Developments in downstream processing of whey for new products

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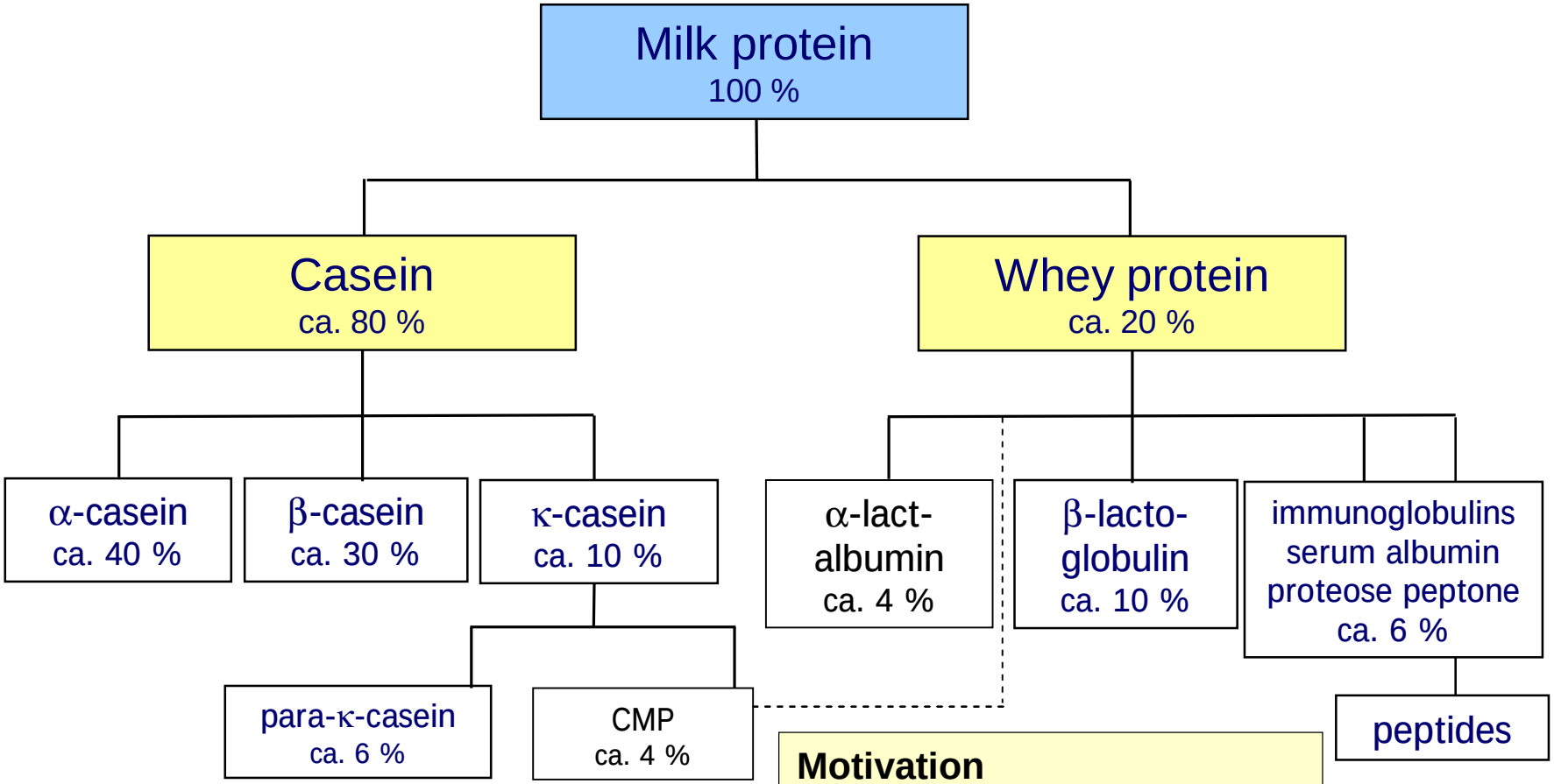
Protein composition of milk



Motivation

- Physiological effects
- Therapeutic effects
- Technological functions

Release of CMP during renneting

k-casein

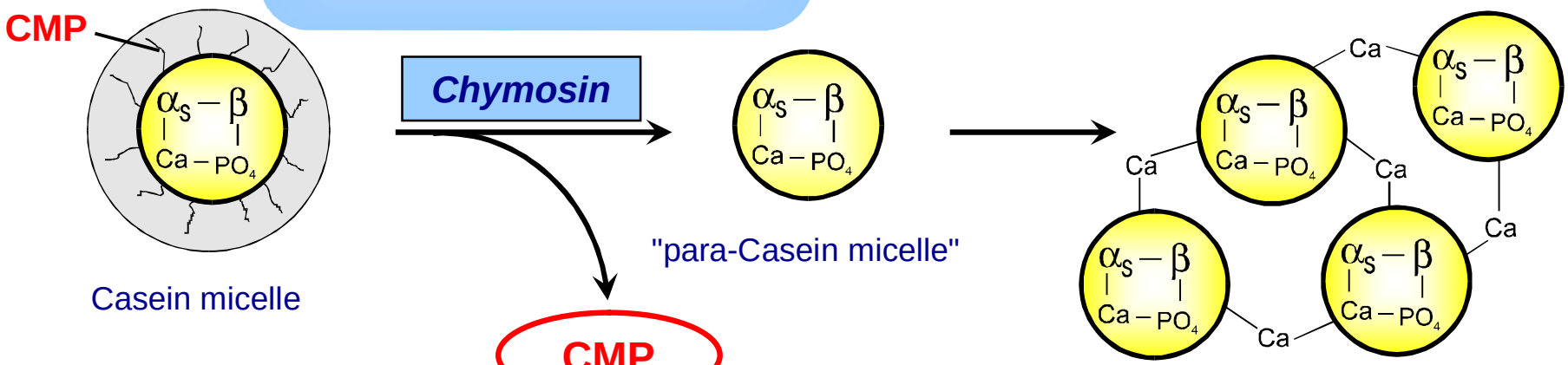
$Pyg_1 \cdots\cdots Phe_{105}\text{-}Met_{106} \cdots\cdots Val_{169}$

