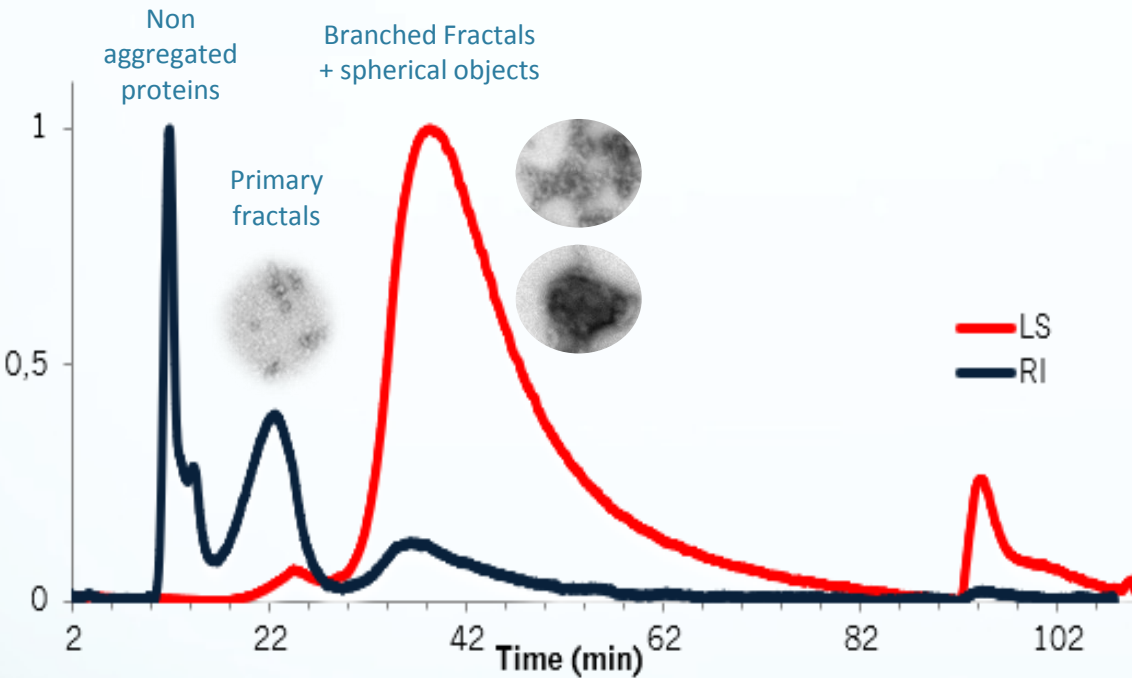
**Relative Scale**





Transmission Electronic microscopy
(negative coloration)

- 1ʳˢᵗ population (10-15min): non aggregated whey proteins

- 2ⁿᵈ population (15-30min): primary fractals

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**5%** proteins ; **10mM** NaCl ; **Static** holding ; **15min** of holding time ; **50/80°C** Preheating/Heating

**Relative Scale**



Transmission Electronic microscopy (negative coloration)

- 1<sup>rst</sup> population (10-15min): non aggregated whey proteins

- 2<sup>nd</sup> population (15-30min): primary fractals

- 3<sup>rd</sup> population (after 30min): mix between large fractals and spherical objects
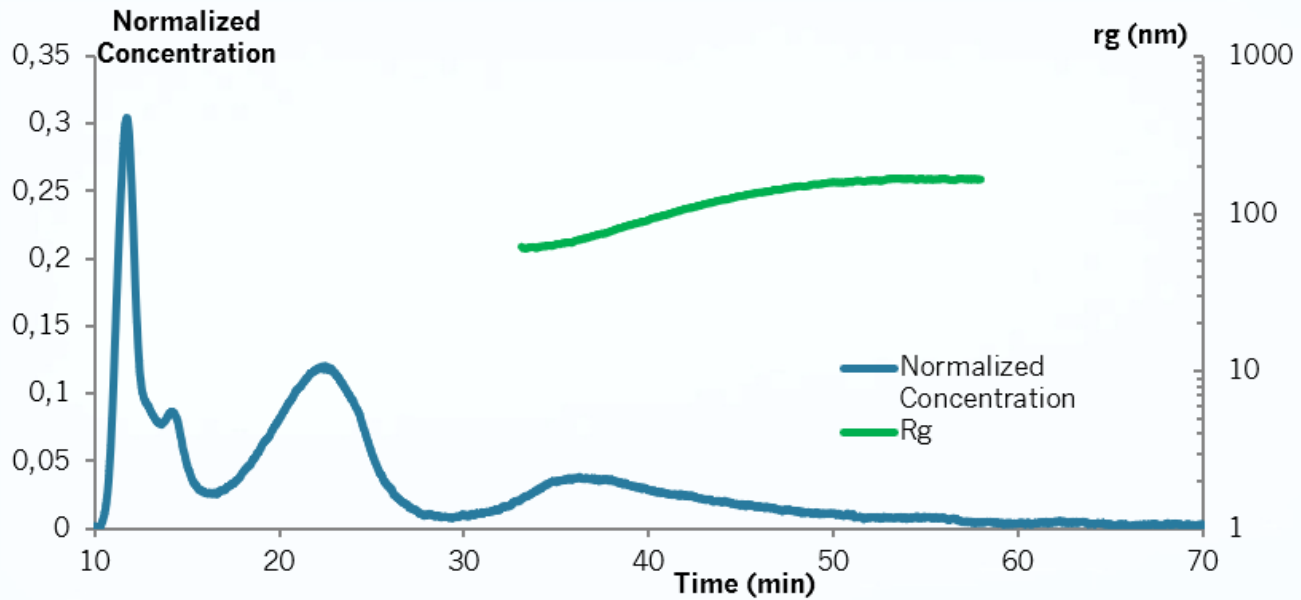
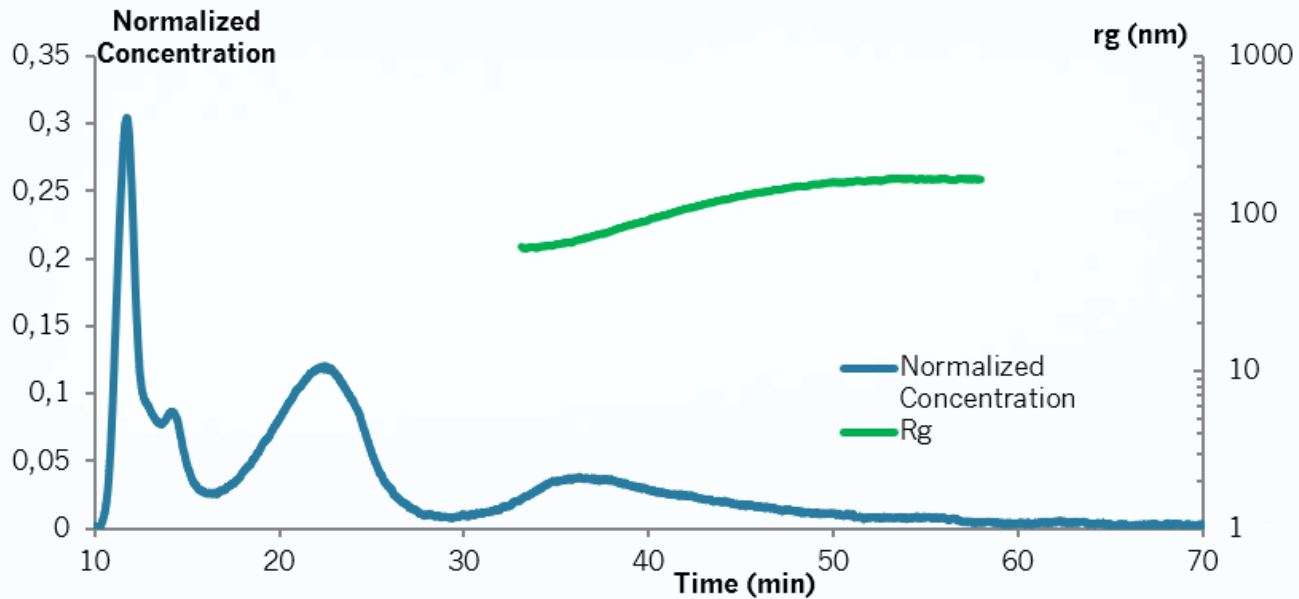| Introduction | Methods | **Results** | Conclusion and perspectives |

