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**TOULOUSE FIELD-FLOW
FRACTIONATION CENTER**



Projet cofinancé par le Fonds Européen de Développement Régional

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FRACTIONATION CENTER

FIELD-FLOW FRACTIONATION TECHNIQUES FOR MILK PROTEIN CHARACTERIZATION

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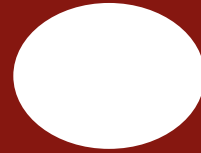
23–27th February 2020

University of Vienna



25-02-20

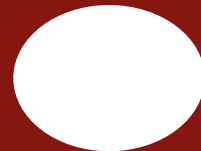
I. Introduction : Milk proteins



II. AsFIFFF analysis of Infant Milk Formulation (IMF)



III. Study of Whey proteins by 2D AsFIFFF-RPLC



IV. Denatured whey proteins studied by Centrifugal-FFF



Conclusion



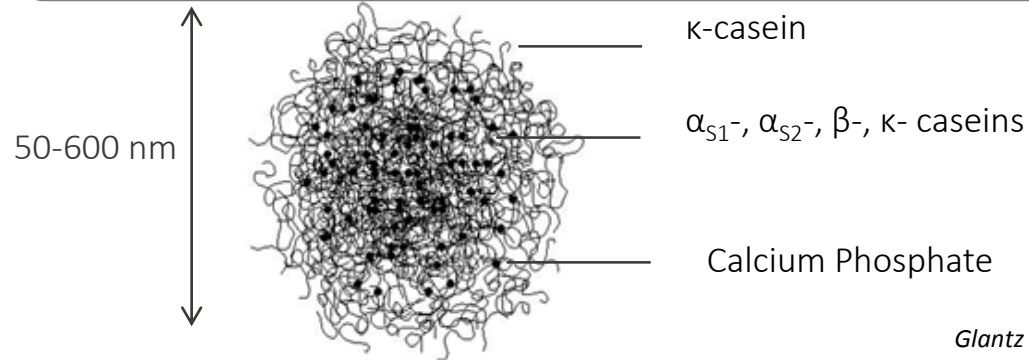


Introduction

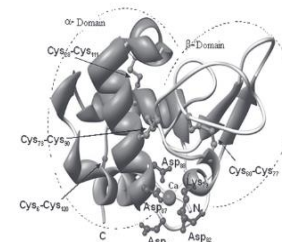
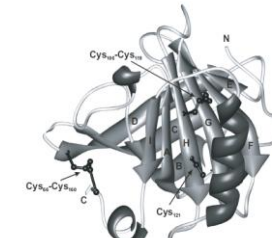


2 categories of milk proteins

Caseins (CN)

*Glantz et al., 2010*

Whey Proteins (WP)

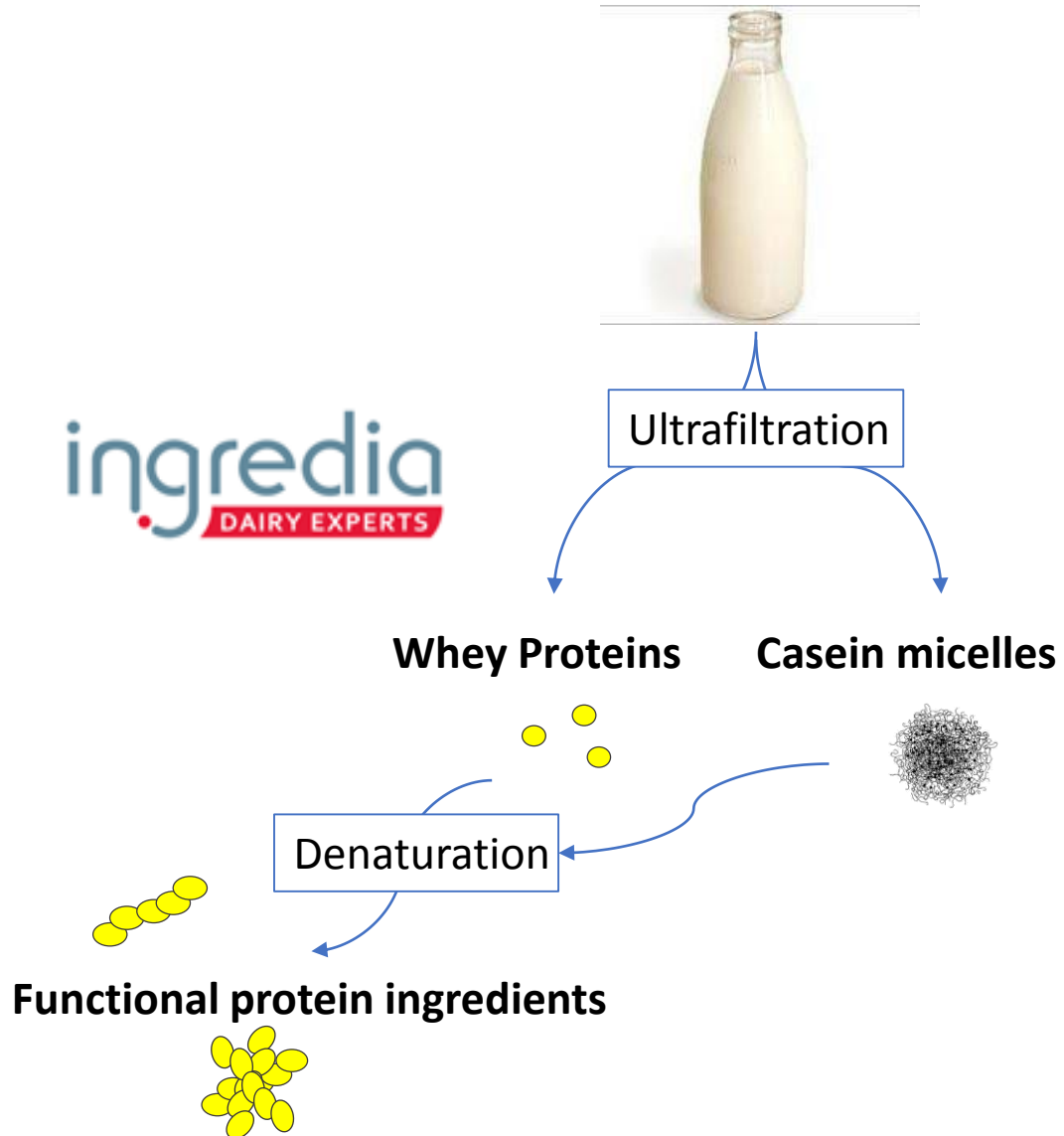
 α -lactalbumin
(α -LA) β -lactoglobulin
(β -LG)Lactoferrin
(LF)

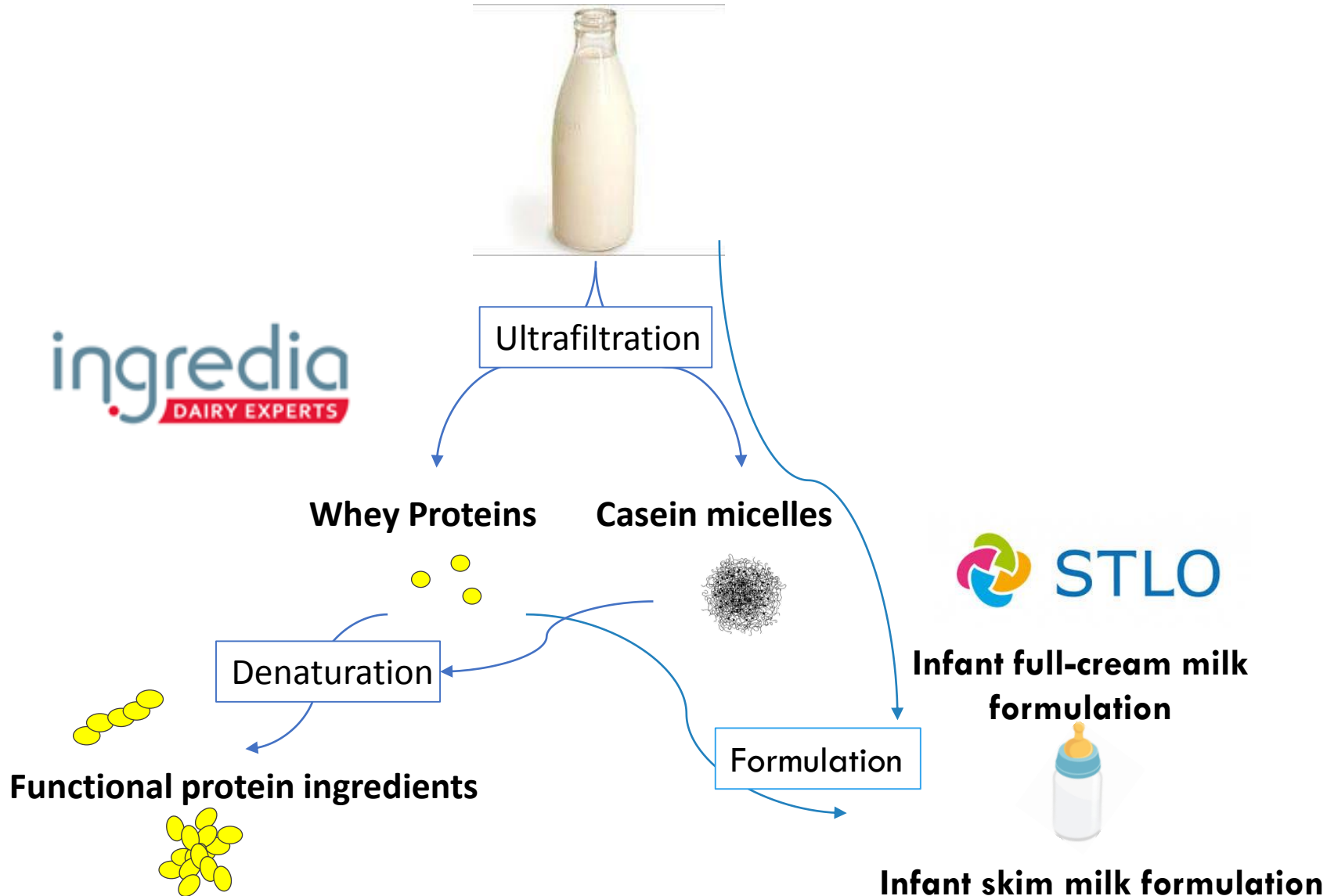
Autres (Immunoglobulin, Serum Albumin...)

Tolkach et al., 2007; Kulozik et al., 2011; Vogel, 2012



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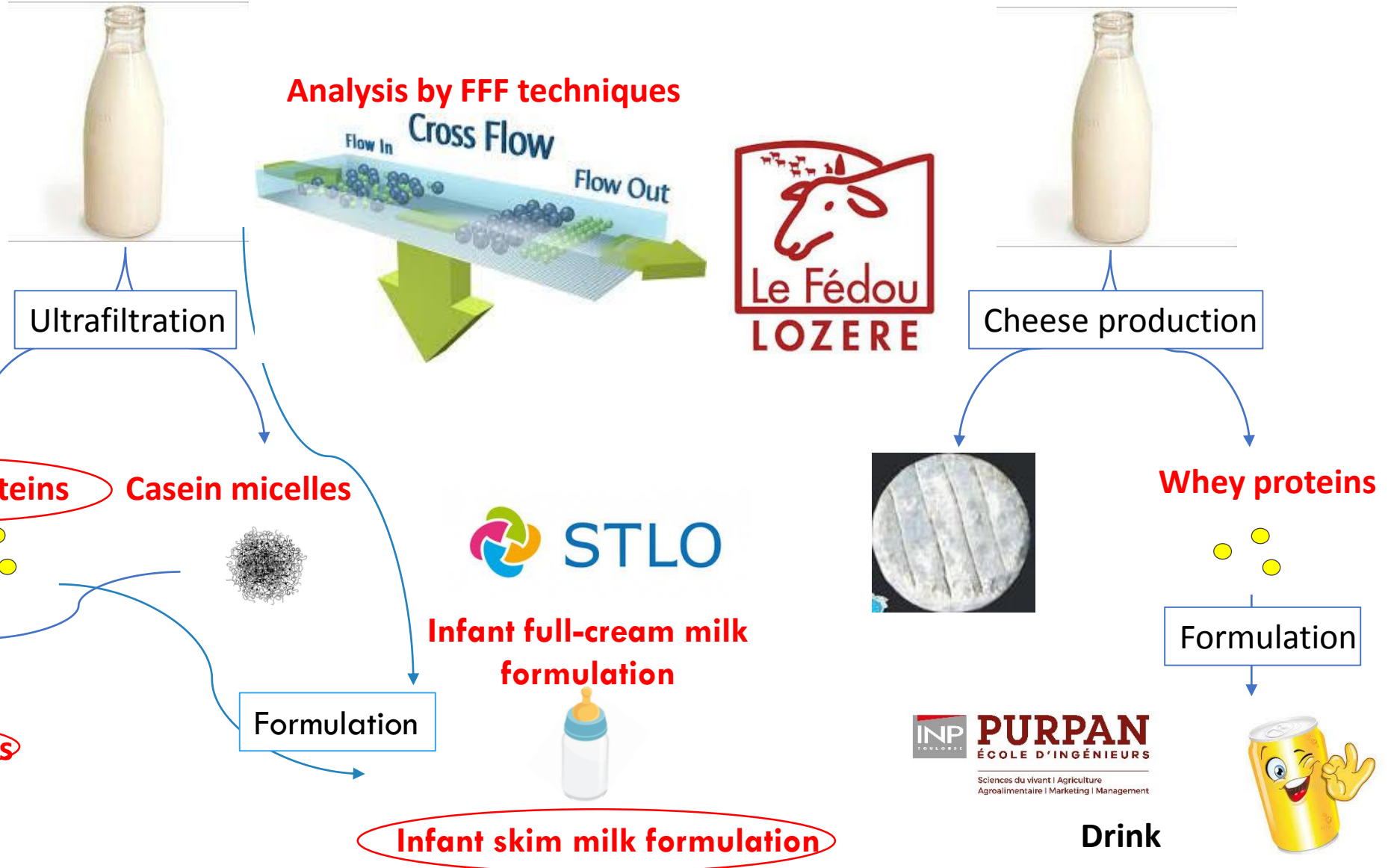




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II

As-FlFFF analysis of Infant Milk
Formulation (IMF)



As-FlFFF analysis of Infant Milk Formulation (IMF)

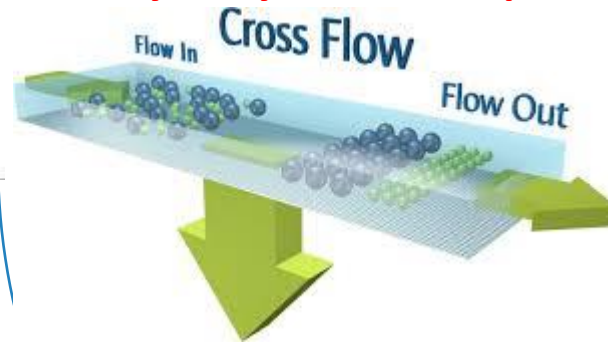
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Analysis by FFF techniques



Ultrafiltration

Cheese production

Whey Proteins

Casein micelles



Infant full-cream milk formulation



Infant skim milk formulation



Whey proteins

Formulation



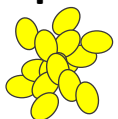
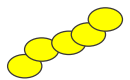
Drink