↓ *Chymosin*

$Pyg_1 \cdots\cdots Phe_{105}$ + $Met_{106} \cdots\cdots Val_{169}$

para-k-casein **CMP**

Milk:	3,3 – 4,5 g/l	κ-Casein
	1,2 – 2,5 g/l	CMP



Casein micelle

"para-Casein micelle"

Aggregation of the casein micelles via Ca^{2+}-bridges

CMP

↓

Whey

15-20% of the total protein content is CMP, the other 85 % are whey proteins

Green Cheese

Amino acid sequence of Caseinmacropeptide [CMP var. A (var. B)]

1 Met - Ala - Ile - Pro - 5 Pro - Lys - Lys - Asn - Gln - 10 Asp - Lys - Thr - Glu - Ile - 15 Pro -

Thr - Ile - Asn - Thr - 20 Ile - Ala - Ser - Gly - Glu - 25 Pro - Thr - Ser - Thr - Pro - 30 Thr -

Thr (Ile) - Glu - Ala - Val - 35 Glu - Ser - Thr - Val - Ala - 40 Thr - Leu - Glu - Asp (Ala) -Ser- 45 Pro -

Glu - Val - Ile - Glu - 50 Ser - Pro - Pro - Glu - Ile - 55 Asn - Thr - Val - Gln - Val - 60 Thr -

Ser - Thr - Ala - 64 Val.

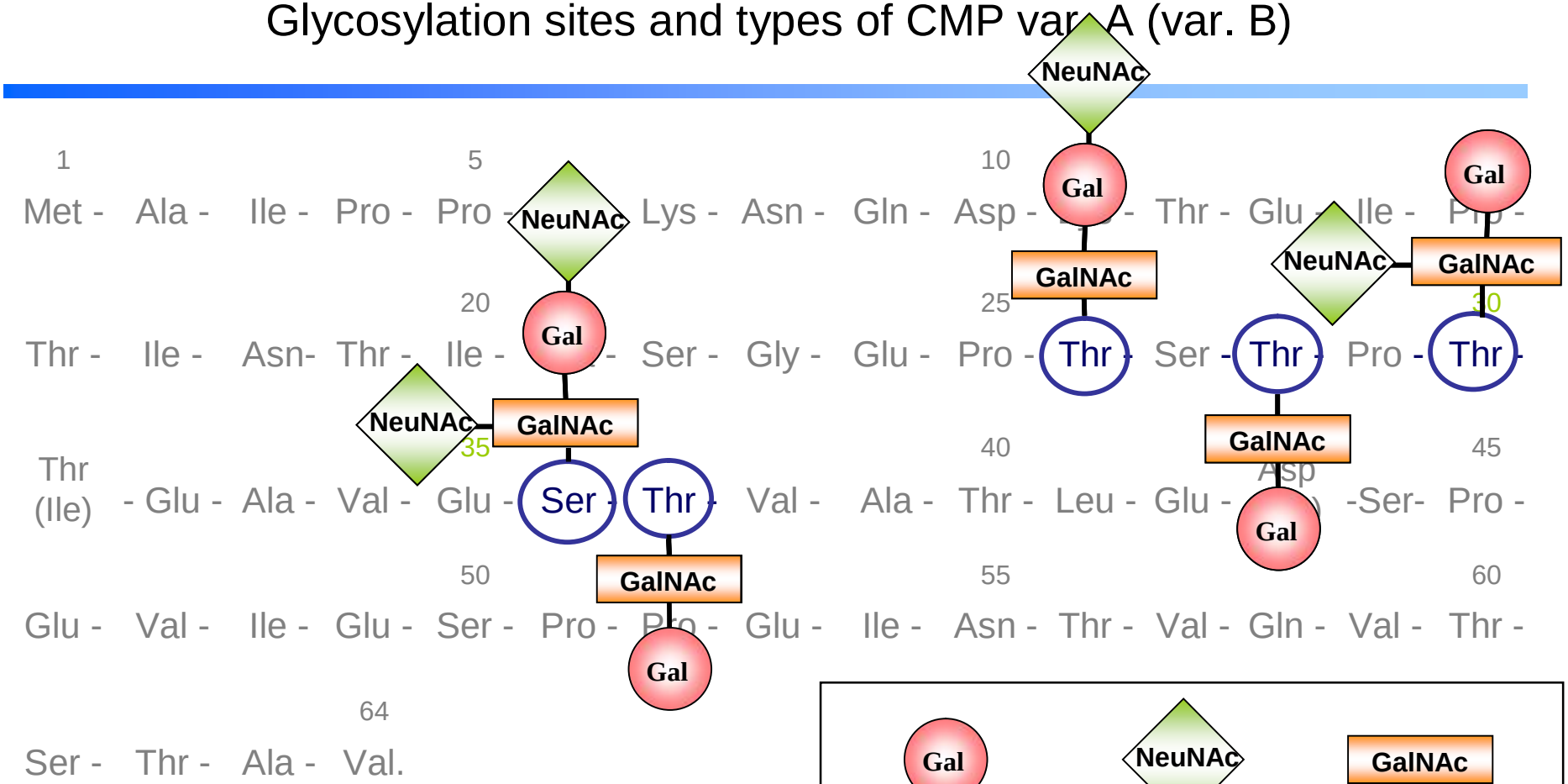
genetic var. A and B

essential amino acid

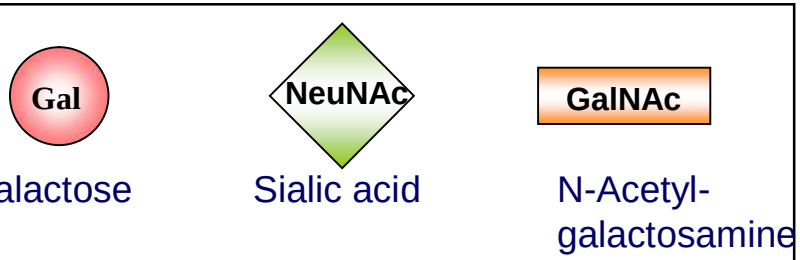
branched-chain amino acid

- High amount of *essential* amino acids
- High amount of *branched-chain* amino acids
- Absence of aromatic amino acids (Phe, Tyr, Trp) **à** acceptable protein source for PKU patients