# First production results

# First production results



| | $\overline{Mw}$ (g/mol) | $\overline{Rg}$ (nm) | Mass fraction (%) |
|---|---|---|---|
| **1st population** | / | / | 30 |
| **2nd population** | $8,5.10^6$ ±14% | / | 40 |
| **3rd population** | $8,3.10^8$ ±1,3% | 79±3% | 30 |

**Objective** : Vary process parameters to obtain largest aggregates

# Dynamic/static holding
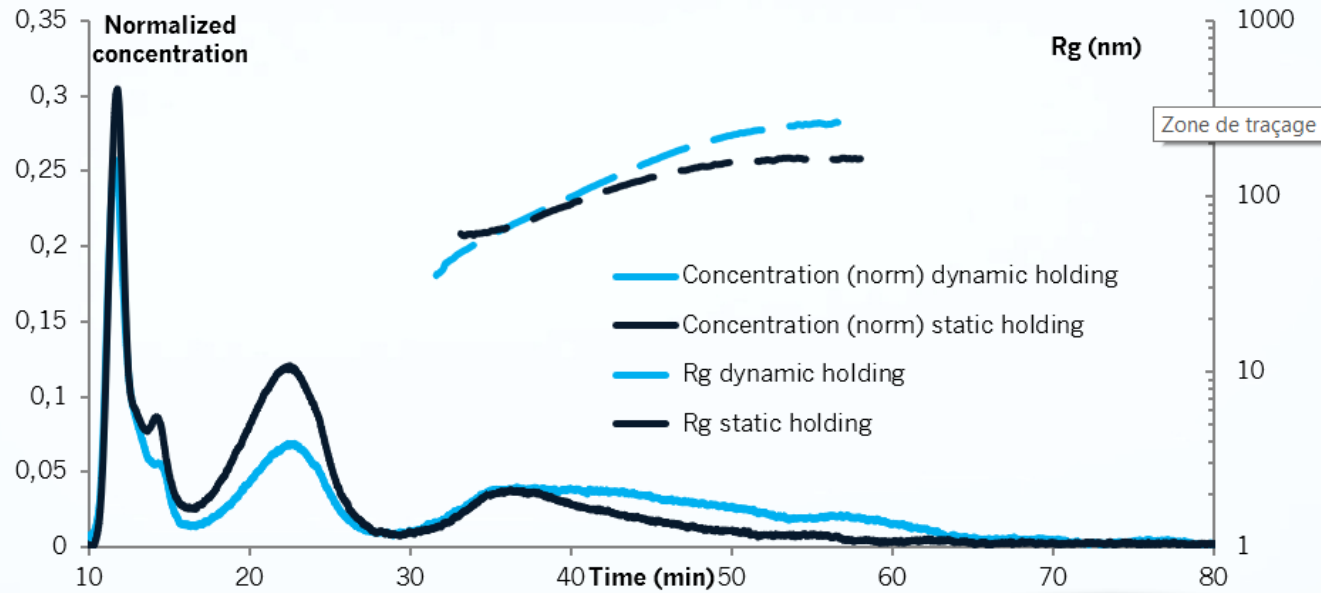
# Dynamic/static holding
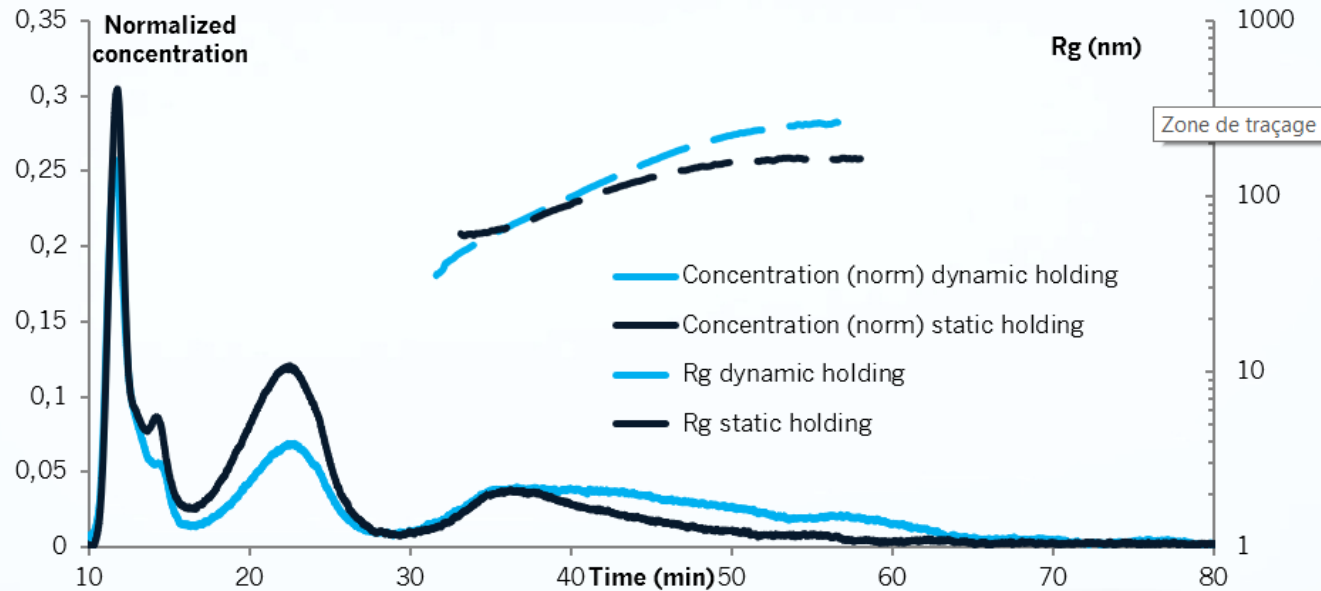