Denaturation

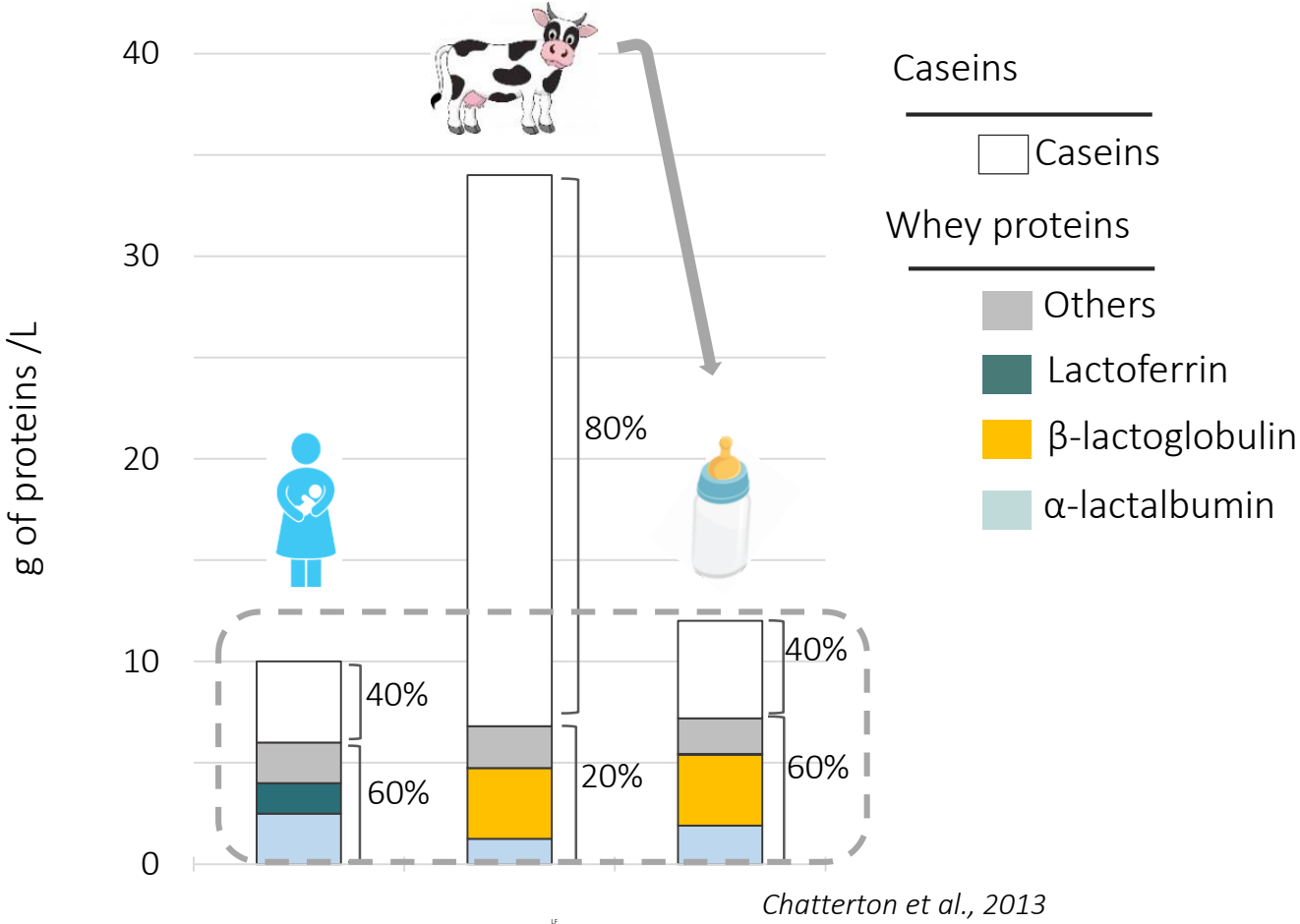
Formulation

Functional protein ingredients



1

Milk composition



The CN:WP ratio and the WP composition have to be modified for the Infant milk formulation (IMF) to mimic the human milk composition



3 Infant Milk Formulation IMFs

Control IMF

CN:WP 40:60

Without modification
of the proportion of
the bovine milk whey
proteins

LF⁺ IMF

CN:WP 40:60

LF quantity closes
to that of human
milk

LF⁺ ALA⁺ IMF

CN:WP 40:60

LF and α -LA
quantities close to
those of human milk

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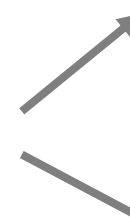


Heat Treatment



67.5°C

80°C



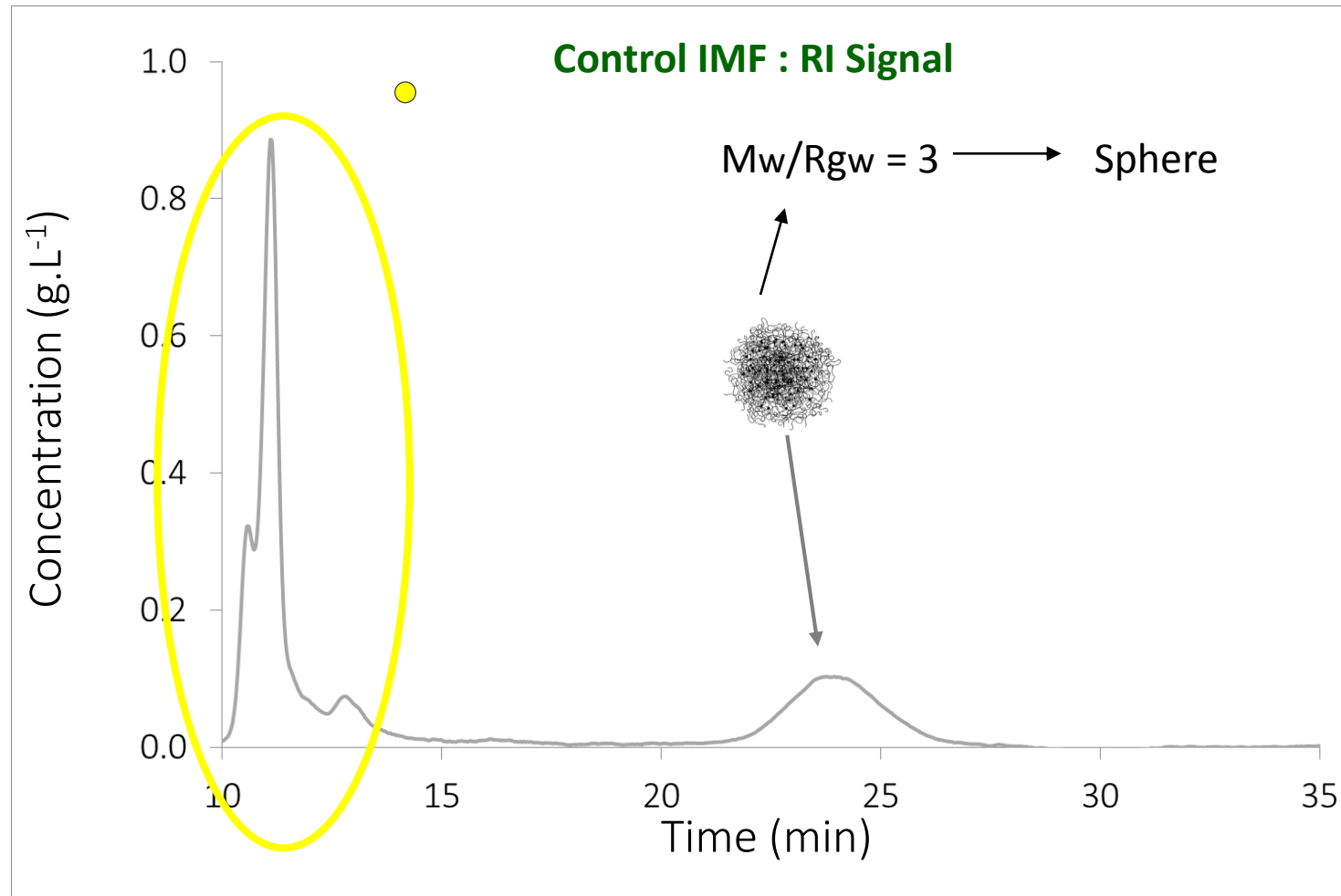
Transmission Electronic
Microscopy

AsFIFFF-MALS-QELS-RI

AF4-MALS-QELS-RI Tractograms of the unheated and heated IMF's at 67.5°C and 80°C : Control IMF

3

- Native WP
- WP aggregate
- Casein Micelle
- Partially desintegrated Casein Micelle



Eluent :
50mM NaCl + 5mM CaCl₂

— Unheated

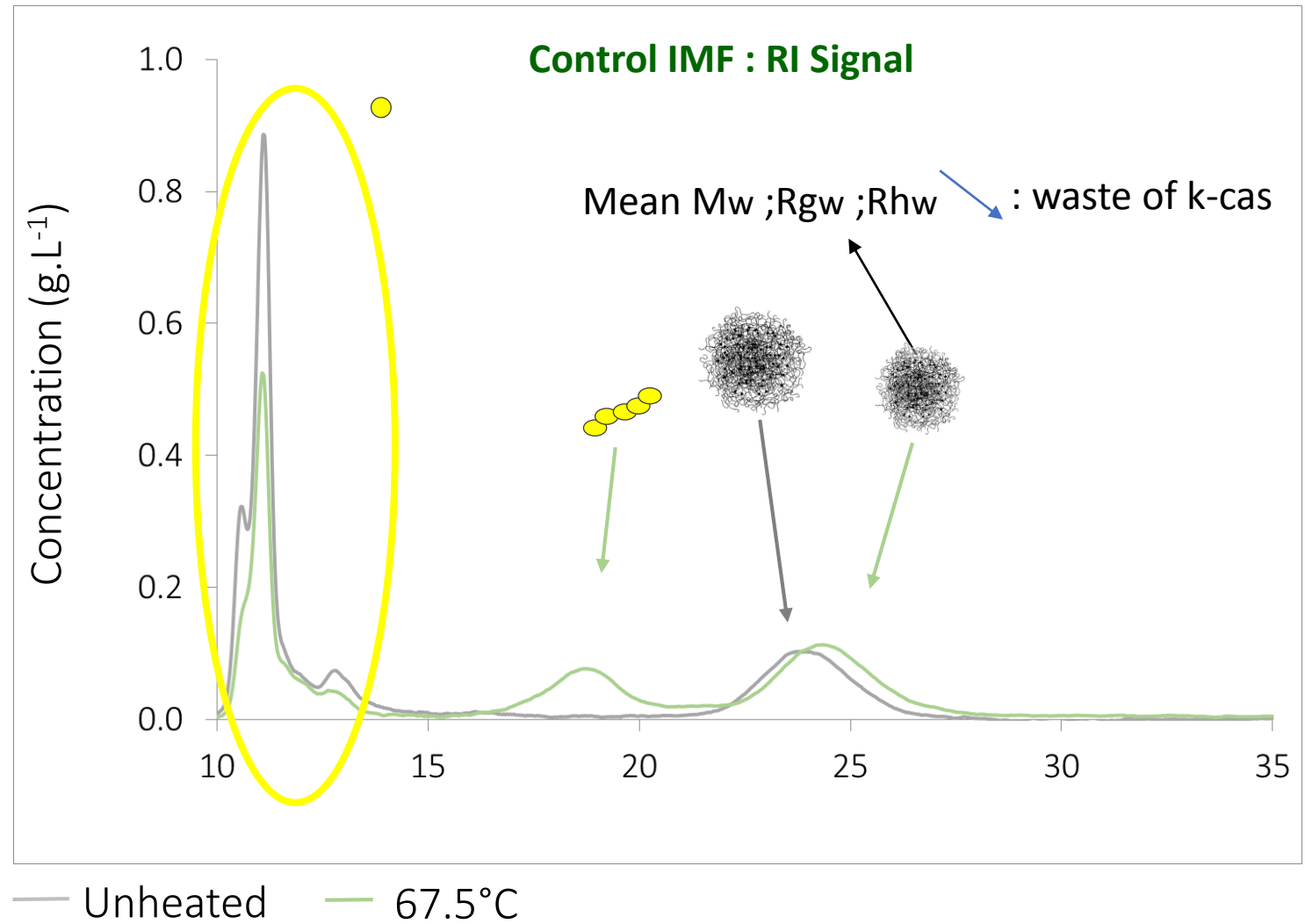


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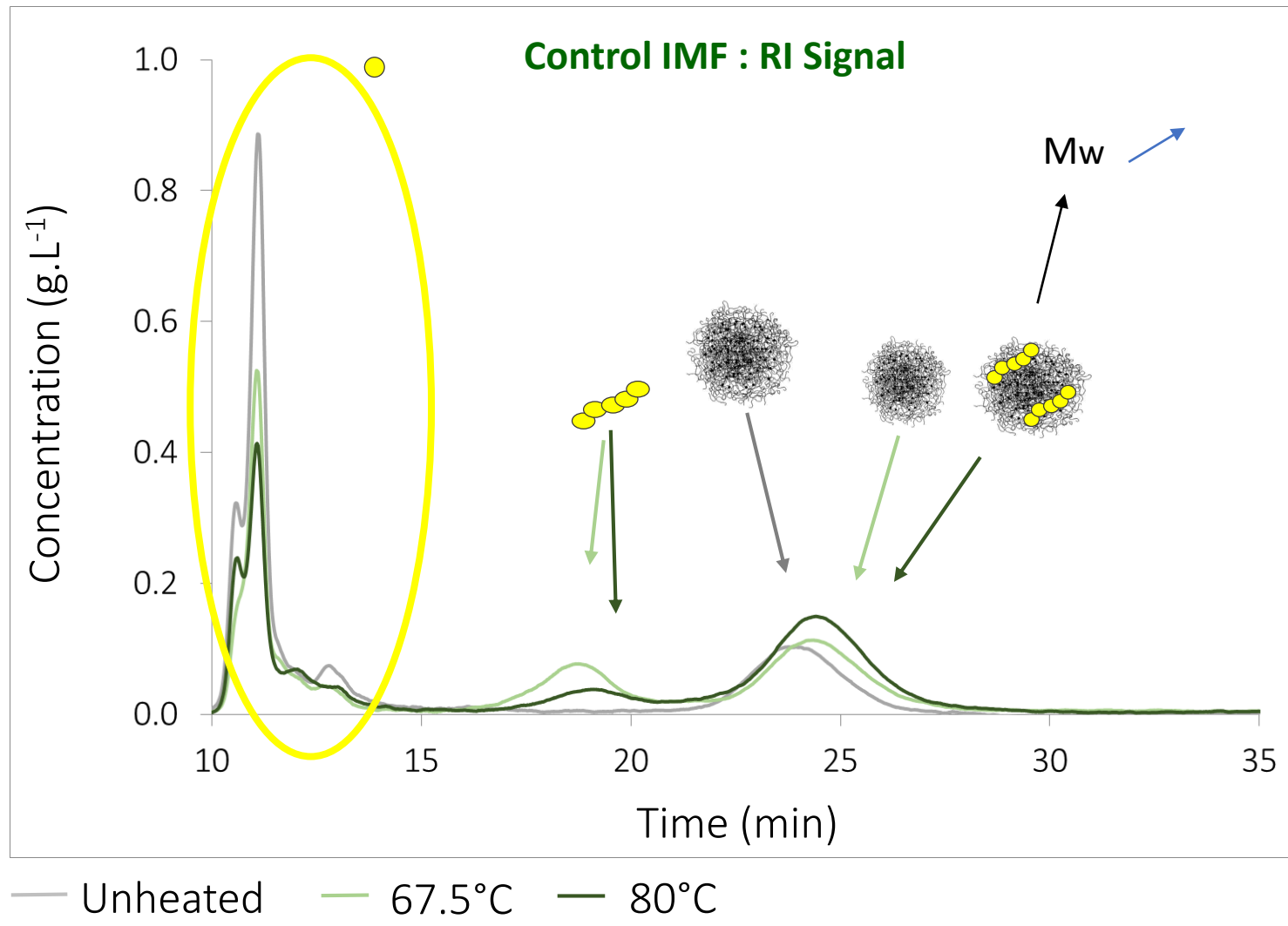


AF4-MALS-QELS-RI Tractograms of the unheated and heated IMFs at 67.5°C and 80°C : Control IMF