Glycosylation sites and types of CMP var. A (var. B)



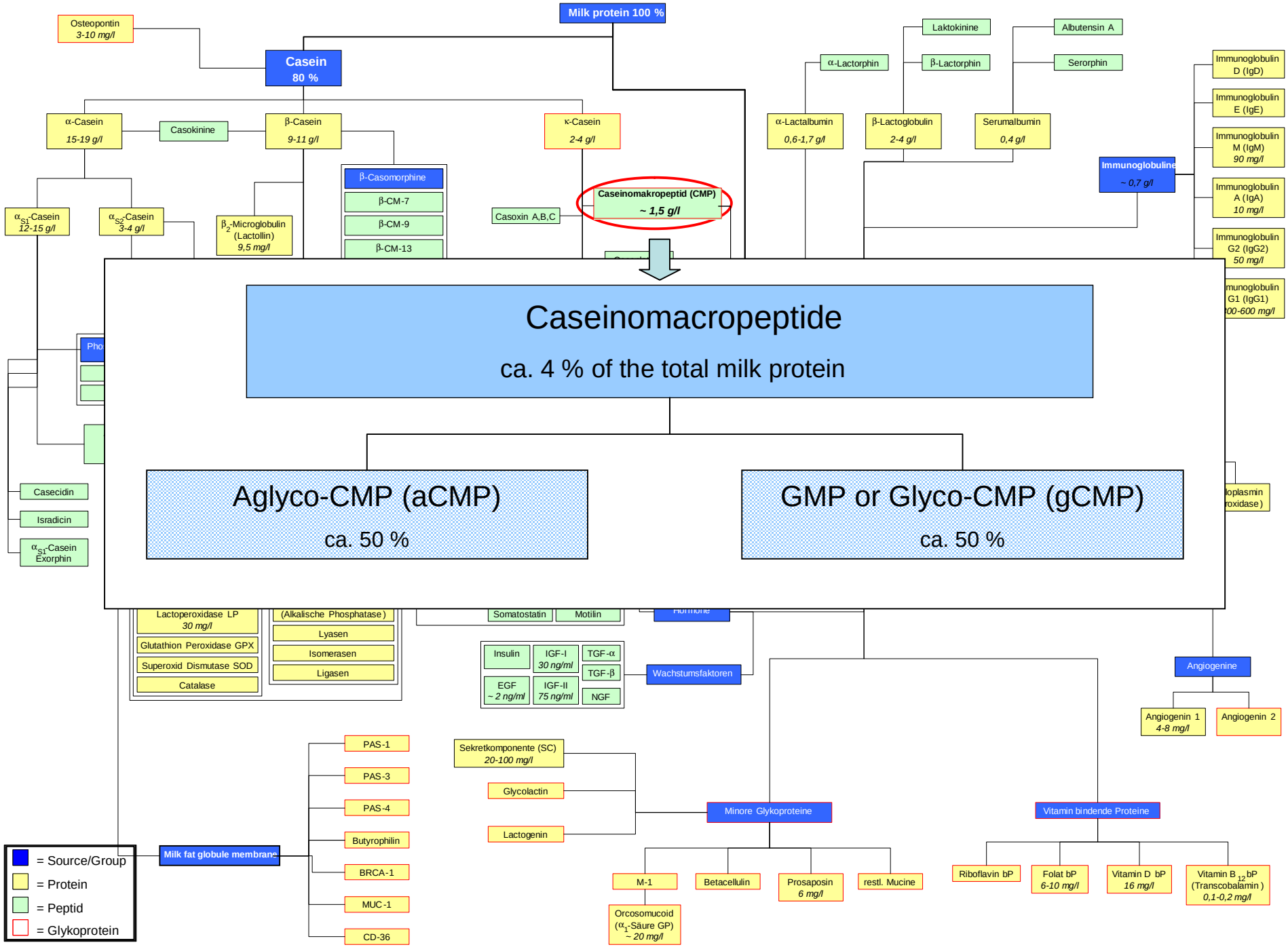
- ≈ 50 % of CMP unglycosylated
- ≈ 50 % of CMP glycosylated (GMP)
- Heat stable in comparison to whey proteins



Galactose | Sialic acid | N-Acetyl-galactosamine

Site of saccharide attachment

variations in:
- point of attachment
- type
- degree of glycosyltn.