Primary fractals aggregate together
to form larger objects
$\overline{Rg}$ =95nm±3%

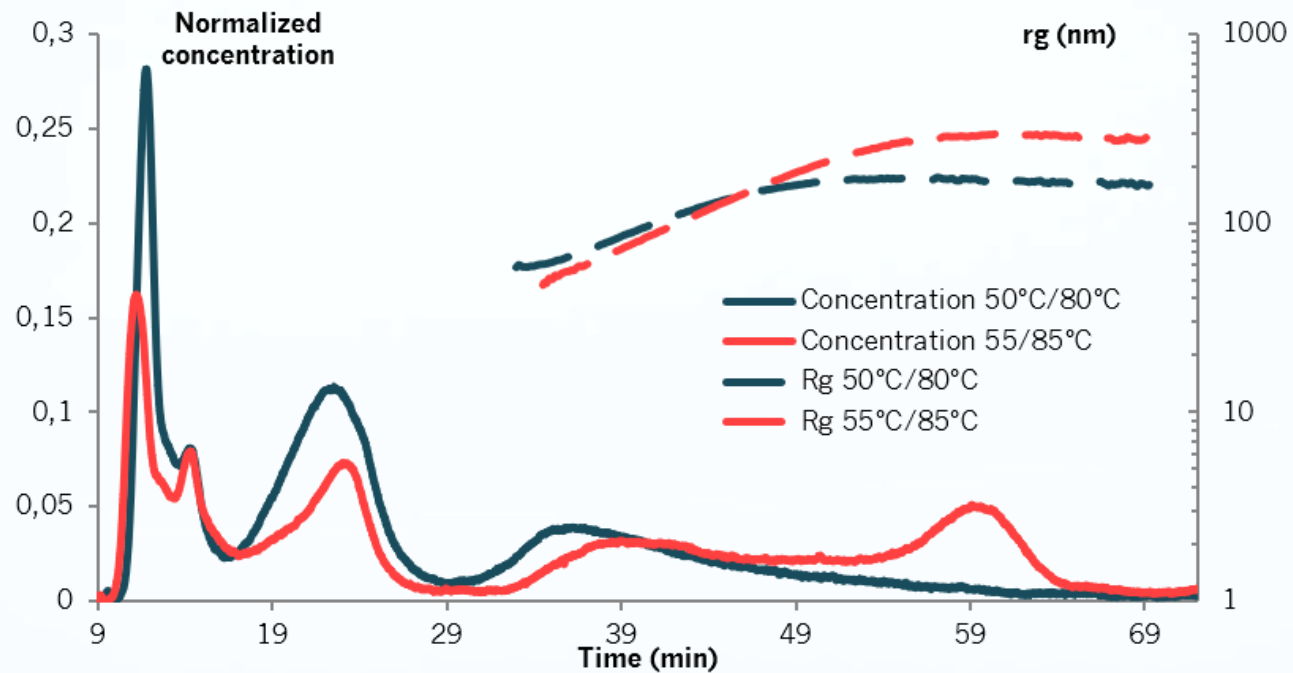Introduction | Methods | **Results** | Conclusion and perspectives