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20

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

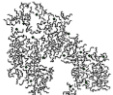


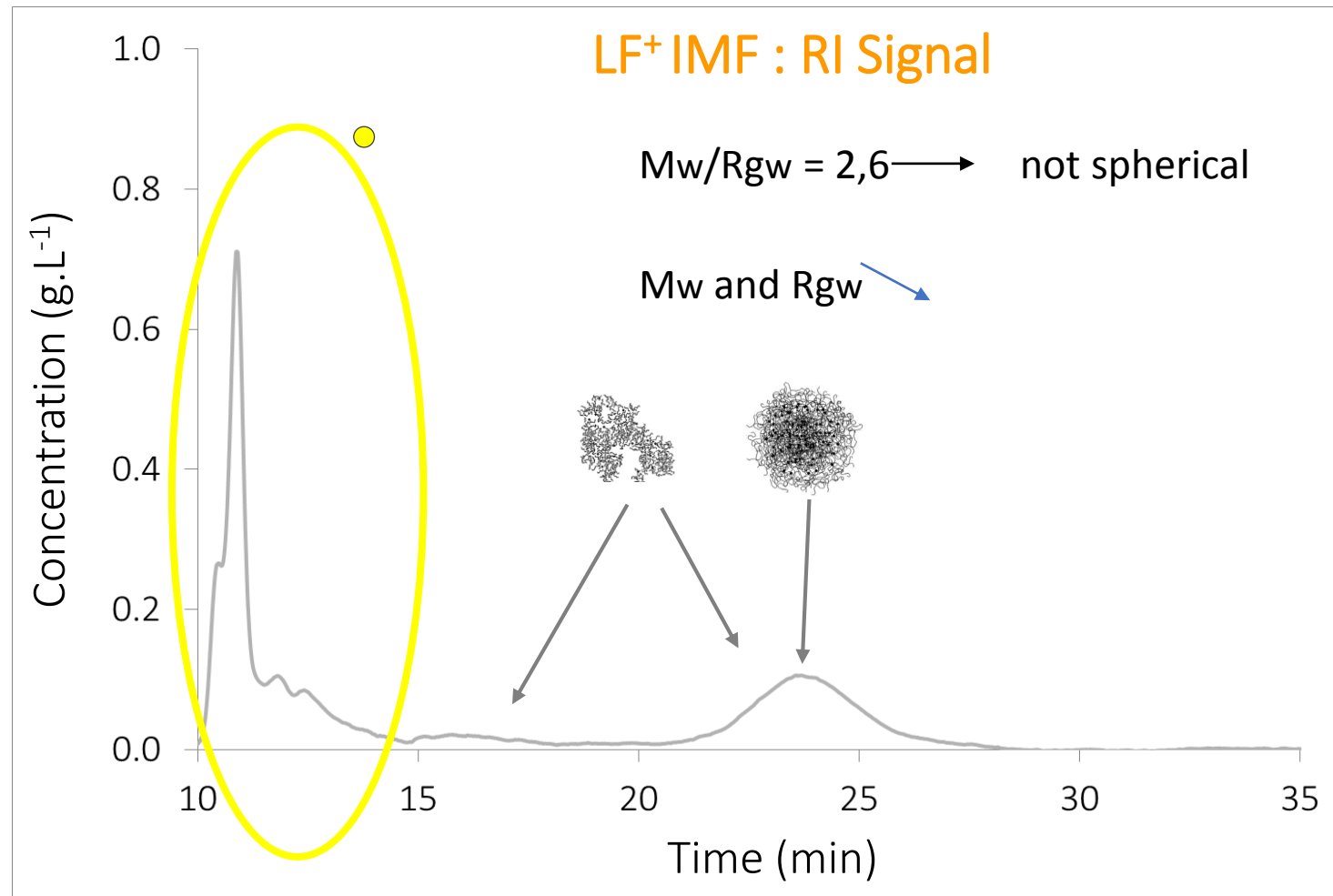
Eluent :
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4

AF4-MALS-QELS-RI fractograms of the unheated and heated IMF's at 67.5°C and 80°C : LF⁺ IMF 21

-  Native WP
-  WP aggregate
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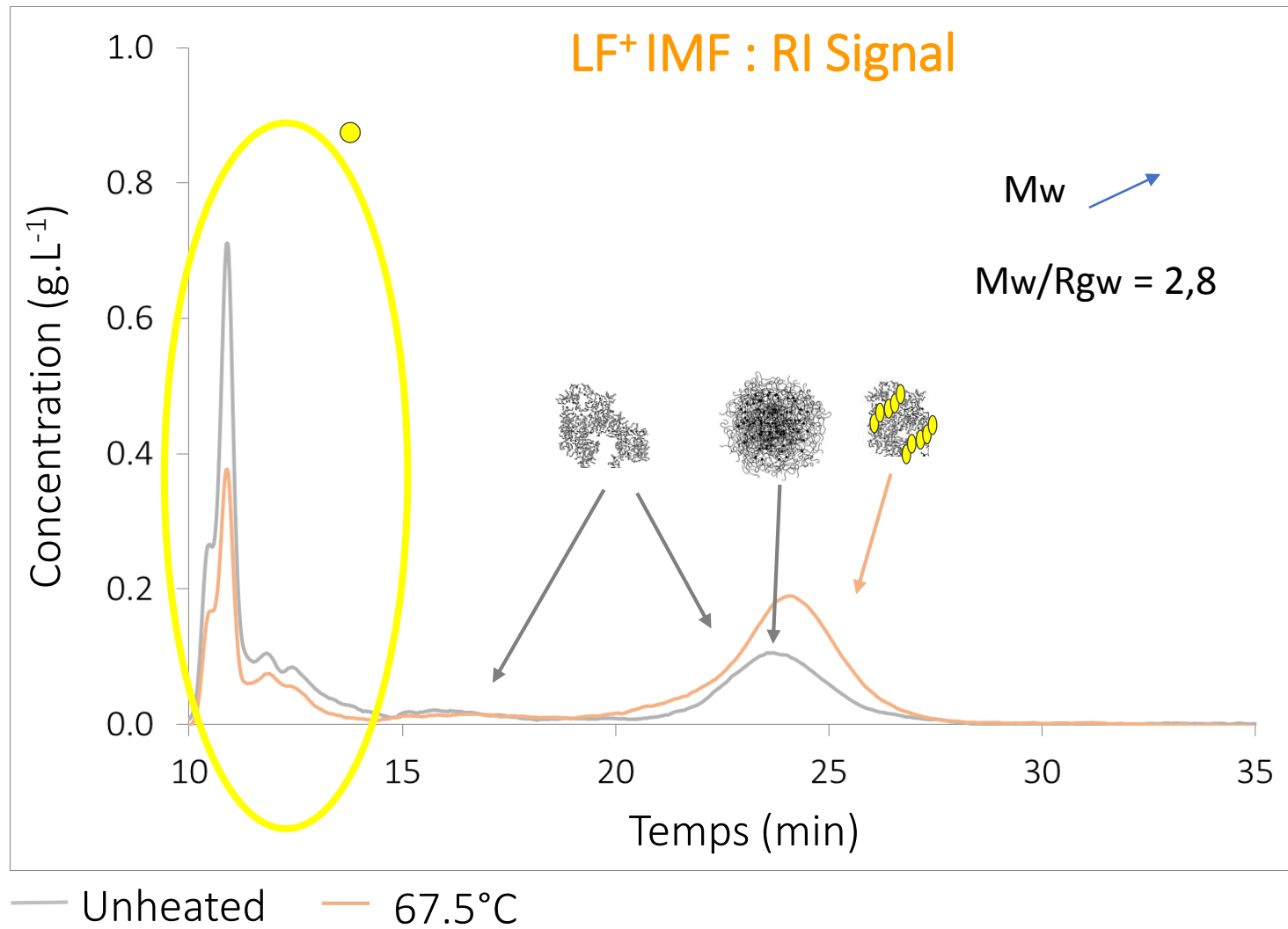


Eluent :
50mM NaCl + 5mM CaCl₂





3

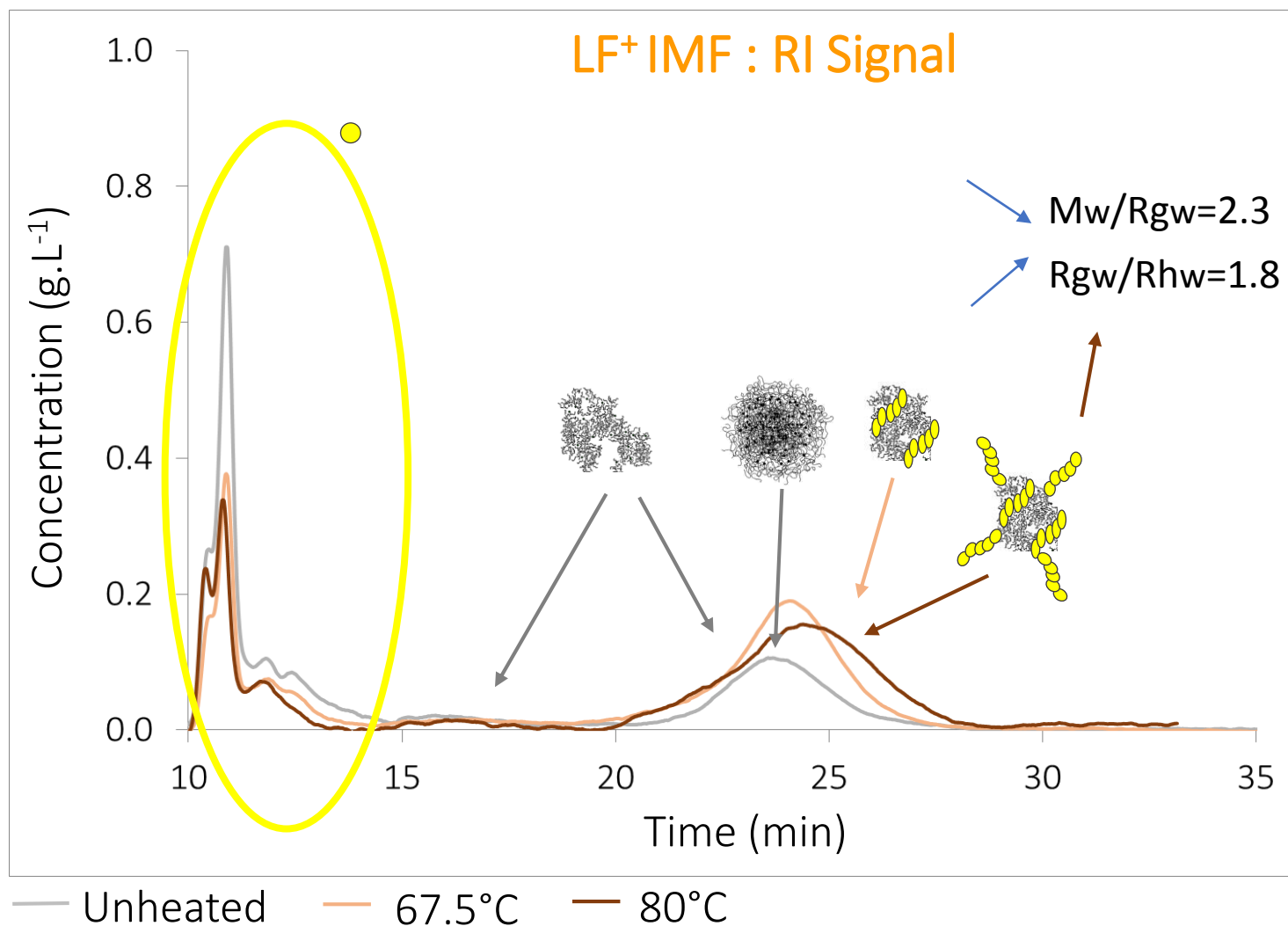
AF4-MALS-QELS-RI fractograms of the unheated and heated IMF's at 67.5°C and 80°C : LF⁺ IMF 22

- Non aggregated WP
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Eluent :
50mM NaCl + 5mM CaCl₂

-  Non aggregated WP
-  WP aggregate
-  Casein Micelle
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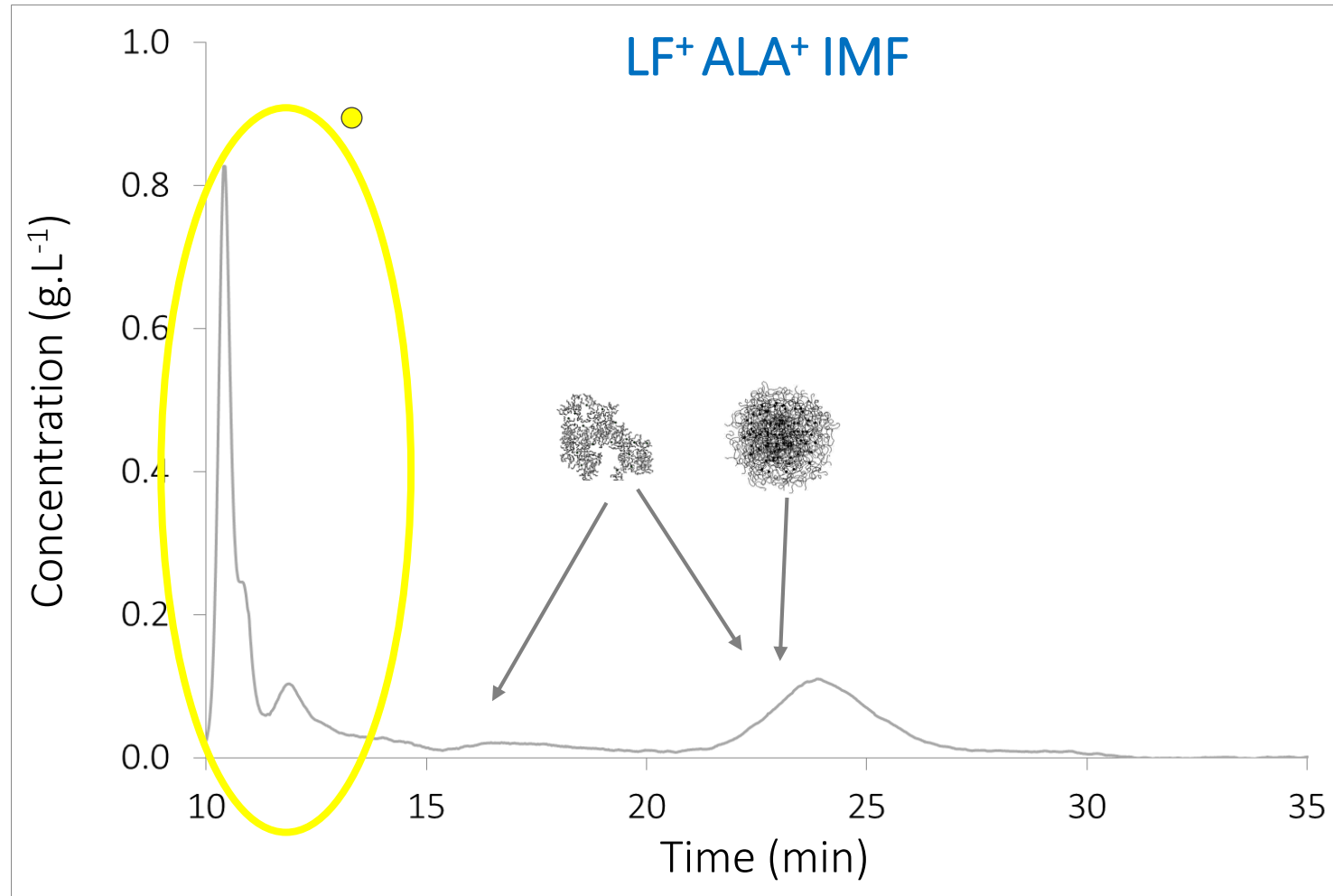
Eluent :
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AF4-MALS-QELS-RI Tractograms of the unheated and heated IMF's at 67.5°C and 80°C : LF⁺ ALA⁺ IMF

4

24

- Native WP
- WP aggregate
- Casein Micelle
- Partially desintegrated Casein Micelle



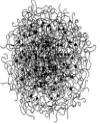
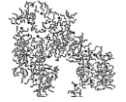
Eluent :
50mM NaCl + 5mM CaCl₂