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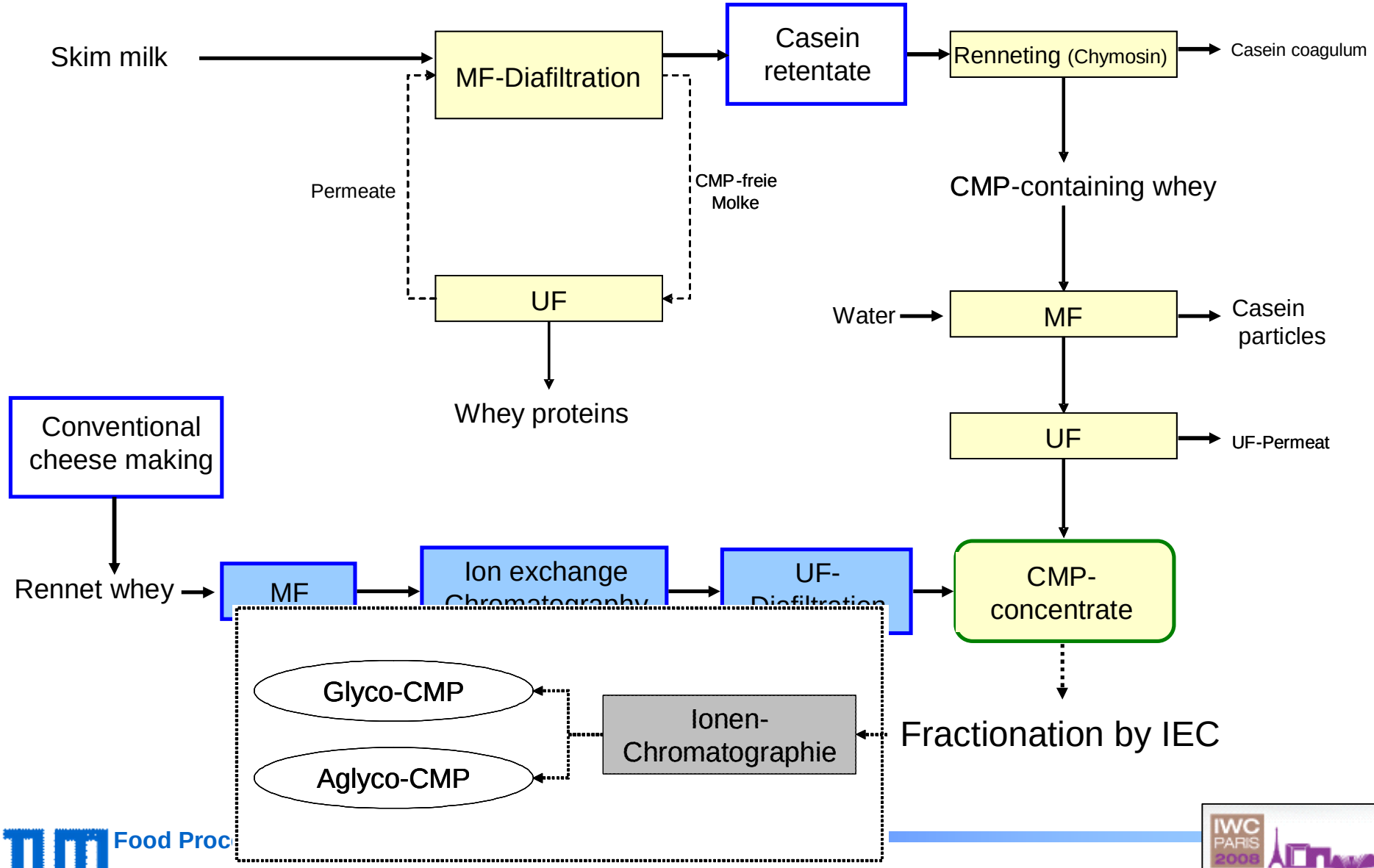
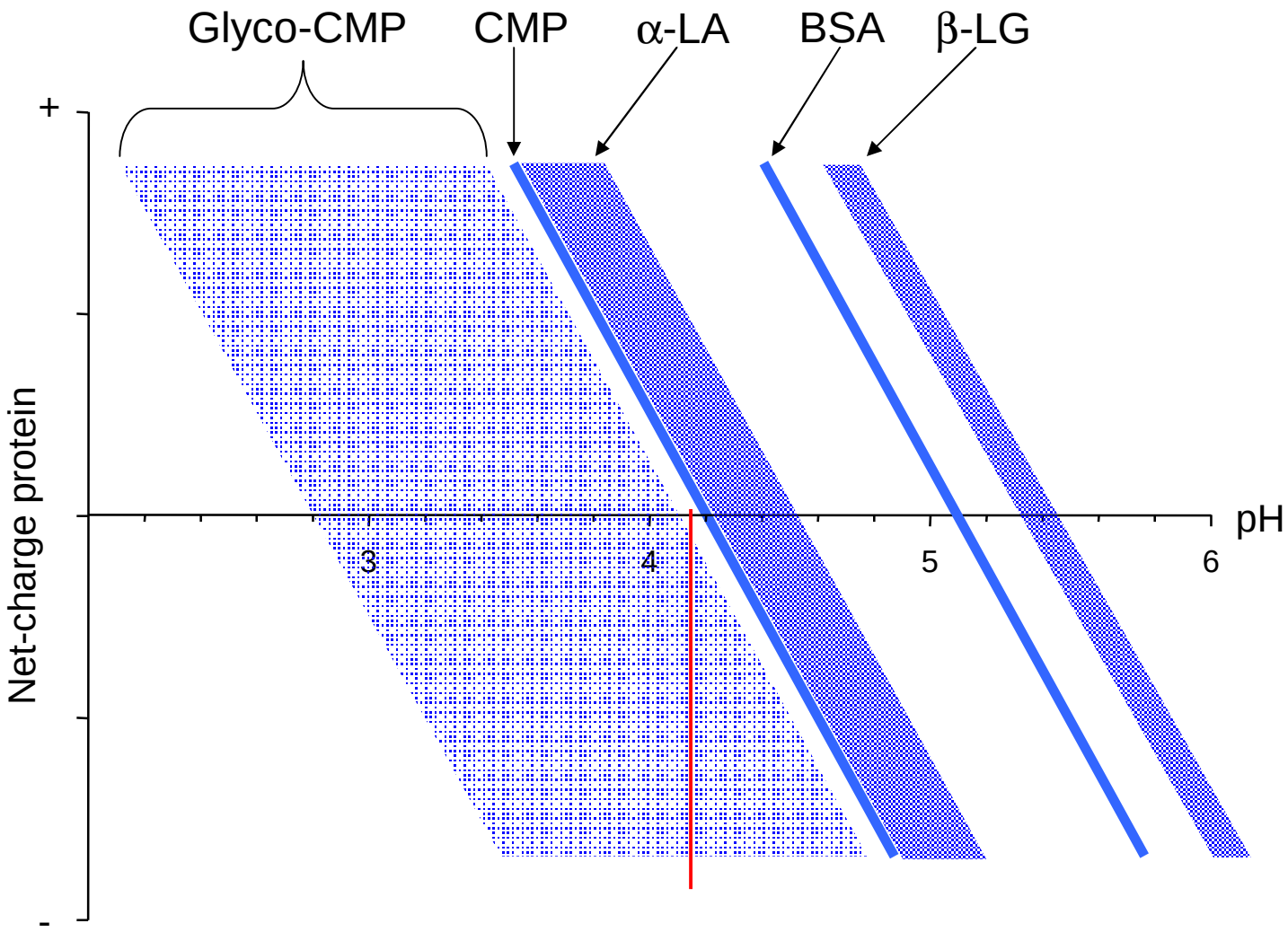
Methods to obtain CMP