# Dynamic/static holding
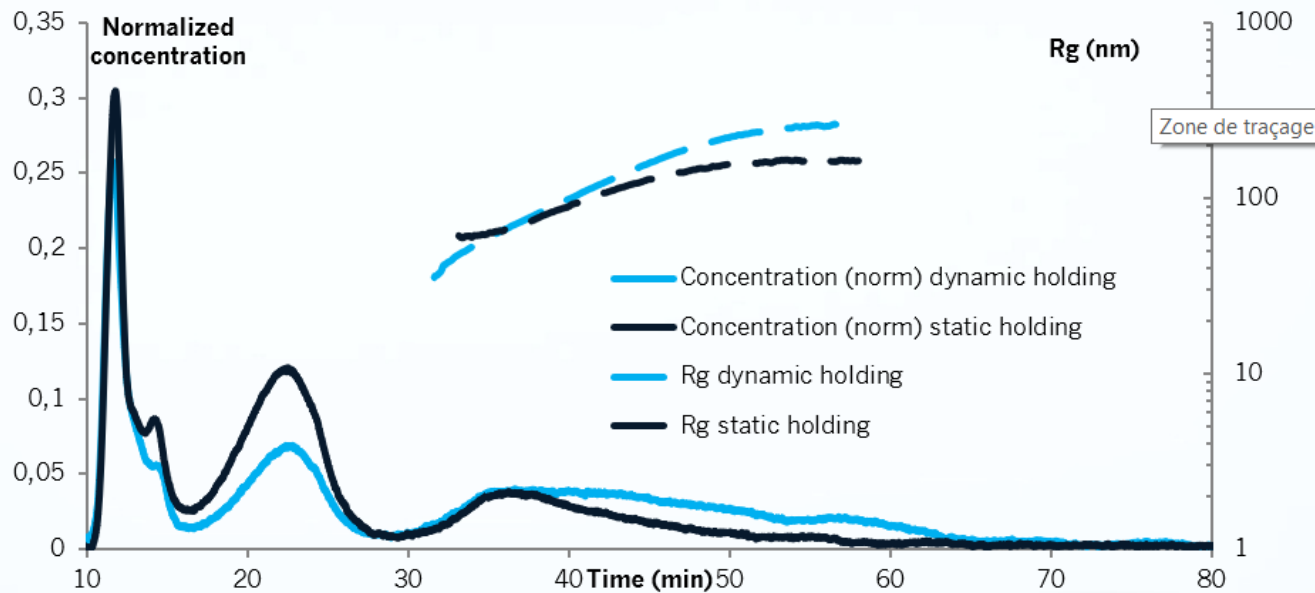


| | Static Mass fraction (%) | Dynamic Mass fraction (%) |
|---|---|---|
| **1st population** | 30 | 30 |
| **2nd population** | 40 | 26 |
| **3rd population** | 30 | 44 |

Primary fractals aggregate together to form larger objects
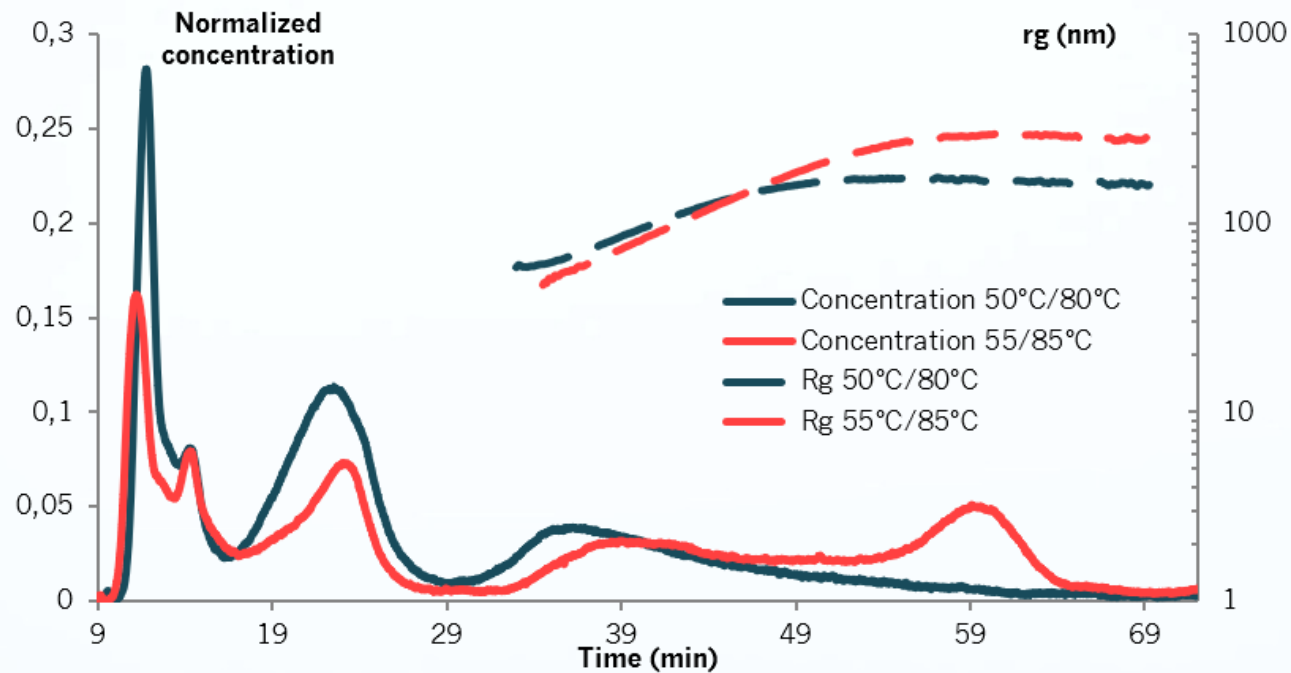$$\overline{Rg} = 95nm \pm 3\%$$

Interaction probability between fractals is more important in dynamic system

Introduction | Methods | **Results** | Conclusion and perspectives

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# Preheating/Heating Temperature Variation
## 50/80° C ⟶ 55/85° C