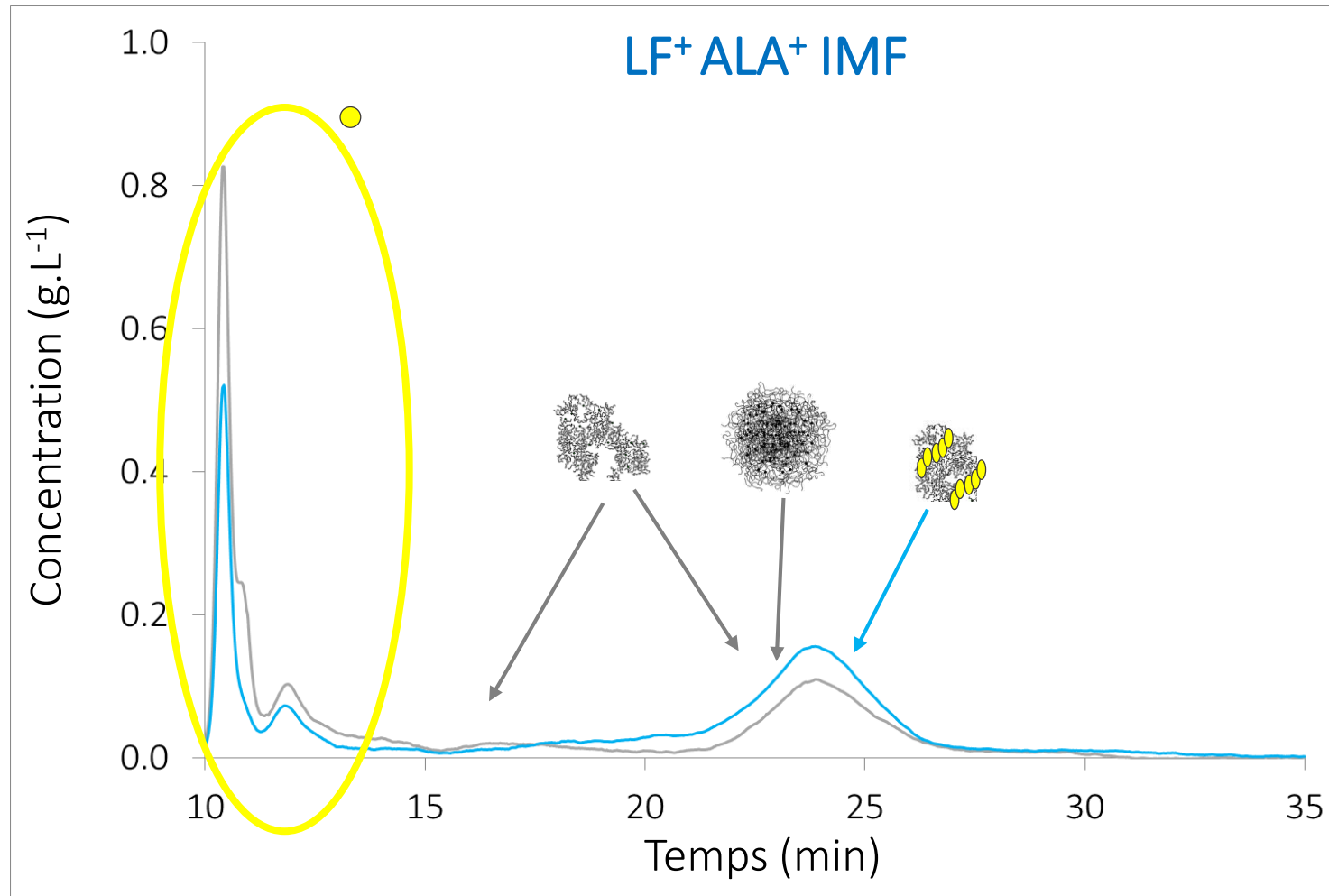
— Unheated



AF4-MALS-QELS-RI Tractograms of the unheated and heated IMF's at 67.5°C and 80°C : LF⁺ ALA⁺ IMF

4





- Non aggregated WP
- WP aggregate
-  Casein Micelle
-  Partially desintegrated Casein Micelle

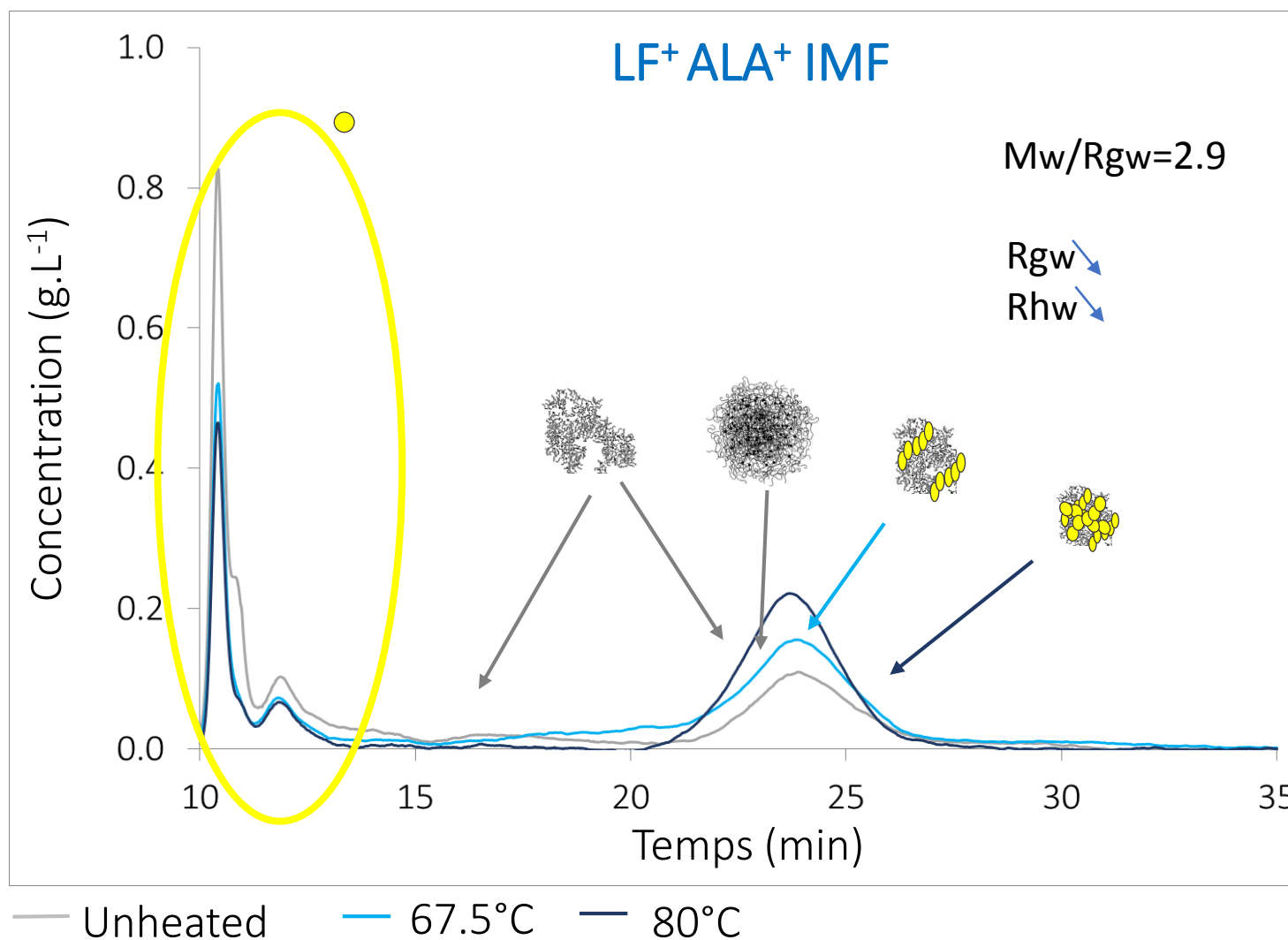


Eluent :
50mM NaCl + 5mM CaCl₂

— Unheated — 67.5°C

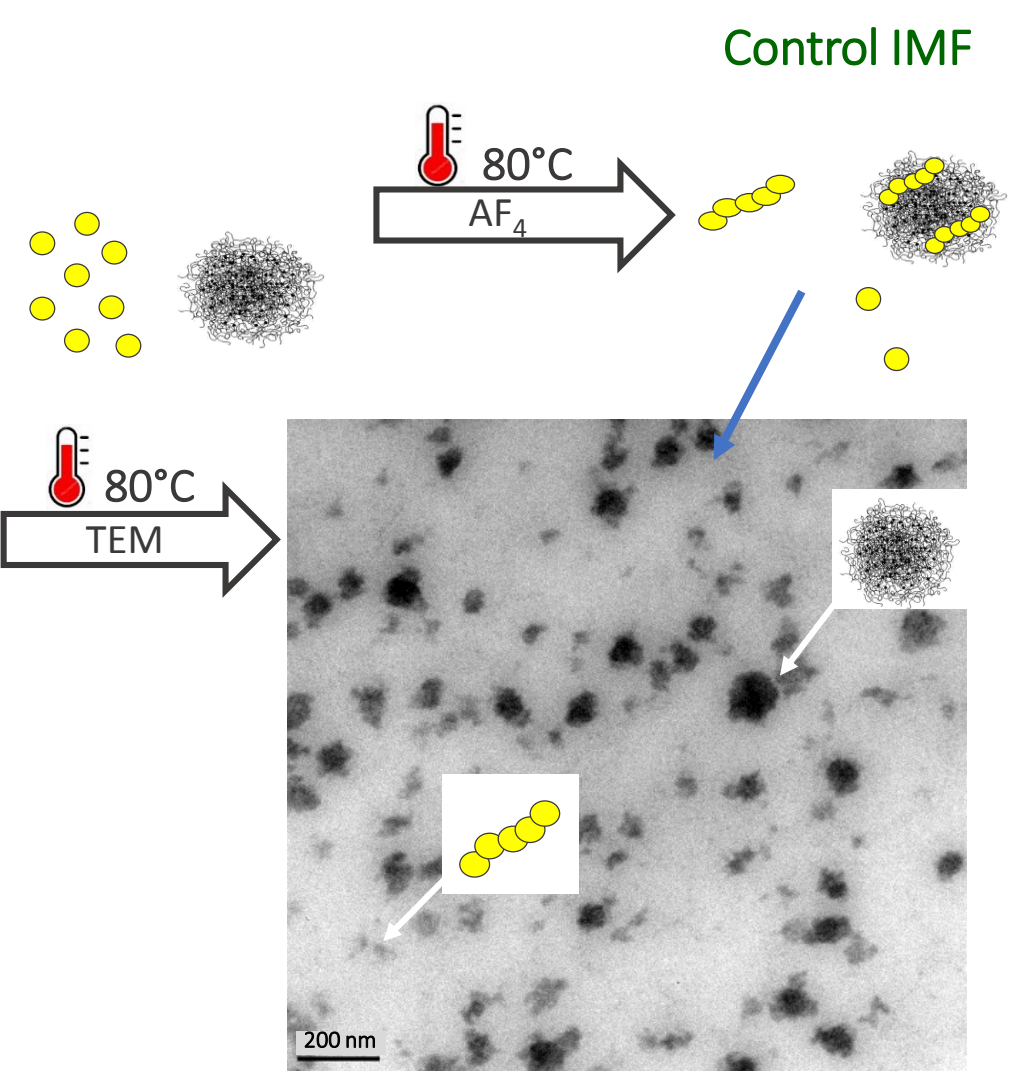


-  Non aggregated WP
-  WP aggregate
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Transmission Electronic Microscopy

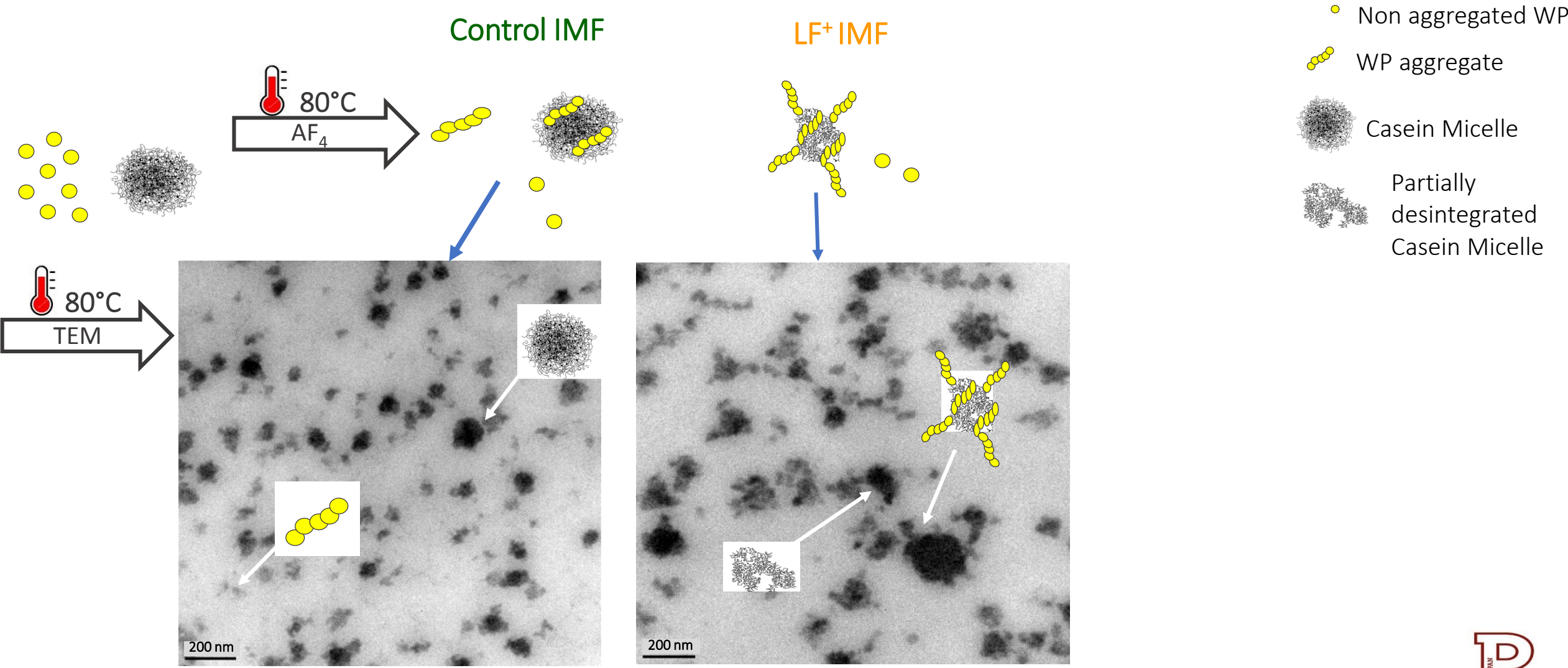
5



- Non aggregated WP
- WP aggregate
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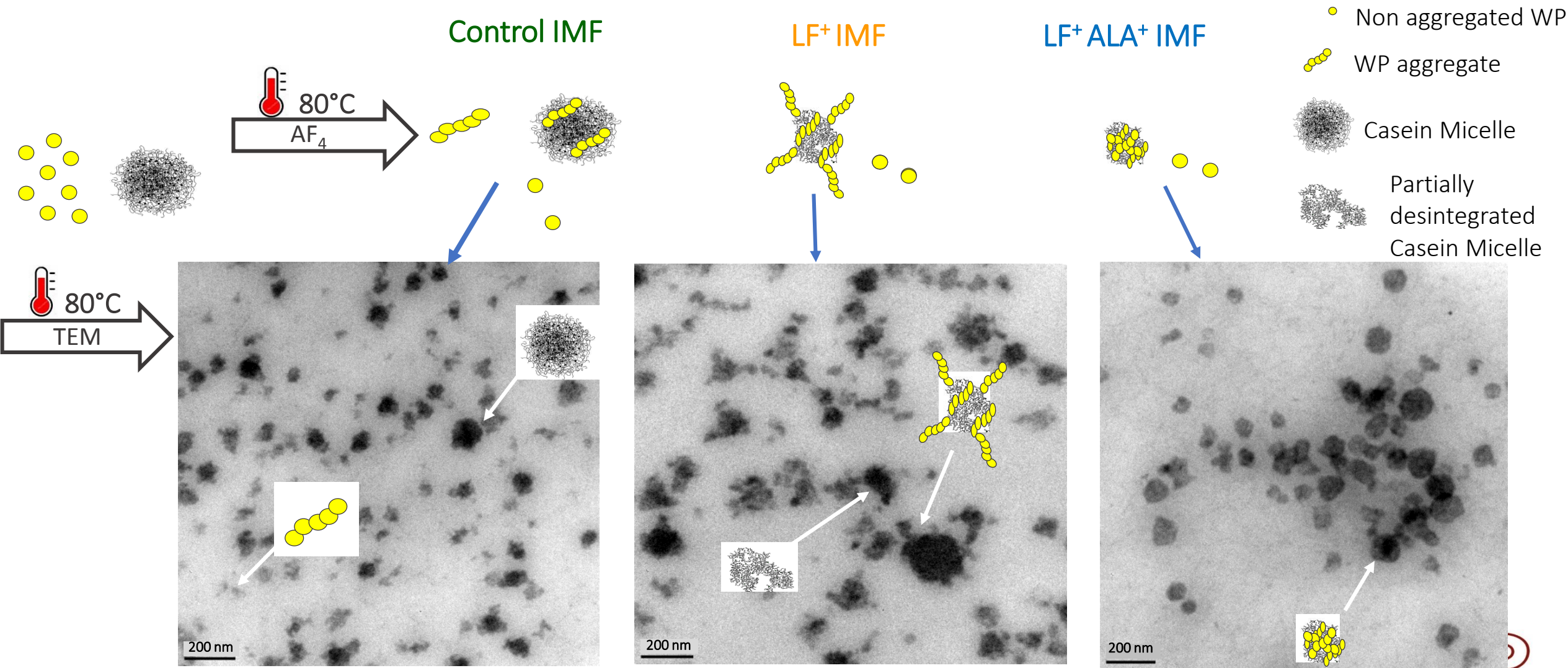
Transmission Electronic Microscopy