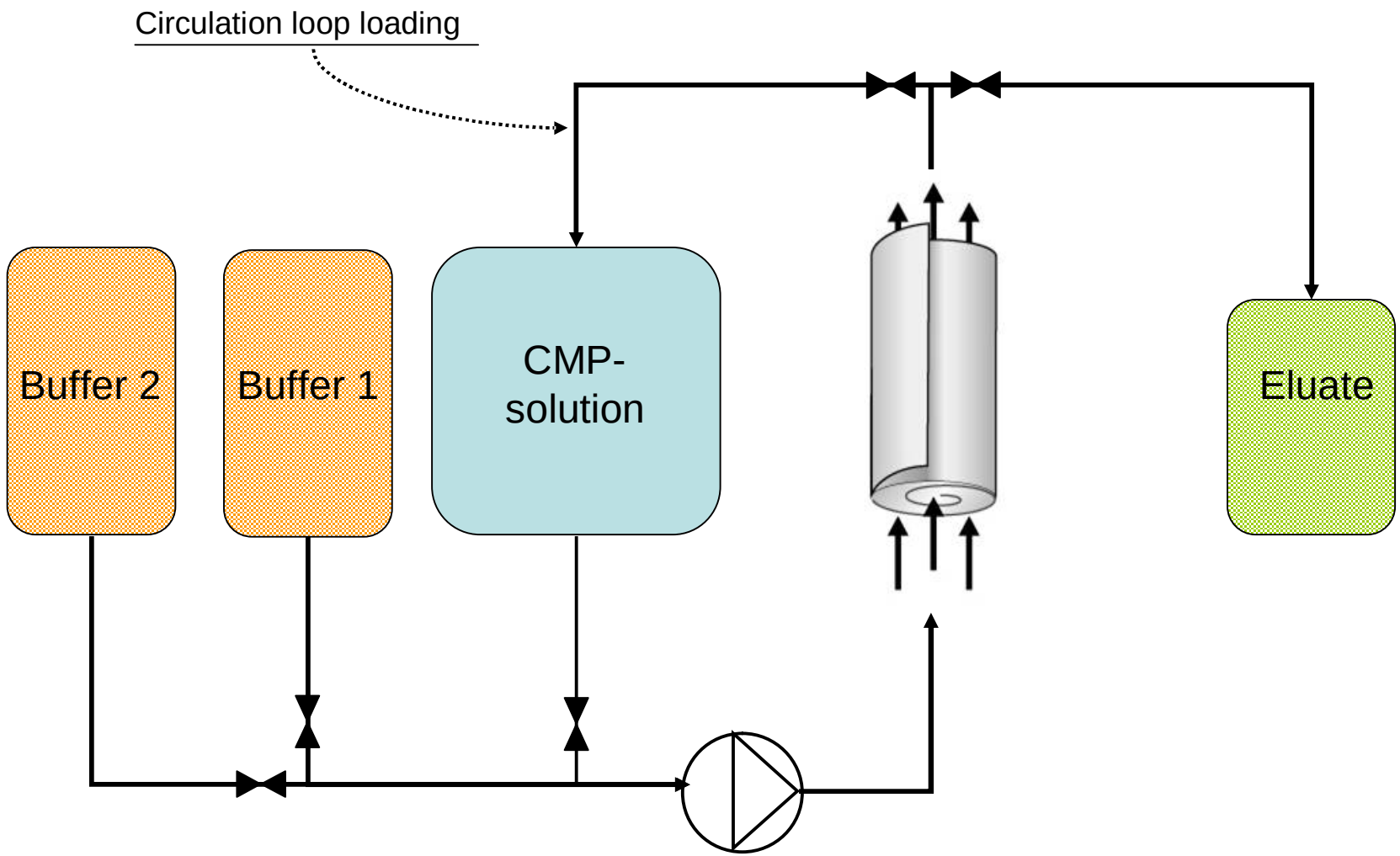


Isoelectric points of whey proteins and CMP

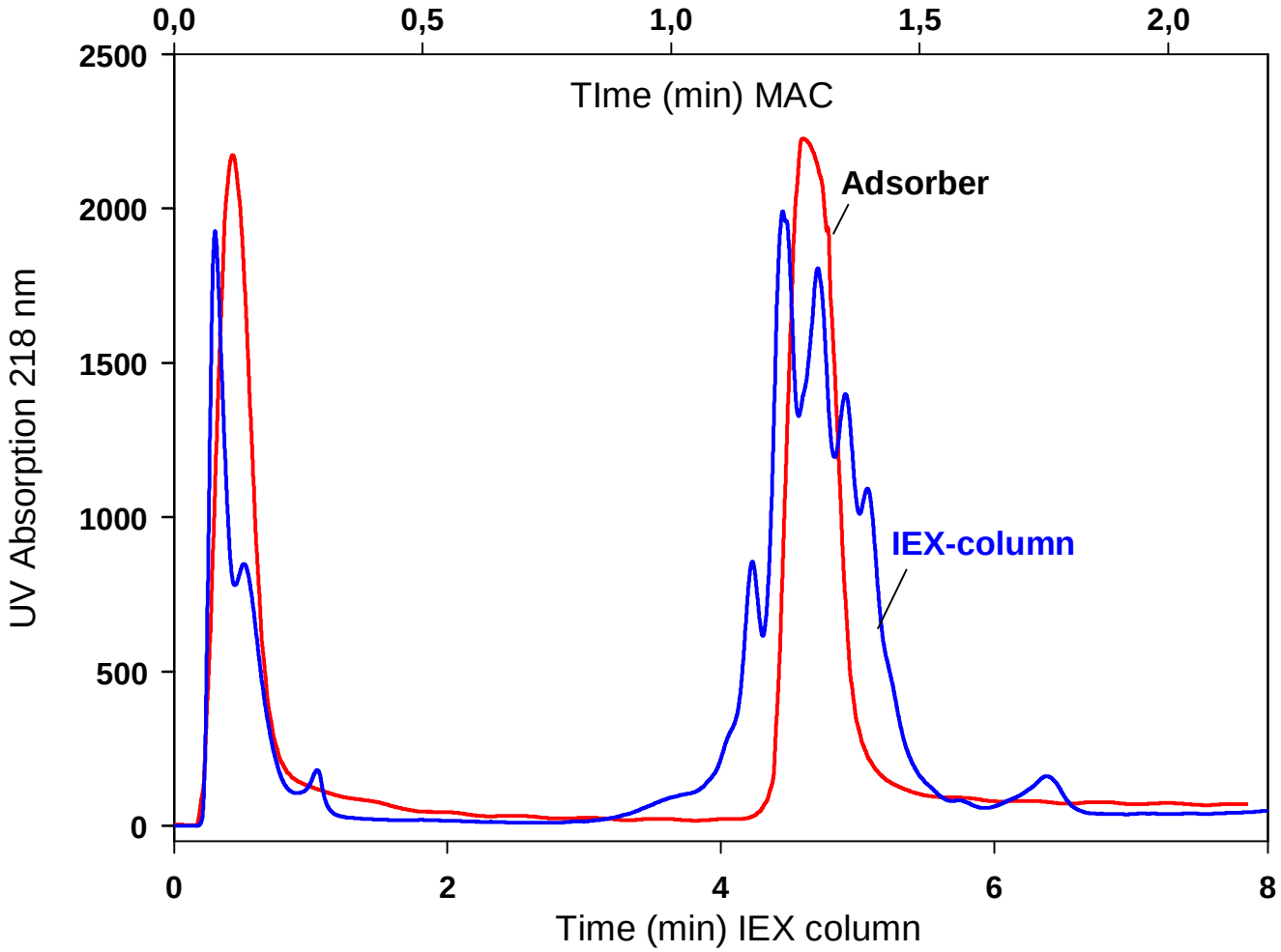


Isoelectric properties of whey proteins (Source: Swiss Protein Database / European Protein Database)