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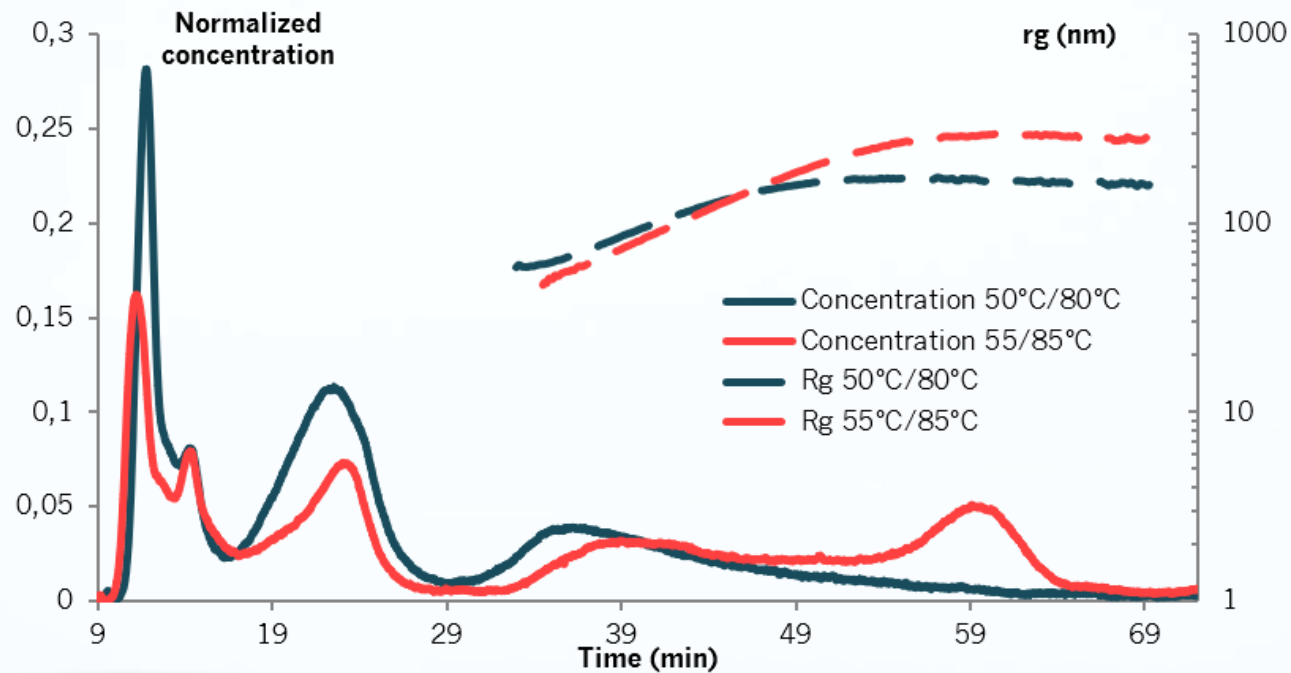New population formation (55-65min) by increasing preheating/heating temperature

$$\overline{Rg} = 285nm \pm 0,7\%$$

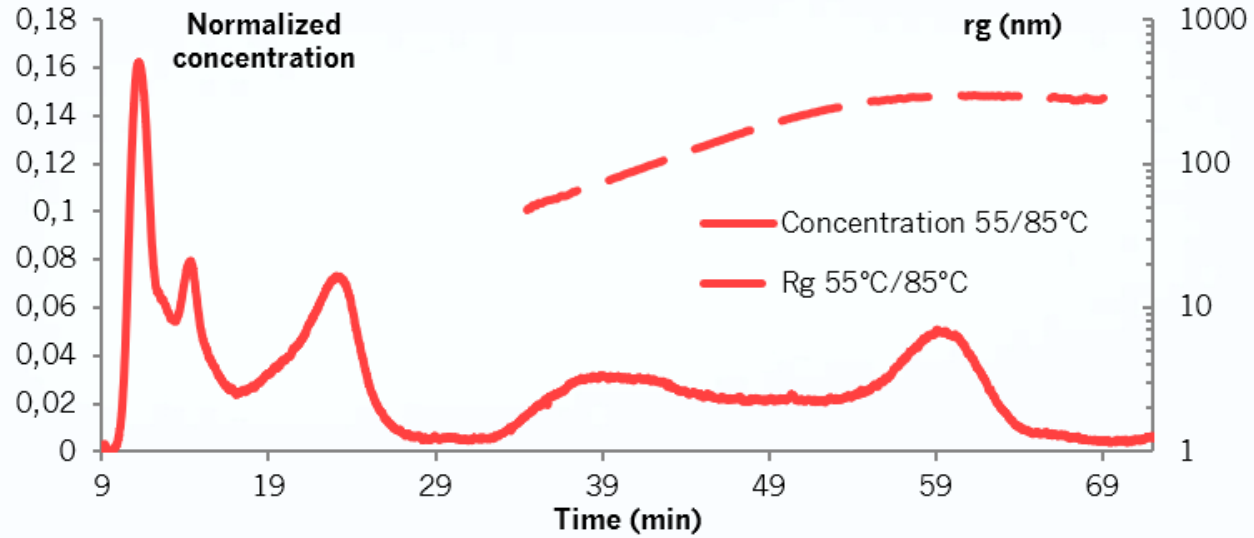| Introduction | Methods | **Results** | Conclusion and perspectives |
|---|---|---|---|

# Preheating/Heating Temperature Variation
## 50/80° C ⟶ 55/85° C



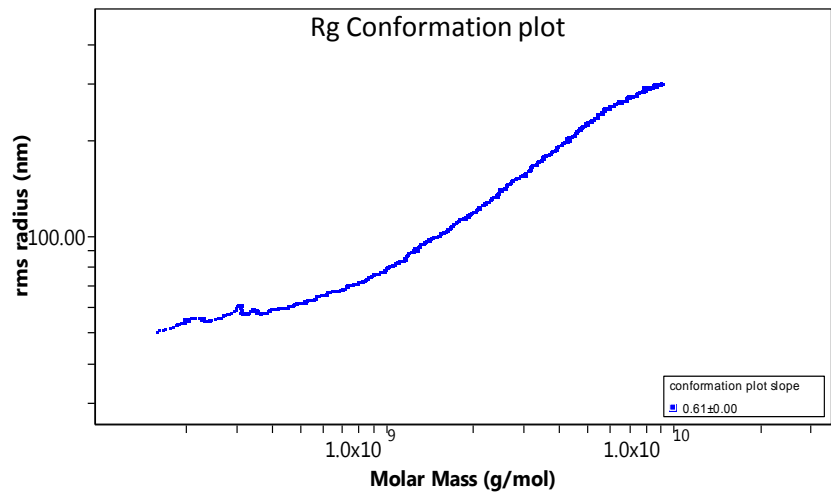New population formation (55-65min) by increasing preheating/heating temperature