5



Transmission Electronic Microscopy

5



III

Study of Whey proteins by 2D
AsFlFFF-RPLC



Study of Whey proteins by 2D-AsF1FFF-RPHPLC

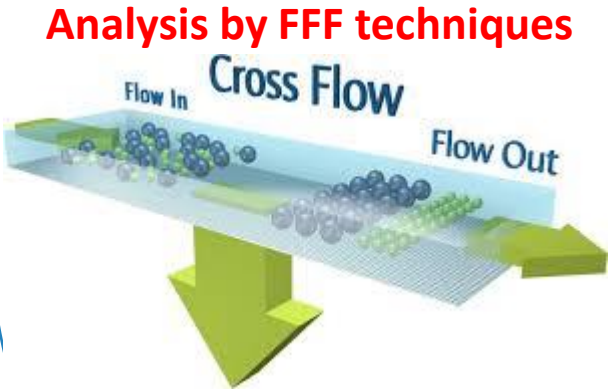
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Whey proteins

Formulation

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Drink



Whey Proteins Analysis by As-FLFFF (1D)

32

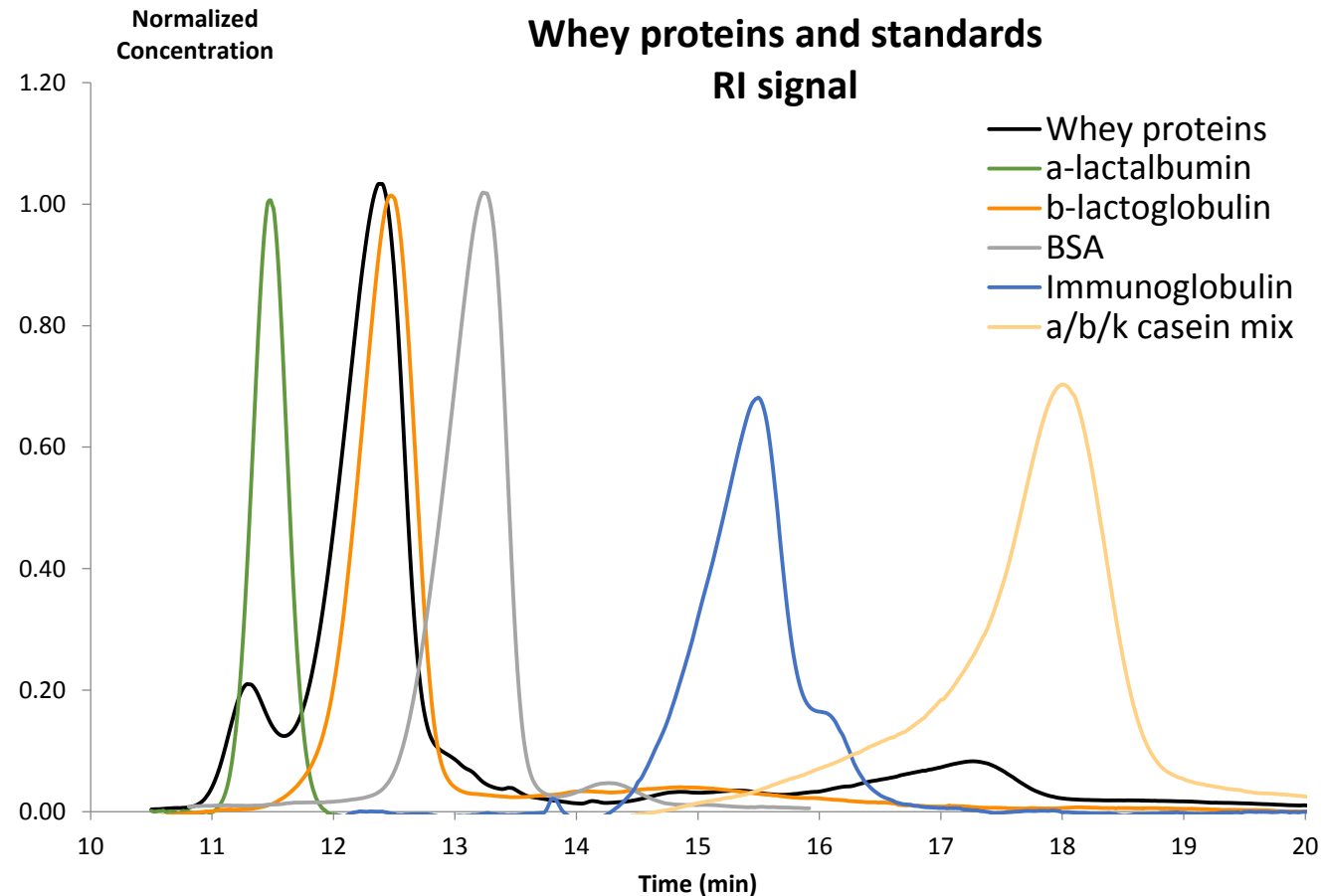
1

Industrial whey protein isolate : they're few percents of caseins inside
Objective : separation of all proteins

Whey Proteins Analysis by As-FIFFF (1D)

1

Industrial whey protein isolate : they're few percents of caseins inside
Objective : separation of all proteins



- The different whey proteins are identified but not very good separated by AsFIFFF
- By HPLC it's not possible to separate caseins from whey proteins



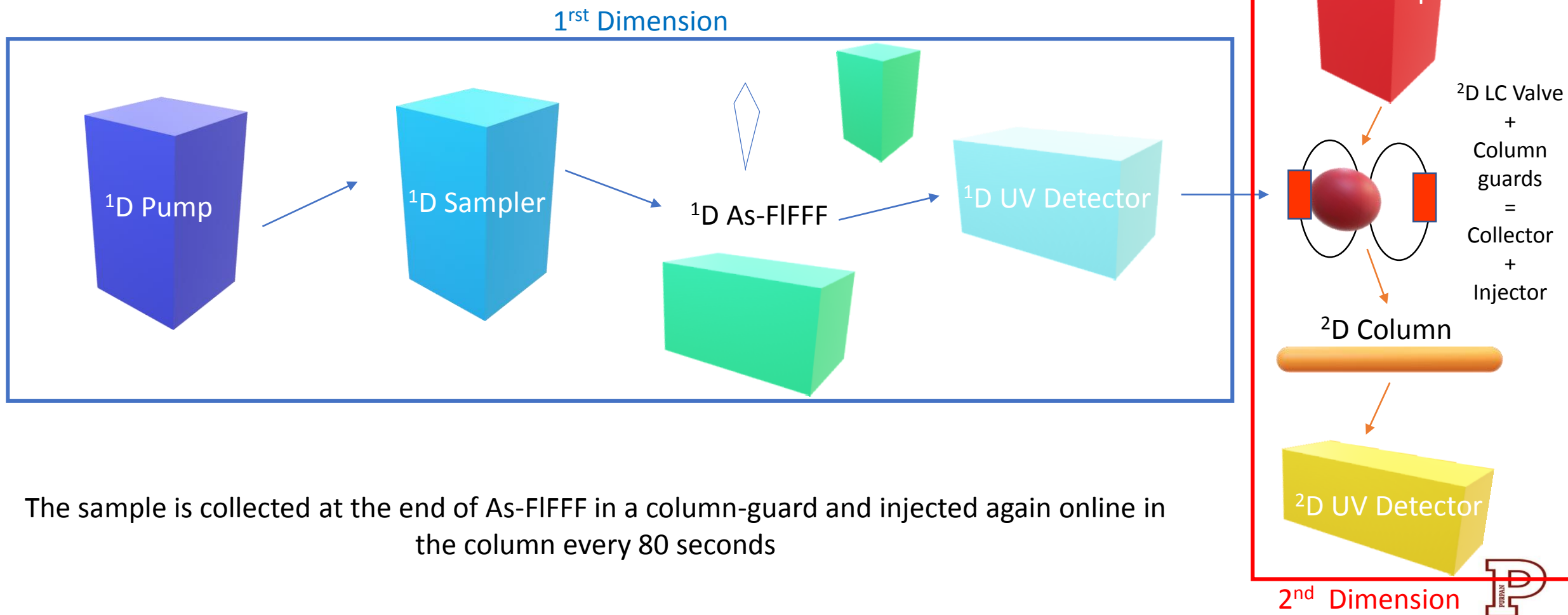
Whey Proteins Analysis by 2D

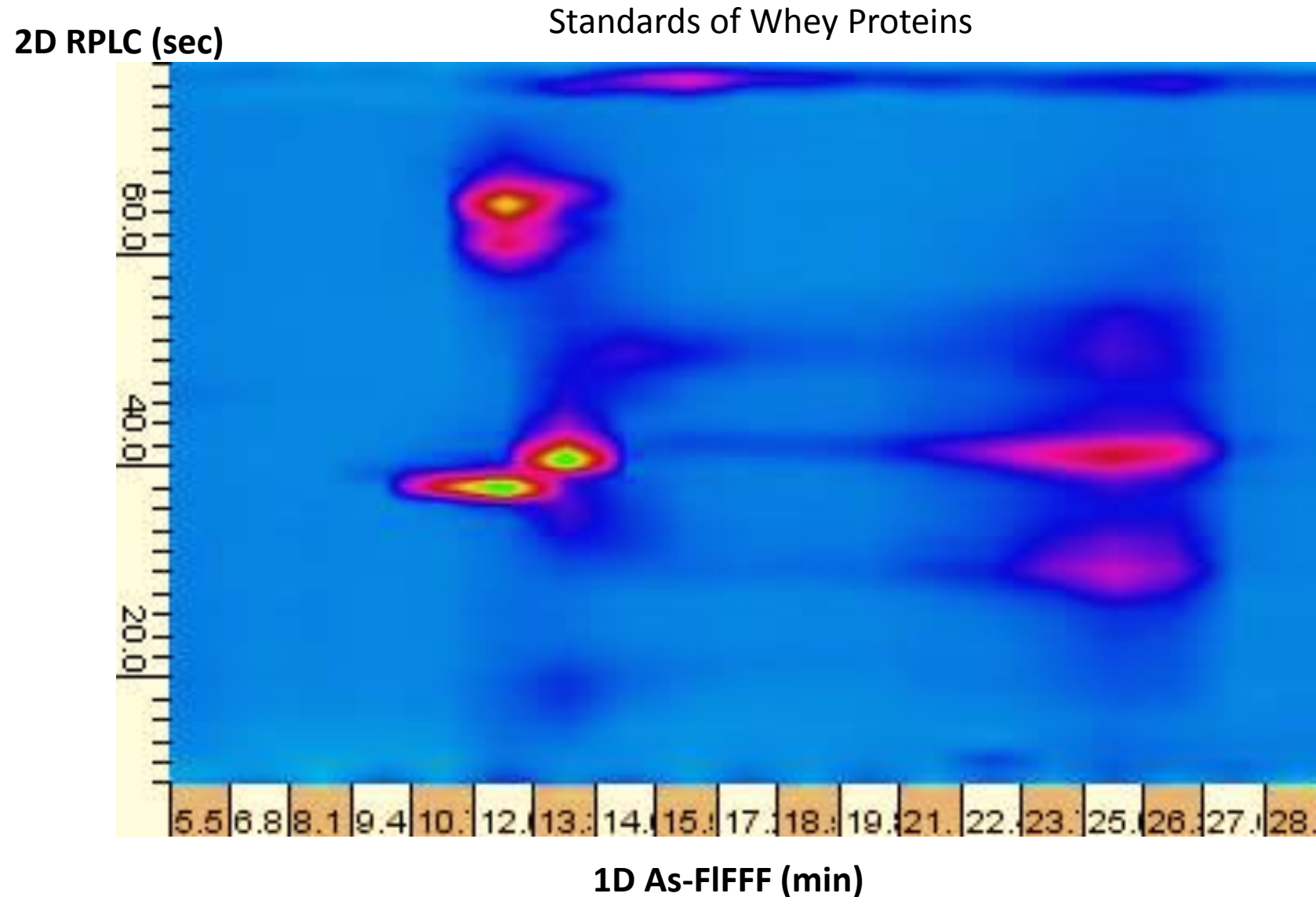
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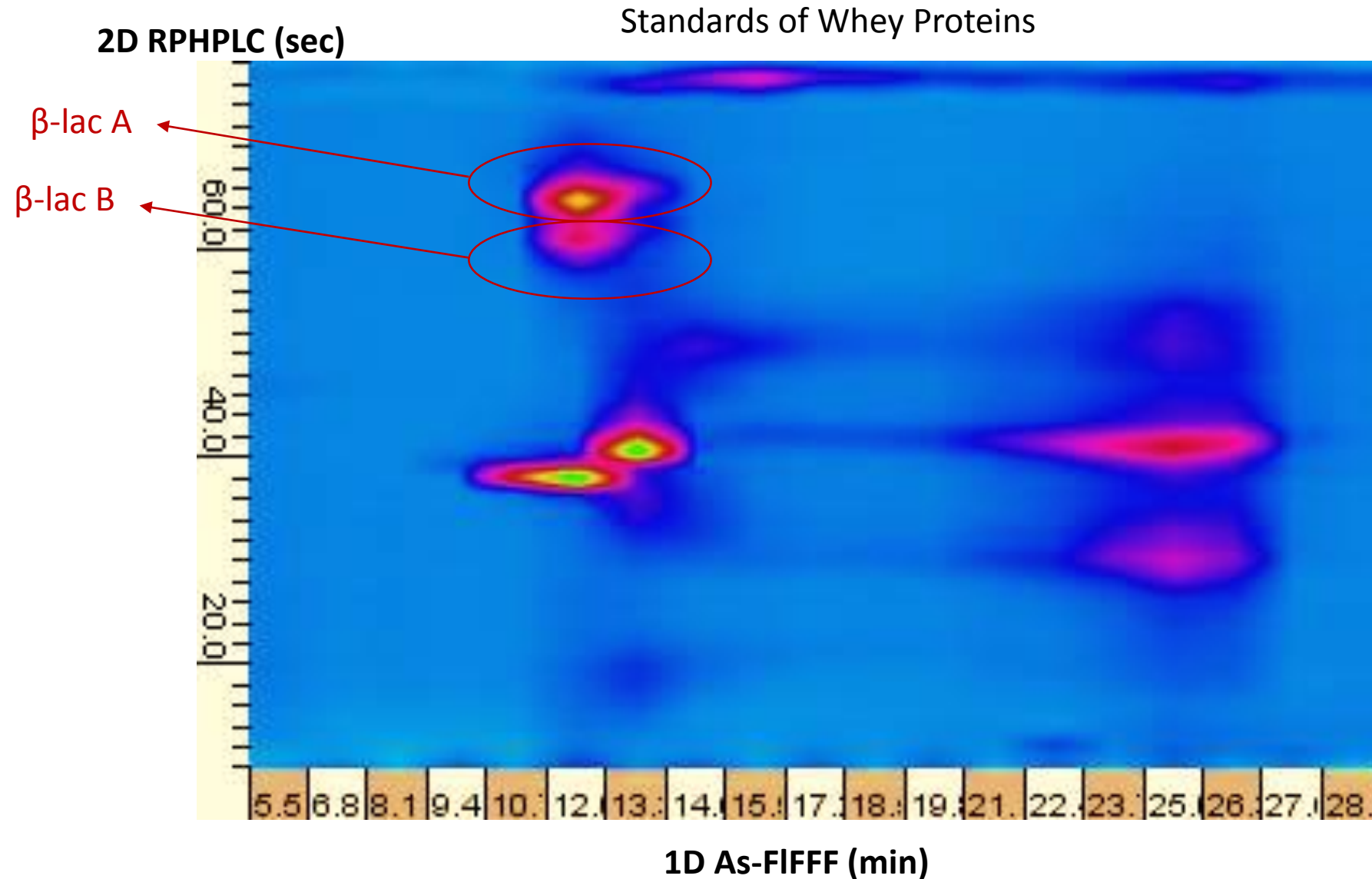
AsFIFFF-RPLC