Schematic of the experimental pilot plant set-up

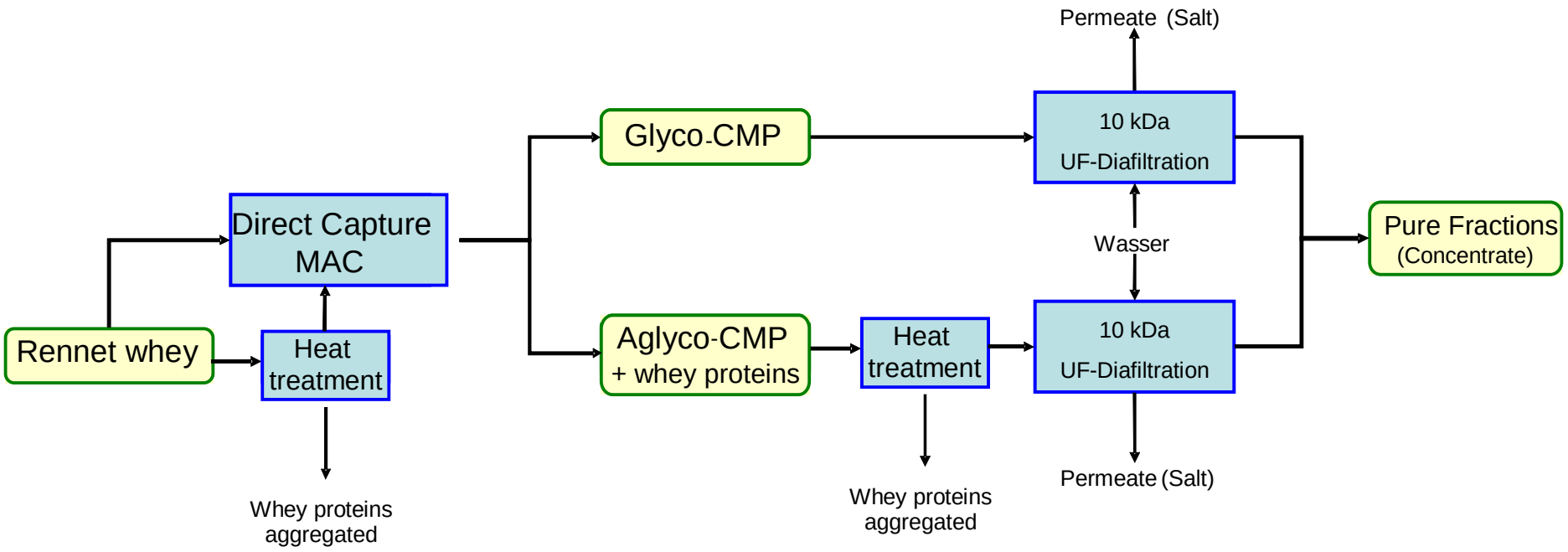


Membrane Adsorber (MAC) – IEX Column in comparison



Variation Phase

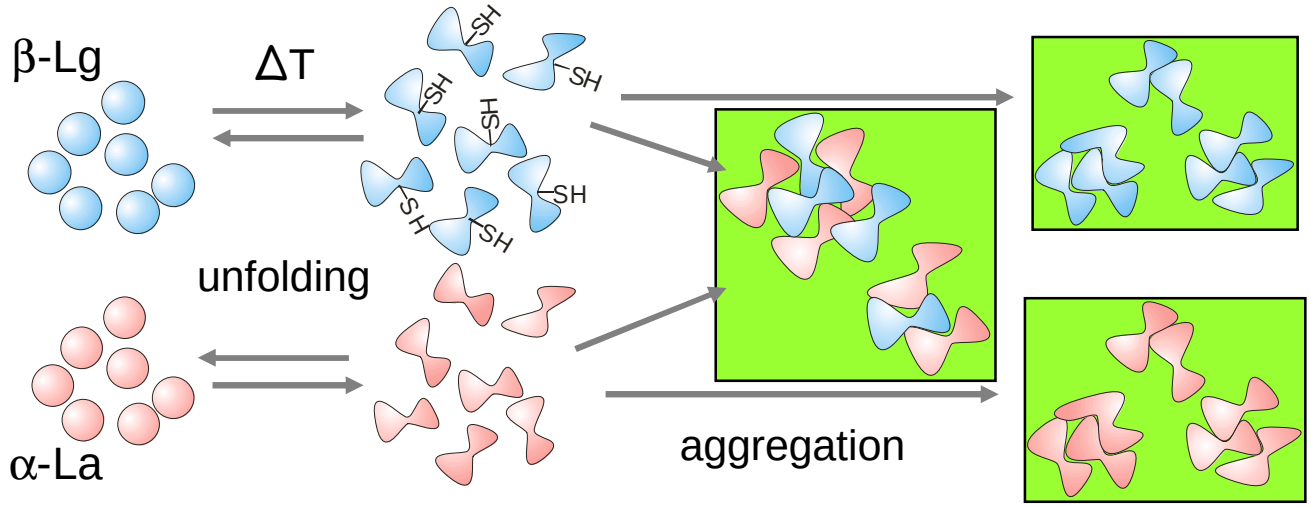
MAC process based on CMP solution



<u>Key data</u>

- Purity eluate: 91 %
- Flow rate: 8 L/min
- Capacity: 7,5 g glyco-CMP/cycle = 0,21 mg/cm²
- Capacity loss from cycle to cycle