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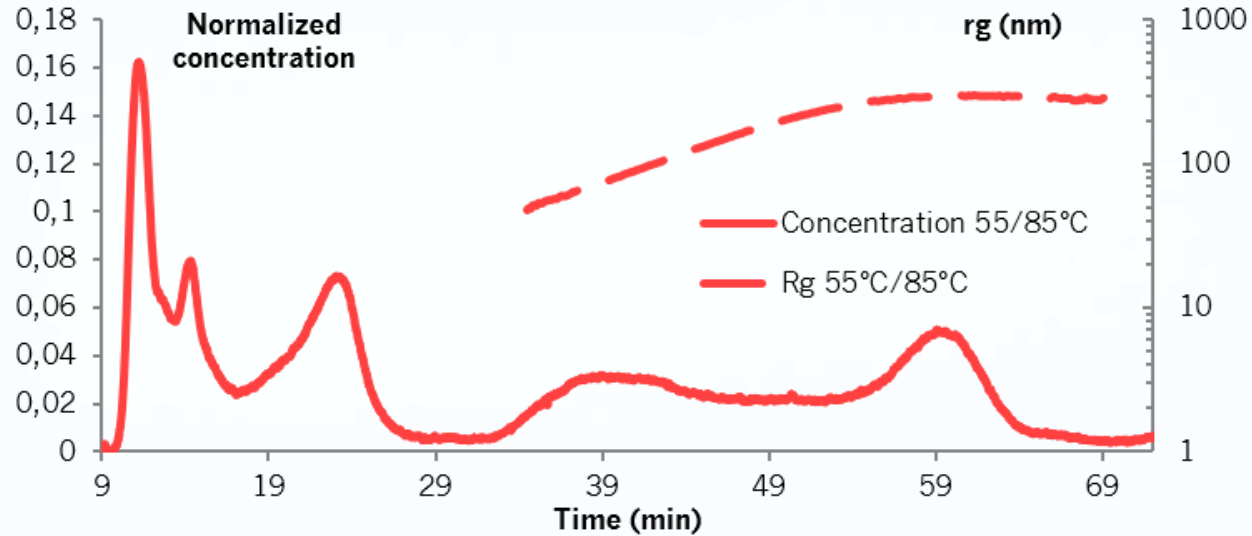Whey proteins and primary fractals concentration reduces to form a 4th largest population

| Introduction | Methods | **Results** | Conclusion and perspectives |

# Fractal Dimension



$$\log(Rg) = \frac{1}{Df} \cdot f(\log(Mw)) \text{ between } 35\text{-}65\text{min}$$