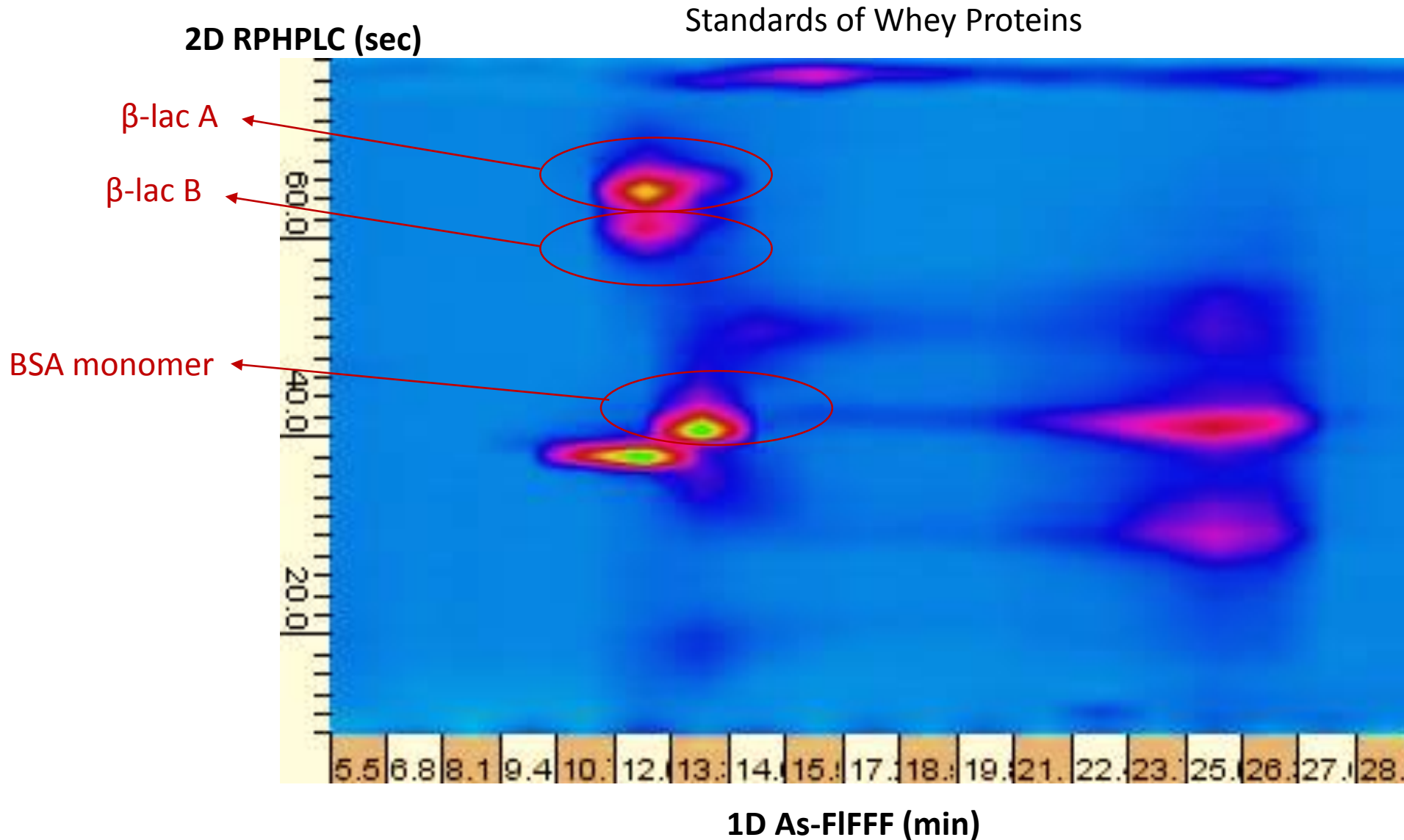
34

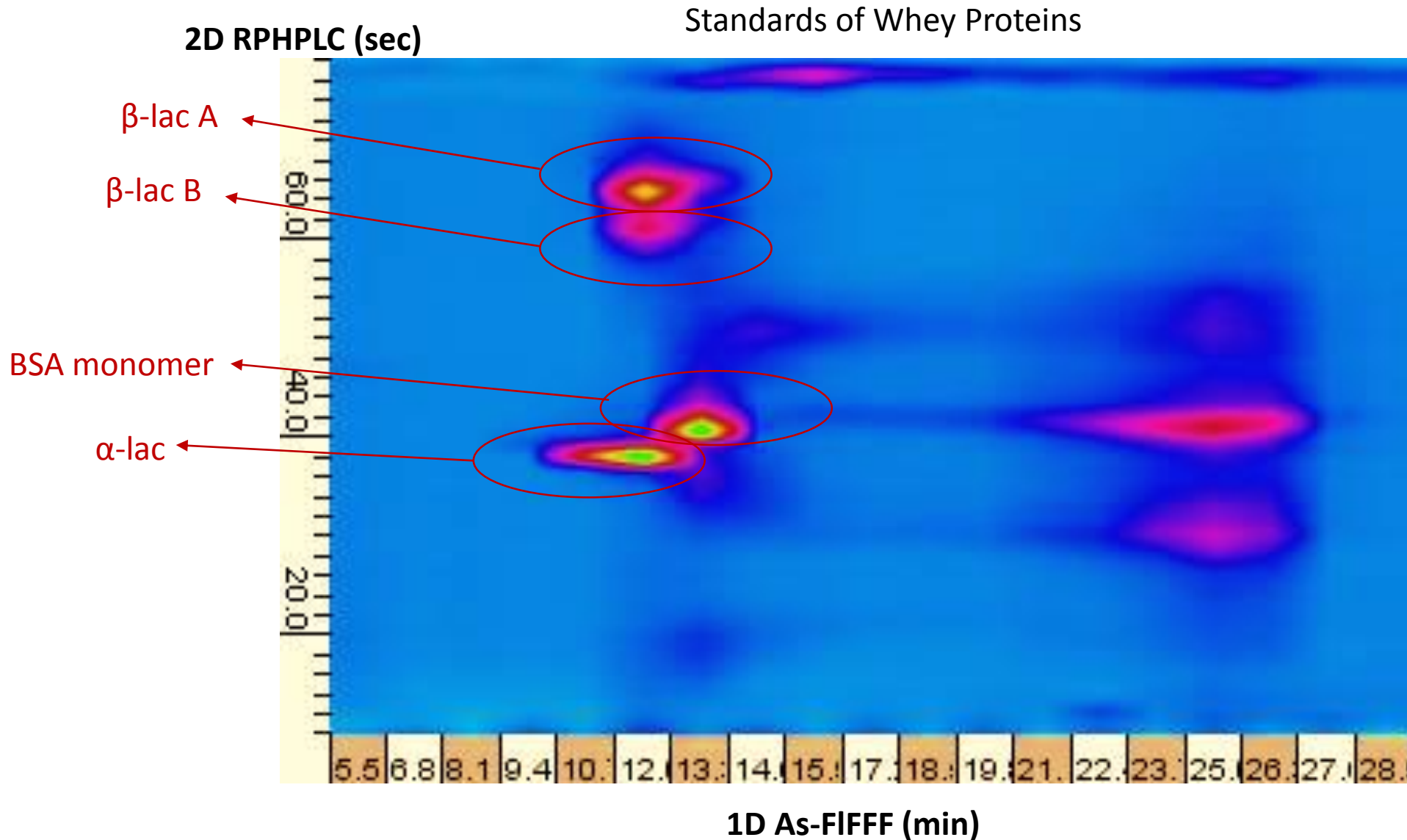
1st dimension : As-FIFFF and 2nd dimension : RPLC
HPLC : Agilent and Thermo system ; As-FIFFF : Wyatt