Whey proteins and their thermal reactivity



Extraction of native α-La/denaturated ß-Lg

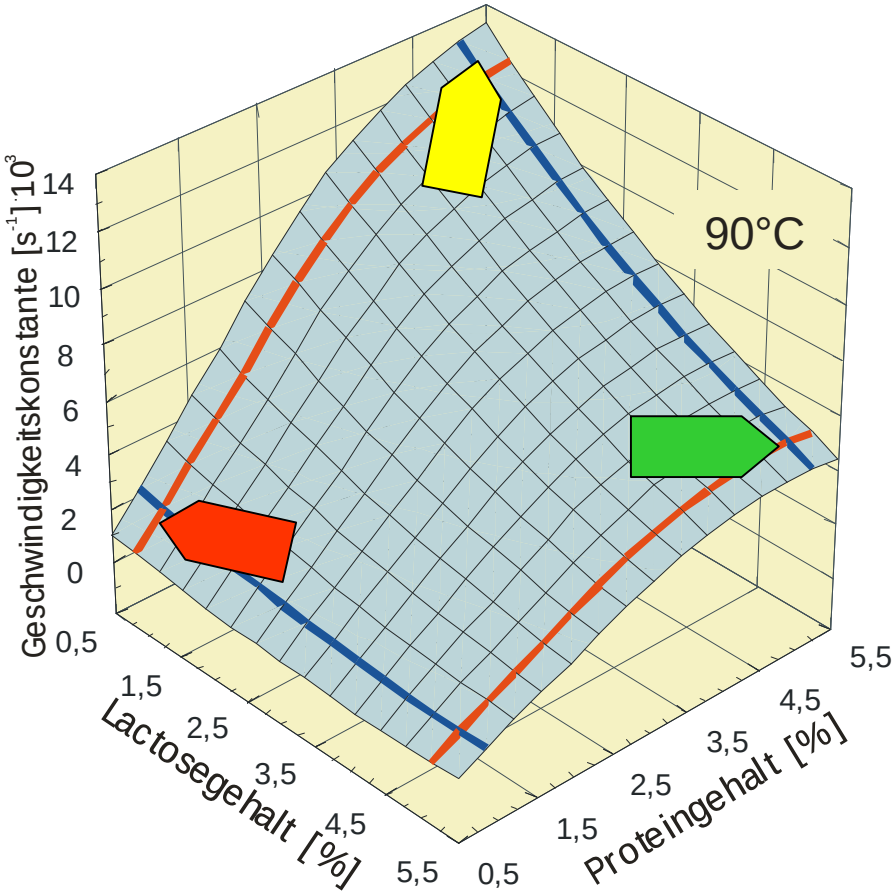
Extraction of native ß-Lg/denaturated α-La

<u>Some influencing factors</u>

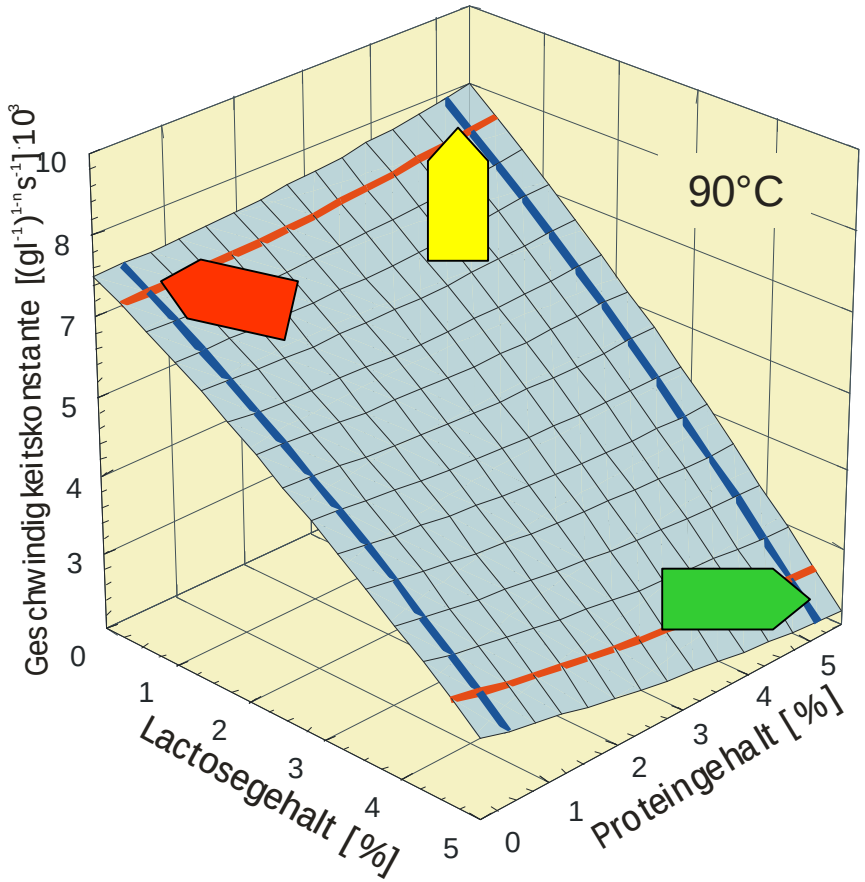
- § Protein content
- § Lactose content
- § Calcium content
- § pH
- § Temperature
- § Treatment time