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The slope increases over time (Df decreases)
$\longrightarrow$ 4th population is less dense than 3rd one
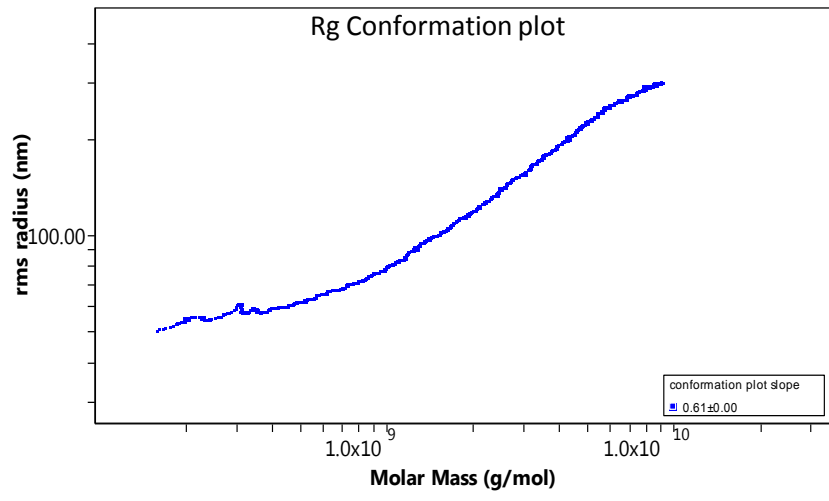Maybe there are less spherical objects?
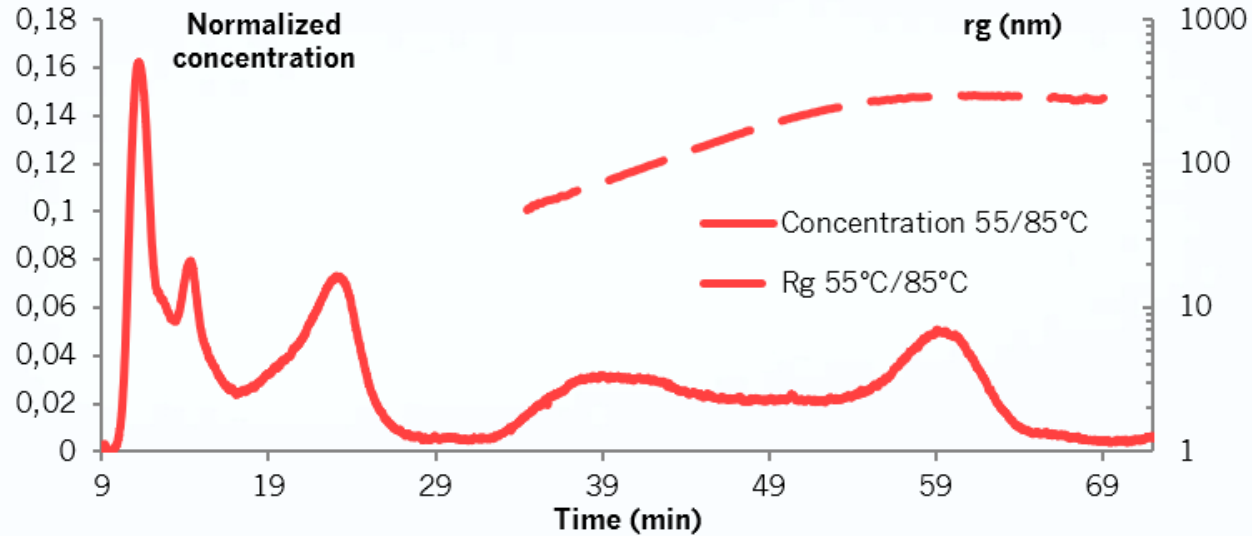
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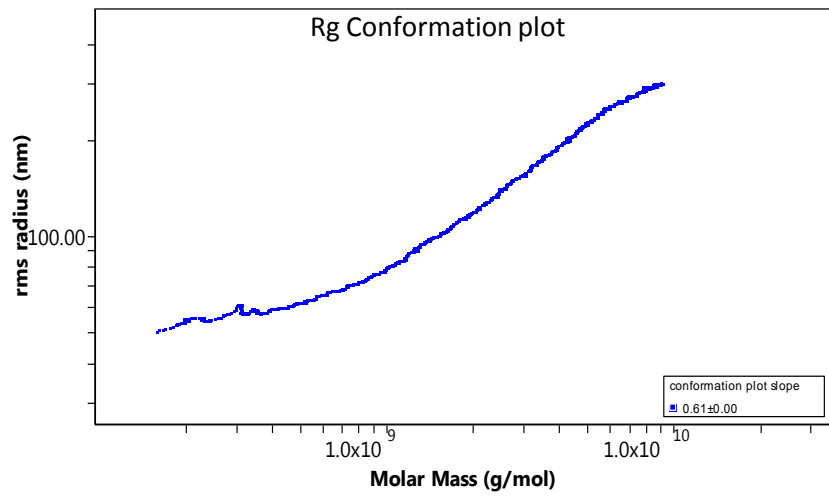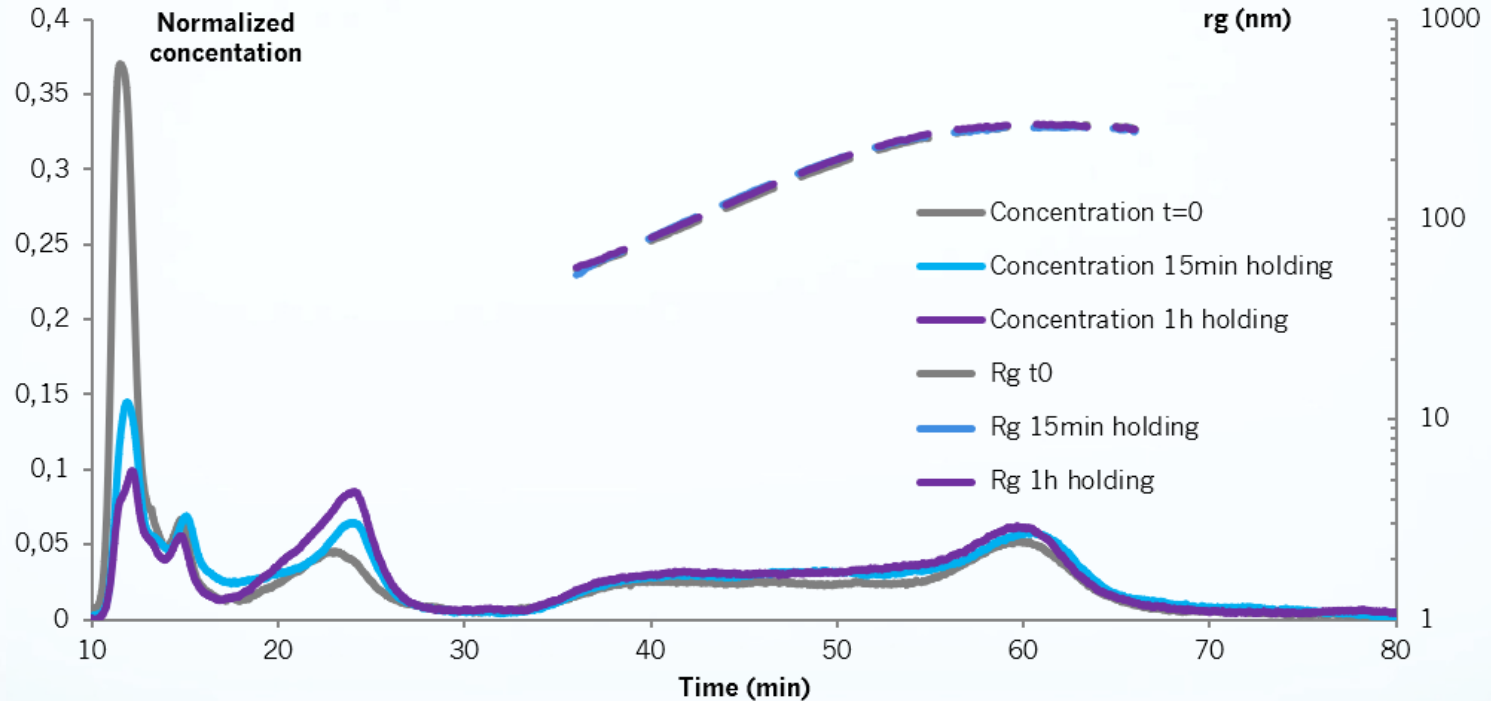Maybe there are less spherical objects?