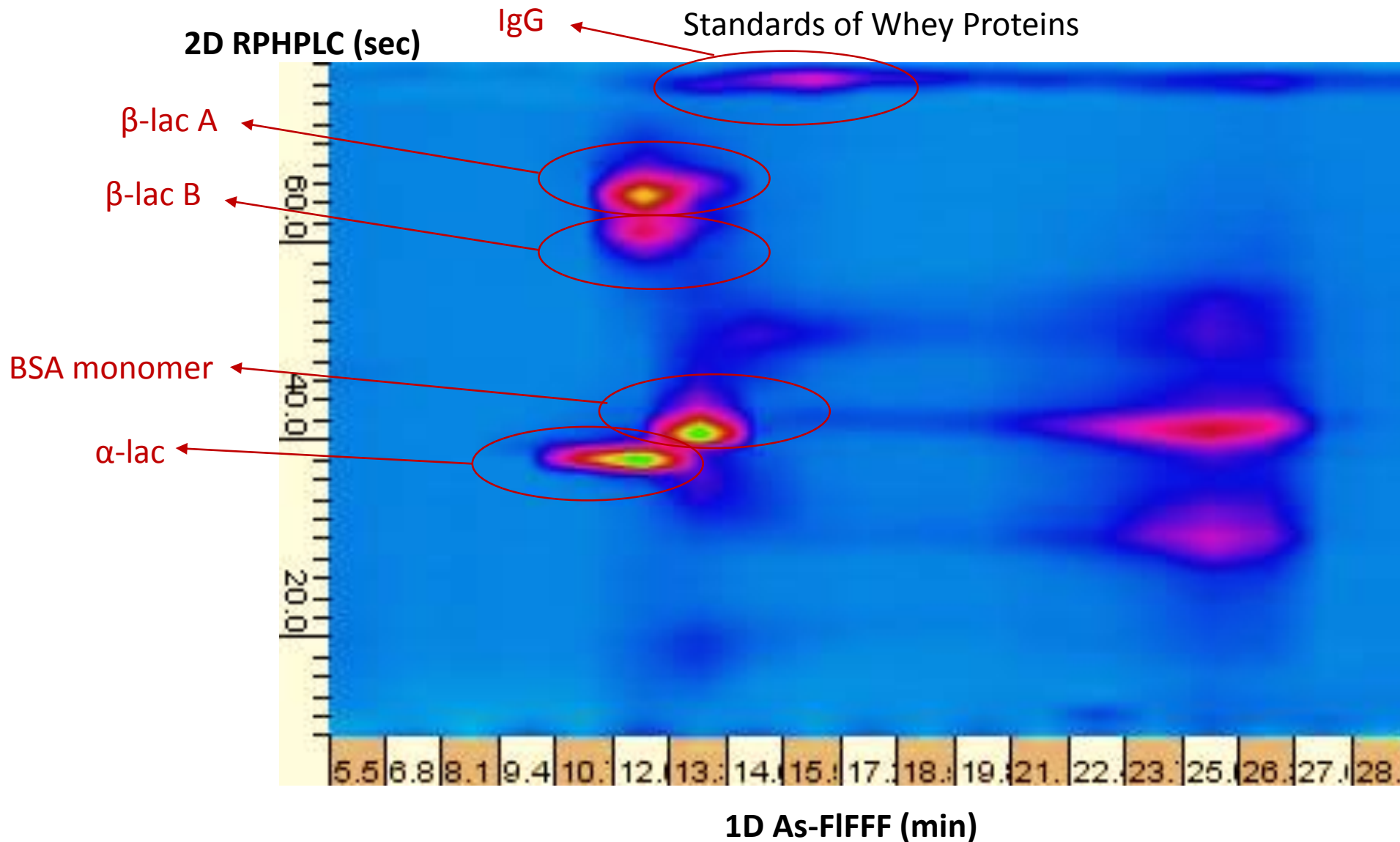






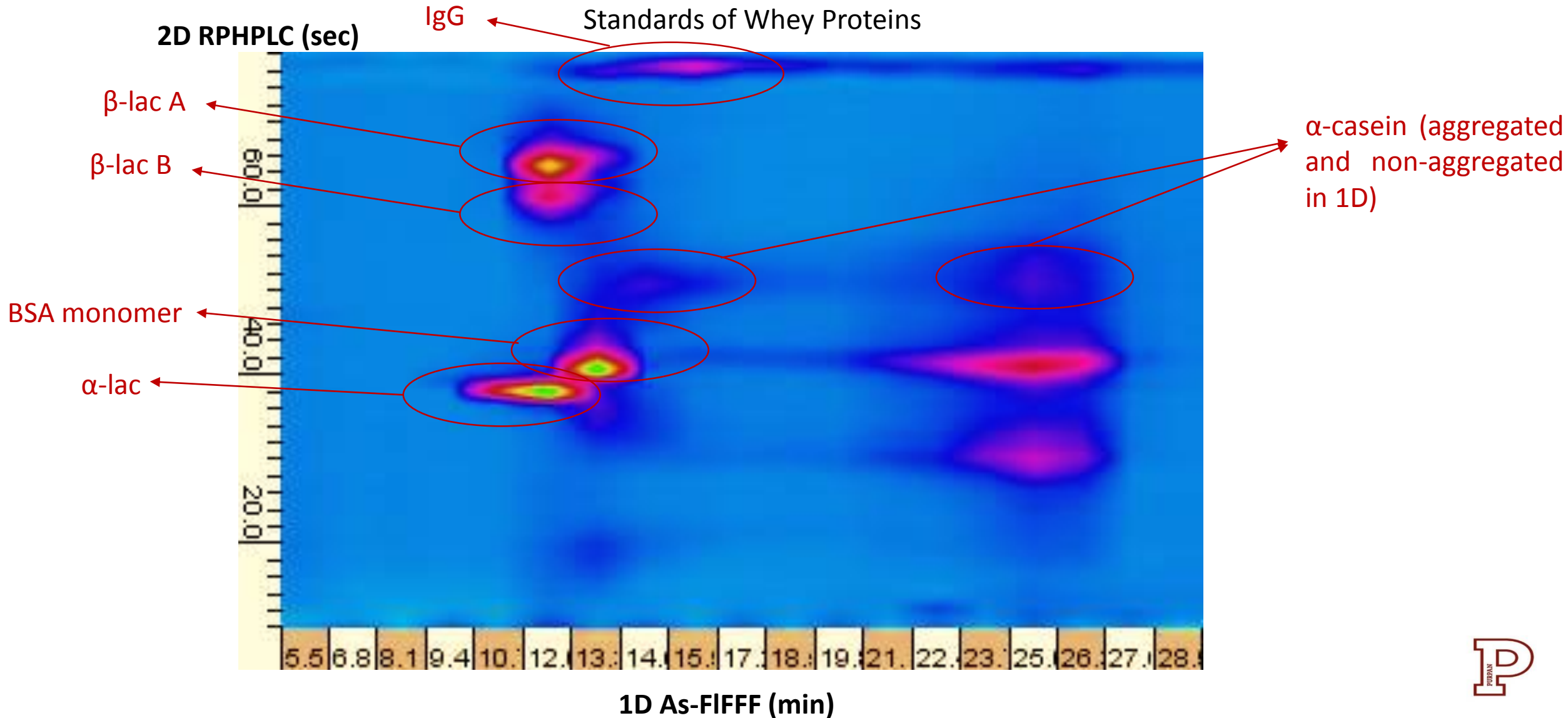


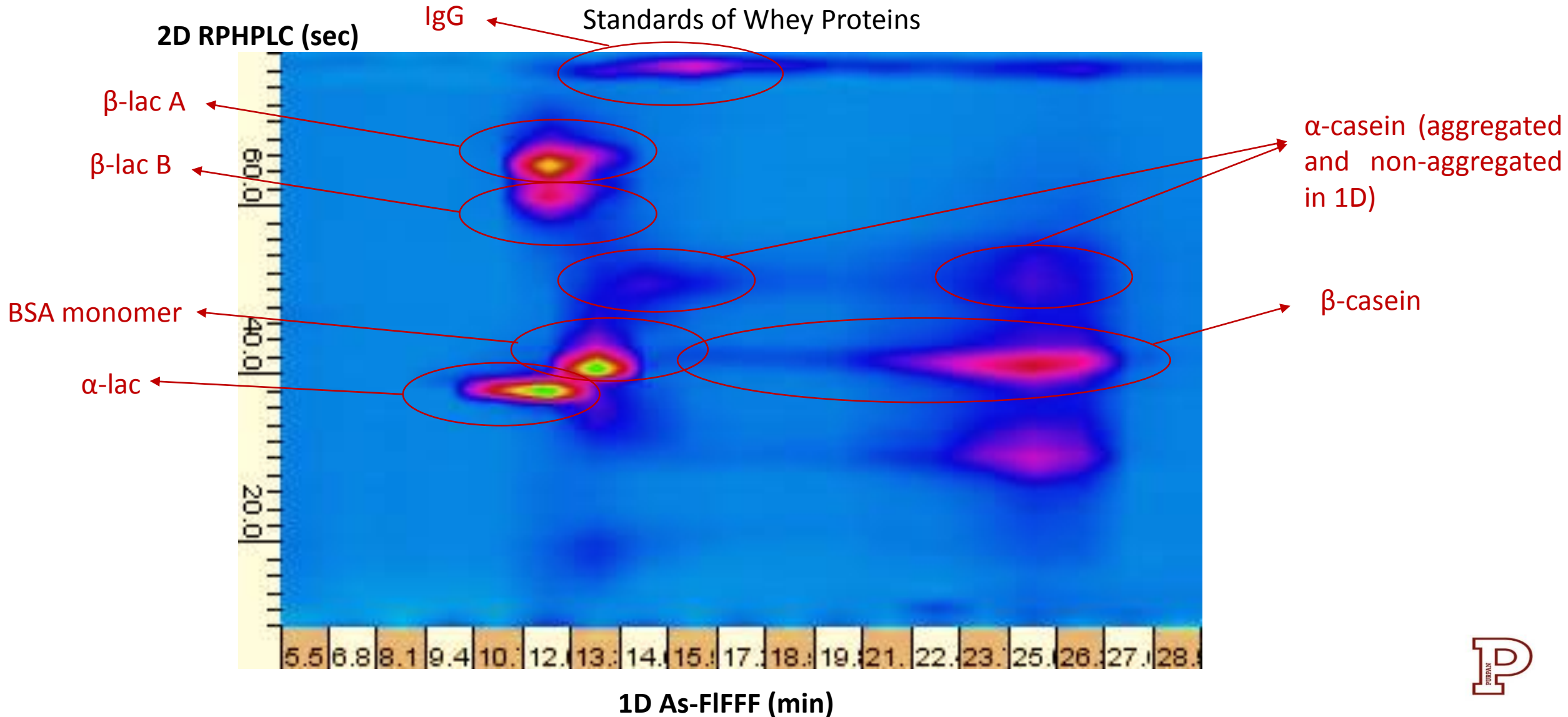


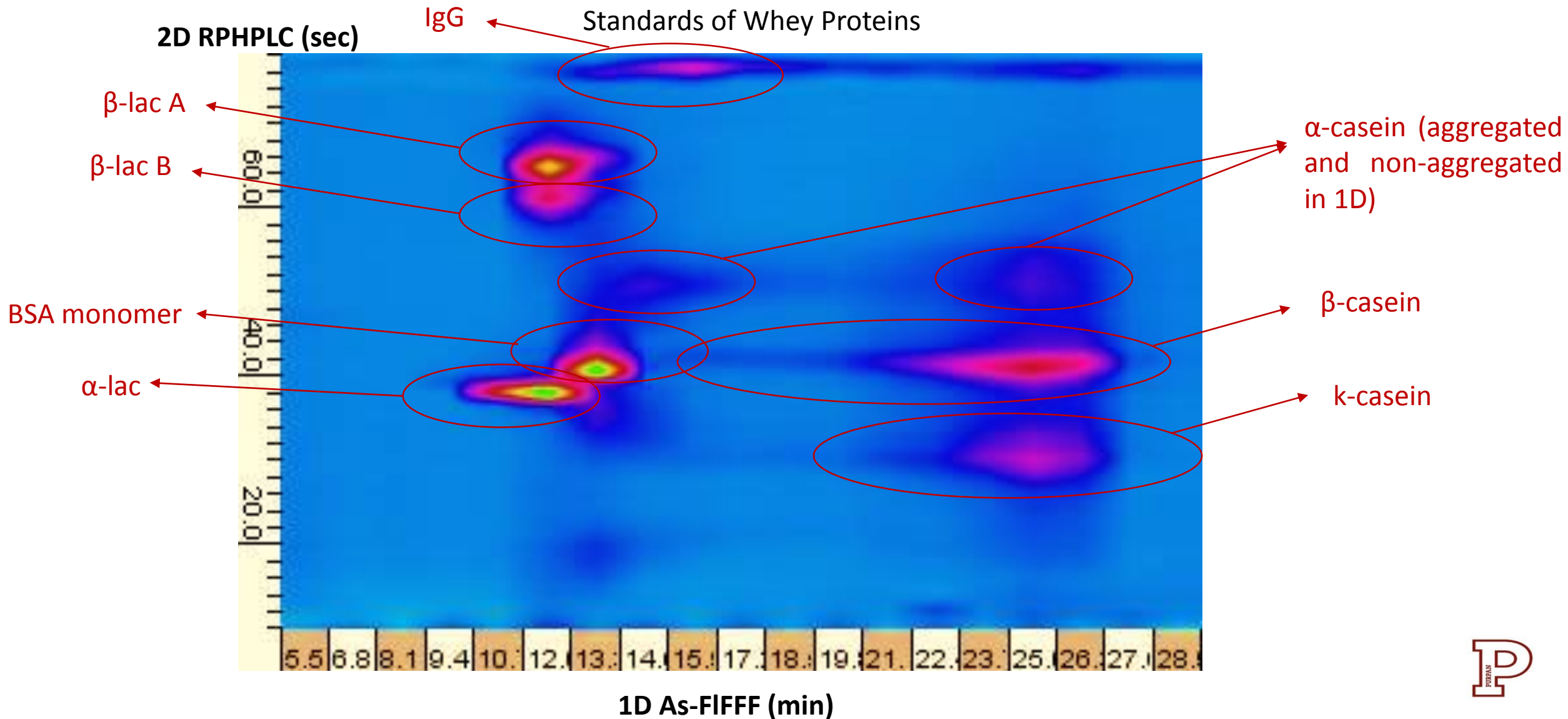


3

Whey Proteins Analysis by 2D-AsF1FFF-RPHPLC : UV signal mapping







IV

Denatured whey proteins studied by
Centrifugal-FFF

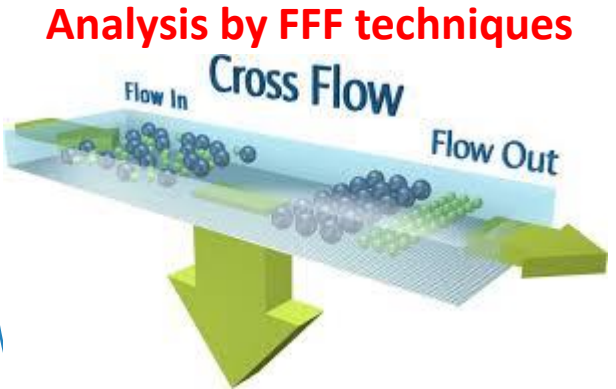


Denatured whey proteins studied by Centrifugal-FFF

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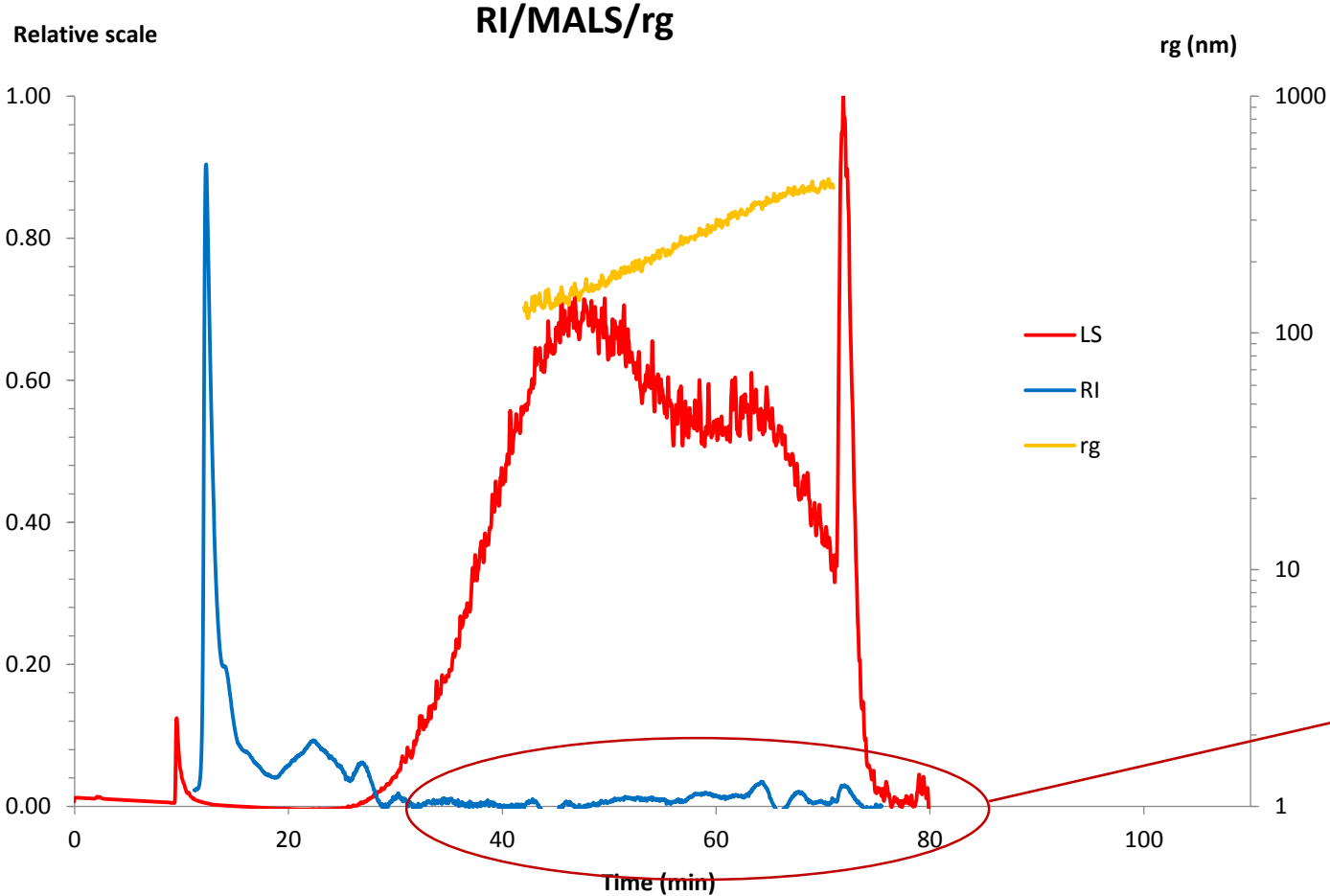
Drink



1

Denatured whey proteins studied by As-FIFFF

Whey proteins heated at high temperature
Eluent : 50mM NaCl



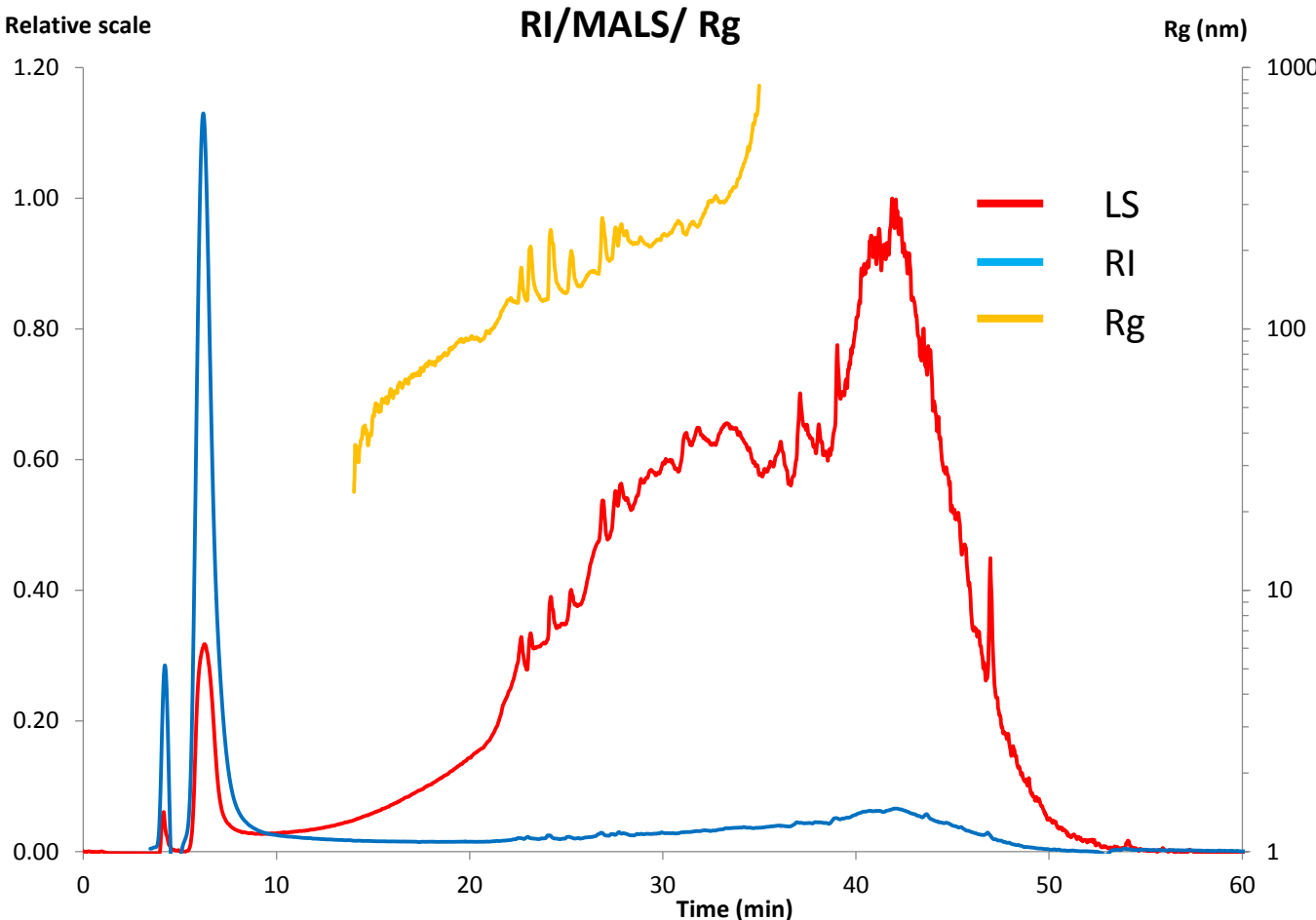
Mass recovery : 44%

There are no big aggregate in concentration



Denatured whey proteins studied by Centrifugal-FFF

Eluent : 50mM NaCl + 0.1% Novachem (detergent)