→ Fraction collection + TEM
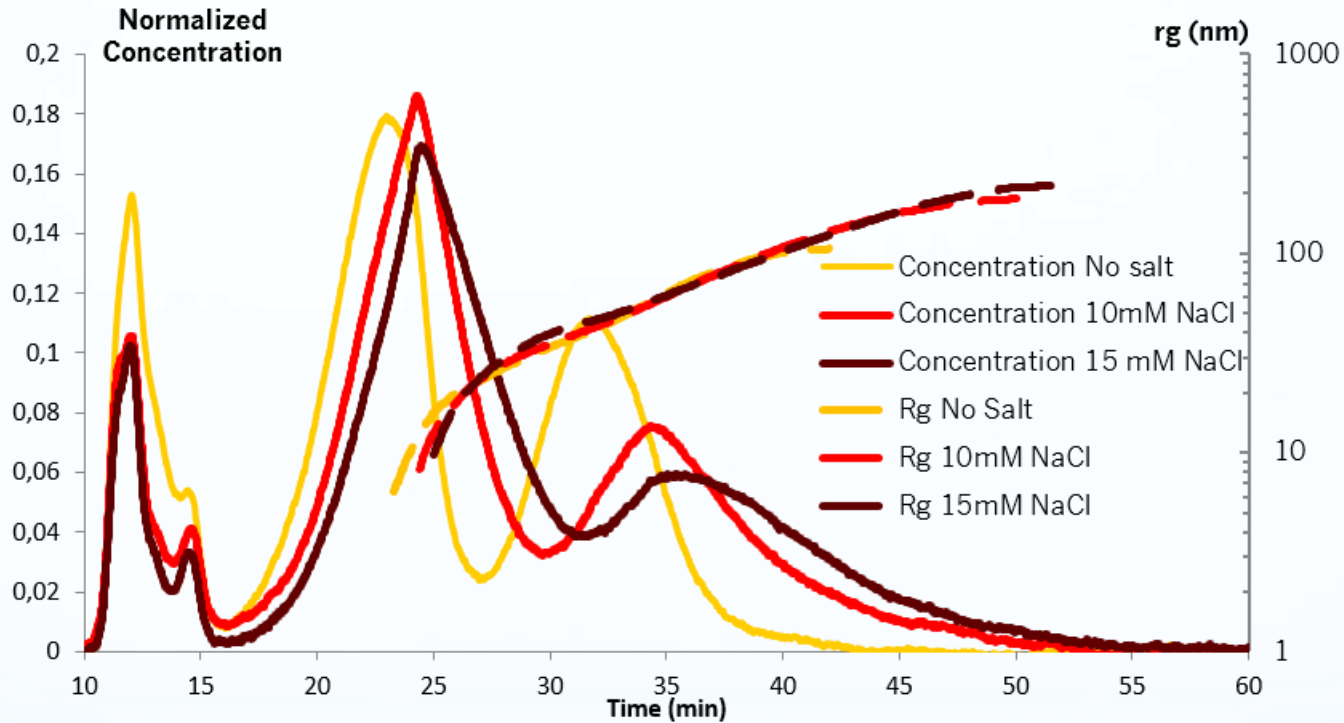
# Holding time variation

Holding time : 0/15min/1h at 80° C



- Last population size does not change with holding time
- Large aggregates are present after heating without holding (t=0)
- During holding, non-aggregated proteins form primary fractals
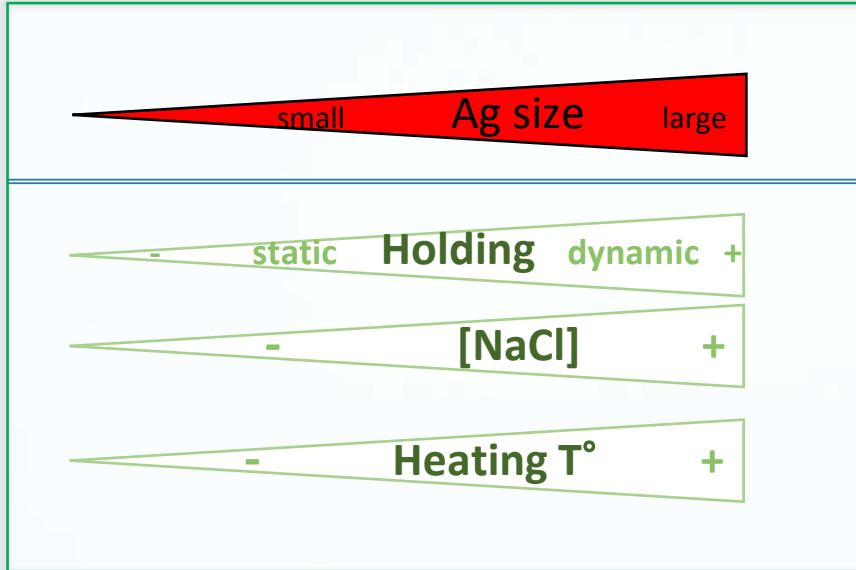  → Work on pre-heating and heating parameters

# Salt Variations
## No Salt/10mM/15mM NaCl



The more salt concentration increases, the larger aggregates are formed

Salt decreases interaction hiding charges: aggregates are produced easier

# Conclusions and Perspectives

small **Ag size** large

- static **Holding** dynamic +

- **[NaCl]** +

- **Heating T°** +

# Conclusions and Perspectives

small **Ag size** large

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- **Heating T°** +

**As-FlFFF**

- Fractionate whey protein aggregates
- Filtration-free injection
- Informations: Radius of gyration / Molar mass / Conformation
- Tool to analyze and compare semi-industrial productions

# Conclusions and Perspectives

small  **Ag size**  large

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- Fractionate whey protein aggregates
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Perspectives :
- Flow rate variations
- Tube dimension variations
- Heating rate variations
- Fraction collection + TEM

| Introduction | Methods | Results | **Conclusions and perspectives** |

# Thank you for your attention