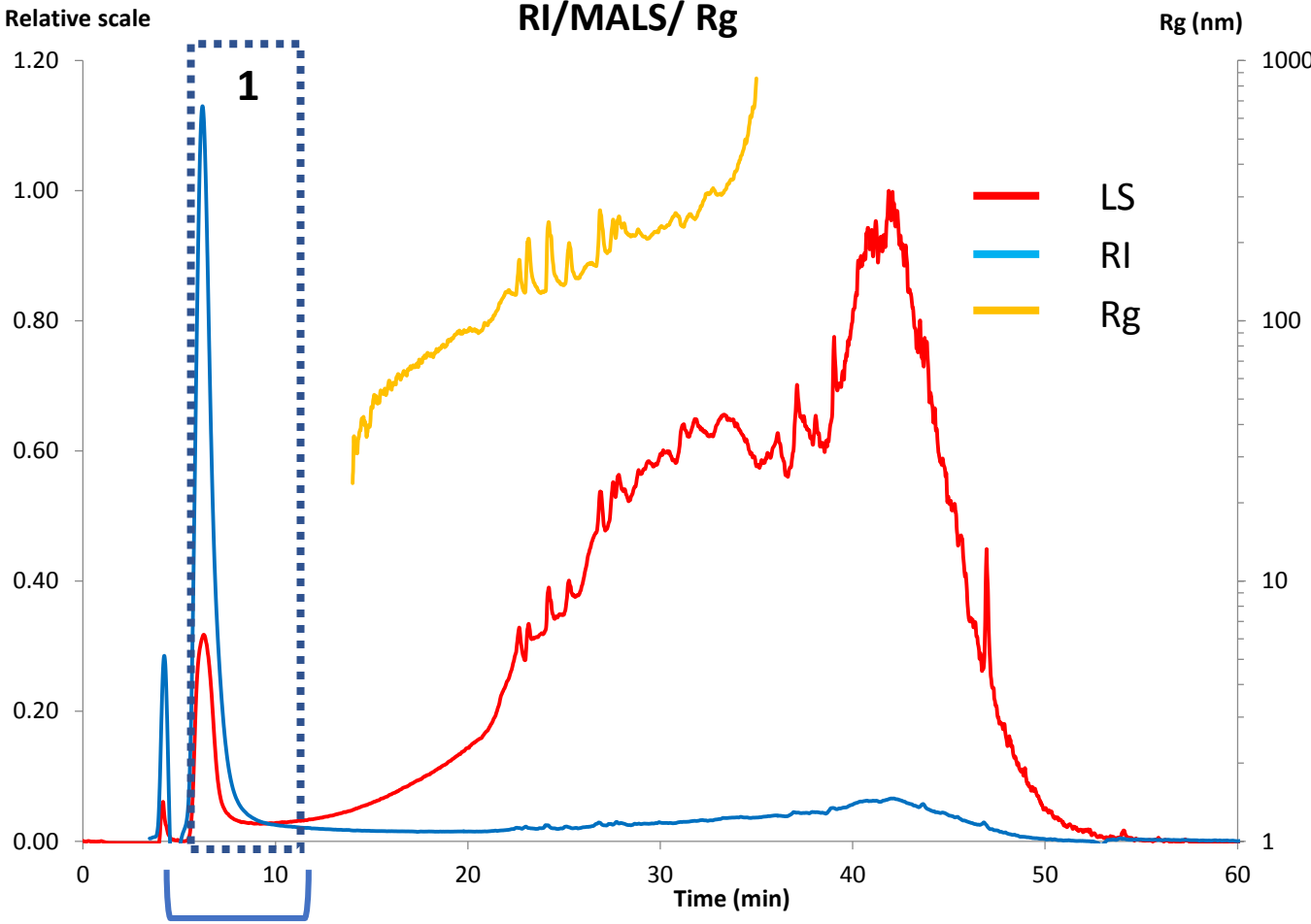


(Postnova system)

Mass recovery of 92% :
No membrane : less interaction
Detergent in the eluent



Eluent : 50mM NaCl + 0.1% Novachem (detergent)



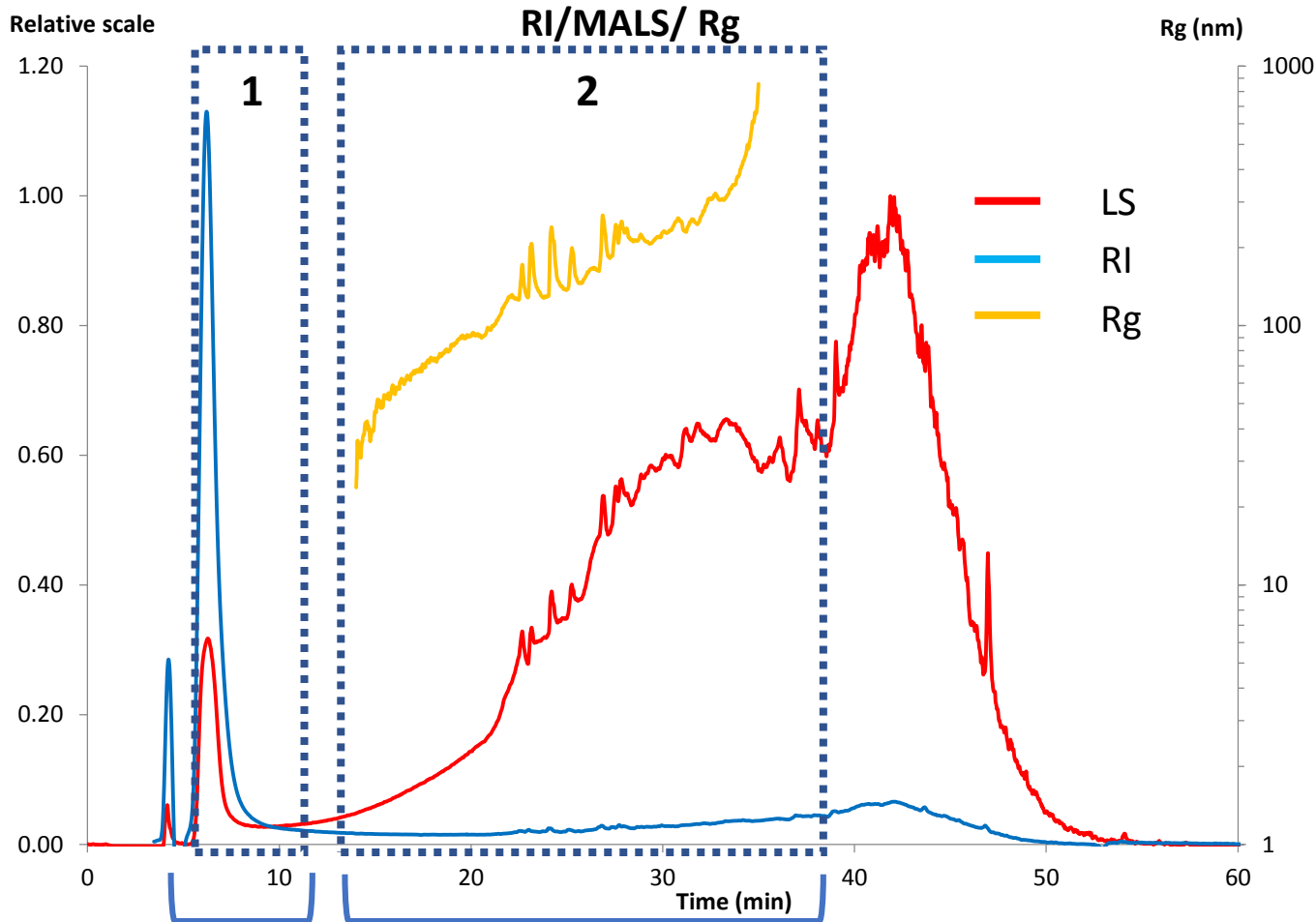
1: Objets less dense and big in the void peak : whey proteins non aggregated

**Mass recovery of 92% :
No membrane : less interaction
Detergent in the eluent**

Mass fractions 51%



Eluent : 50mM NaCl + 0.1% Novachem (detergent)



1: Objets less dense and big in the void peak

2: Rg from 25 to 850nm

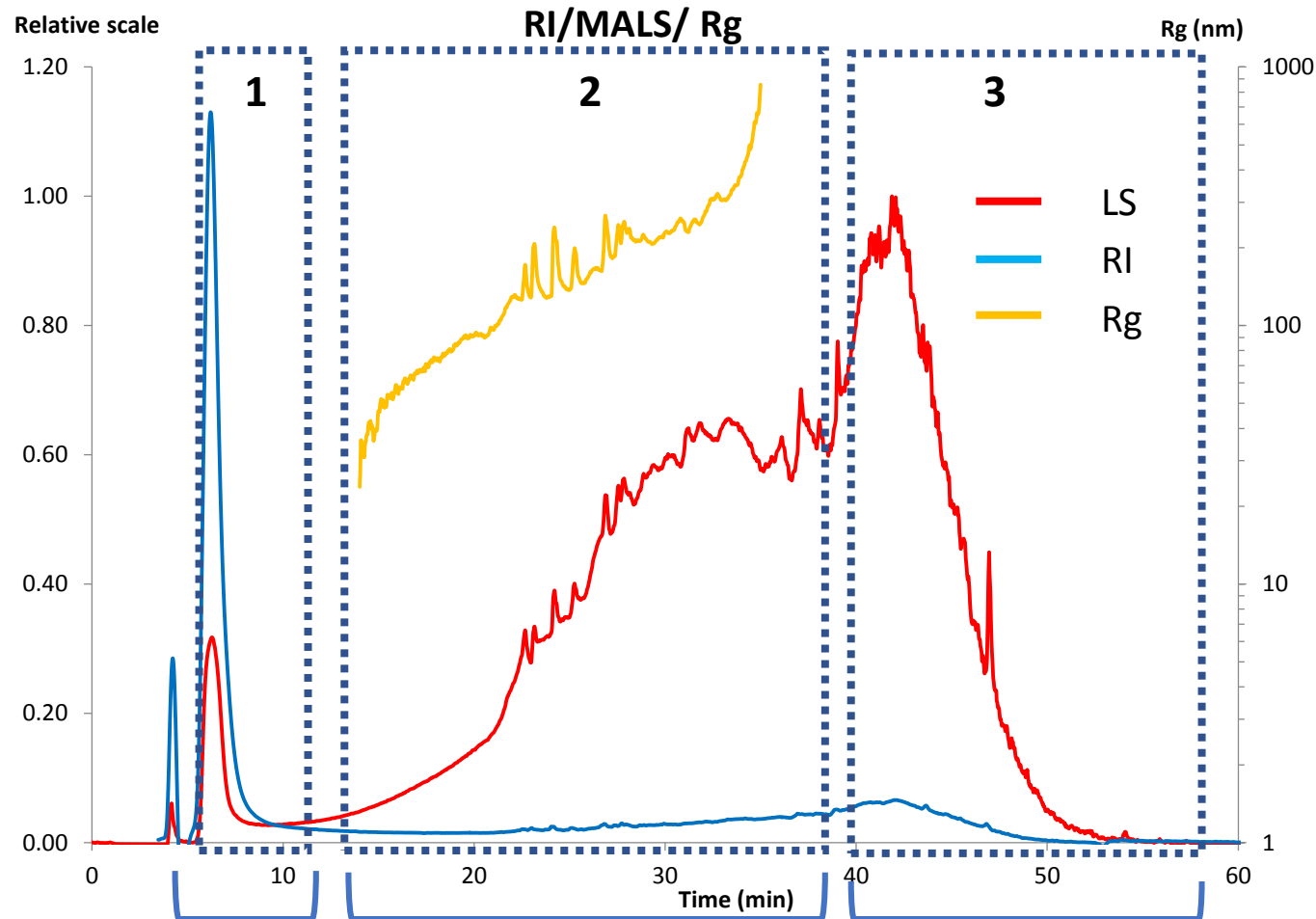
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Mass fractions

51%

23%

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2: Rg from 25 to 850nm

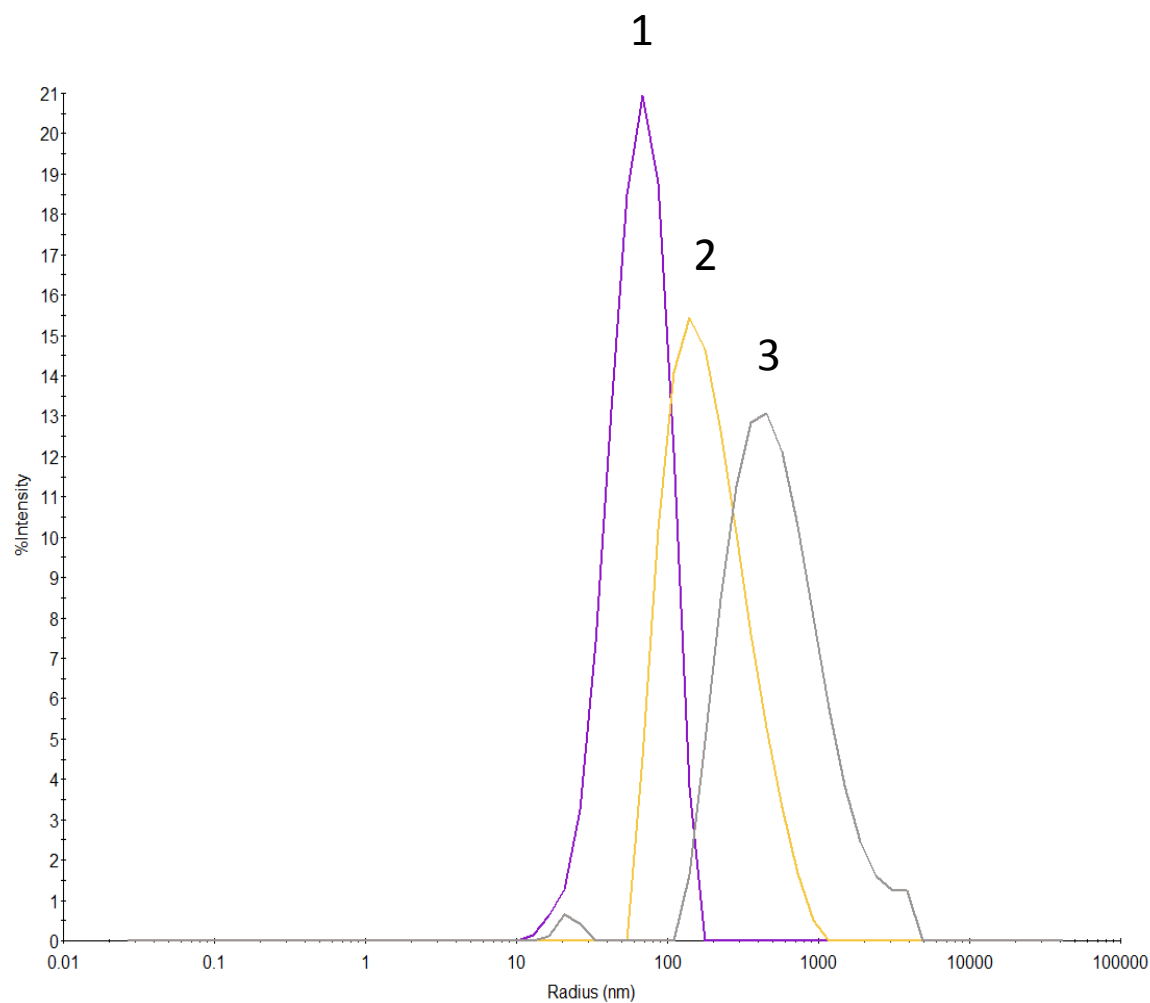
3: More than 1 μ m : MALS can't calculate the size**Fraction collection to do batch DLS****Mass recovery of 92% :****No membrane : less interaction****Detergent in the eluent**

Mass fractions

51%

23%

26%



1 Mean Rh: **68 nm**
Between **10 nm to 200 nm**

2 Mean Rh : **219 nm**
Between **45 nm to 1,2 μ m**

3 Mean Rh : **692 nm**
Between **100 nm to 4,5 μ m**

Conclusion and outlook

- 1st study : casein-WP pasteurized structures are different in function of the WP composition in IMF and heat temperature :
 - Addition of lactoferrin in IMF destabilize the casein micelles
 - Addition of Lactoferrin form structures with ams around the micelle after heat treatment at 80°C
 - Addition of Lactoferrin + α -lac form smaller structures with WP aggregates inside the micelles at 80°C
- Digestibility of this structures? Scale-up for formulation and pasteurization : Structural difference?



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- 2nd study $\xrightarrow{\quad}$: 2D-AsFlFFF-RPHPLC can separate all proteins of WP and casein mix

Quantitative experiment with calibration curve and industrial mix study



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Digestibility of this structures? Scale-up for formulation and pasteurization: Structural difference?

- 2nd study : 2D-AsFlFFF-RPHPLC can separate all proteins of WP and casein mix

Quantitative experiment with calibration curve and industrial mix study

- 3rd study : Centrifugal-FFF allow to have a better mass recovery for denatured WP



Acknowledgmen

t :
Amira Halabi, Amélie Deglaire,
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Alain Baniel, Frédéric Violleau

The TFFFC team: Estelle N'Tsiba, Audrey Ric,
Mireille Gaucher, Kévin Pascotto



Projet cofinancé par le Fonds Européen de Développement Régional



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Thank you for your
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Annexes

IMFs	Heat treatment parameters	Elution time ranges (min)	\overline{M}_w (10^8 g.mol^{-1})	\overline{R}_g (nm)	\overline{R}_h (nm)	Shape factors	df_{app}
Control	Unheated	20-28	6.4	138	96	1.4	3.0
	67.5°C	22-28	5.8	121	80	1.5	2.6
	80°C	22-28	6.8	126	82	1.5	2.7
LF ⁺	Unheated	20-28	4.7	123	91	1.4	2.6
	67.5°C	20-28	6.0	128	85	1.5	2.8
	80°C	20-28	7.3	157	87	1.8	2.3
LF ⁺ α-LA ⁺	Unheated	20-28	4.7	142	91	1.6	2.5
	67.5°C	20-28	6.0	153	82	1.9	2.7
	80°C	20-28	6.2	123	78	1.6	2.9