Heat induced aggregation of α-Lactalbumin and β-Lactoglobulin

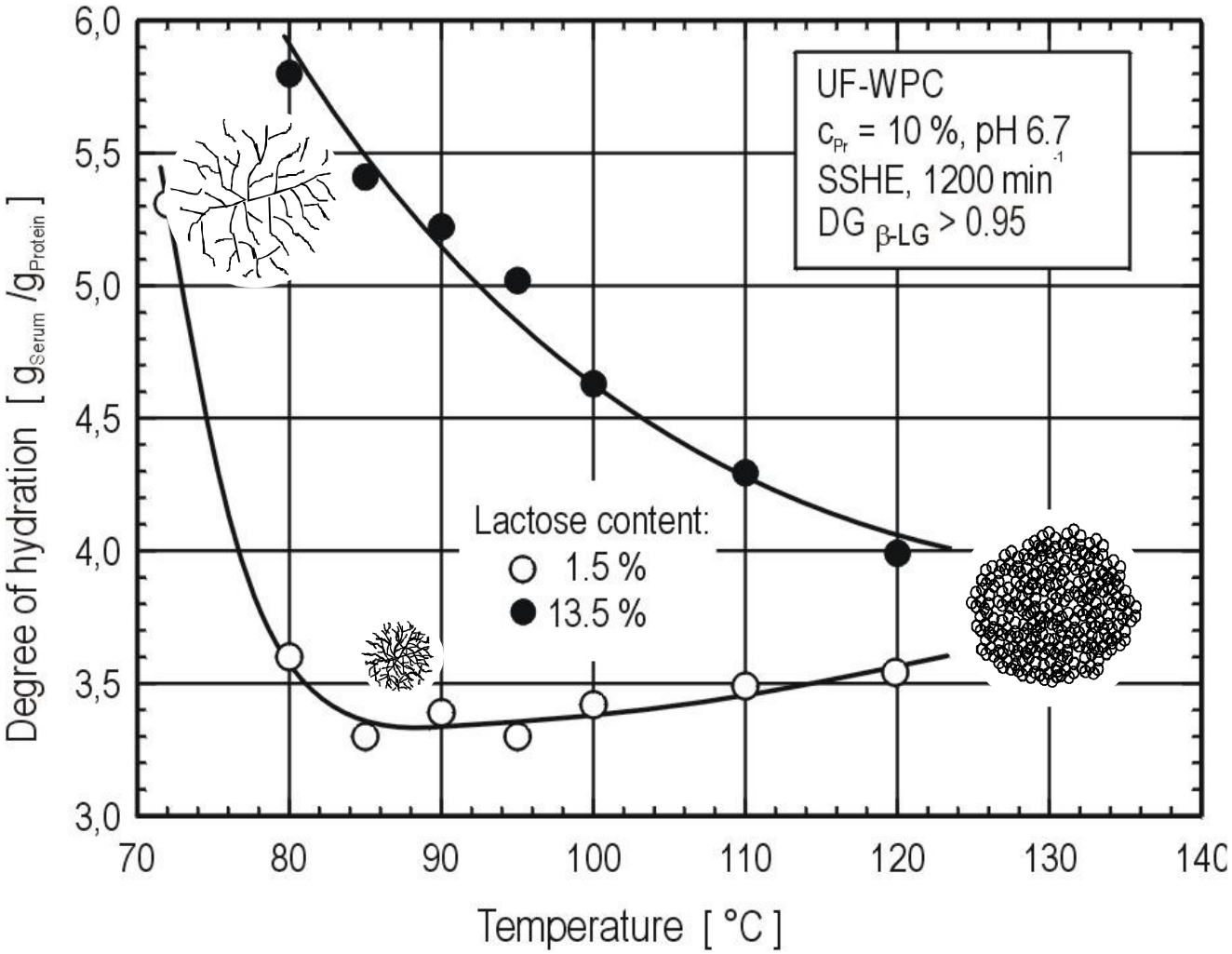
α-Lactalbumin, n = 1



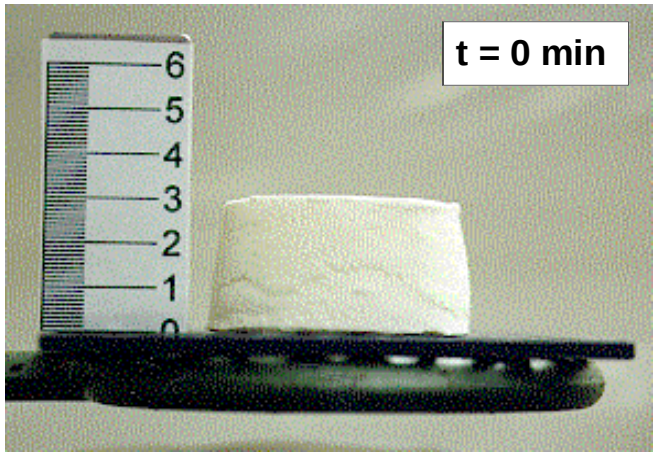
β-Lactoglobulin, n = 1,5



Co-aggregates: Effect of temperature on the particulates´ serum binding capacity

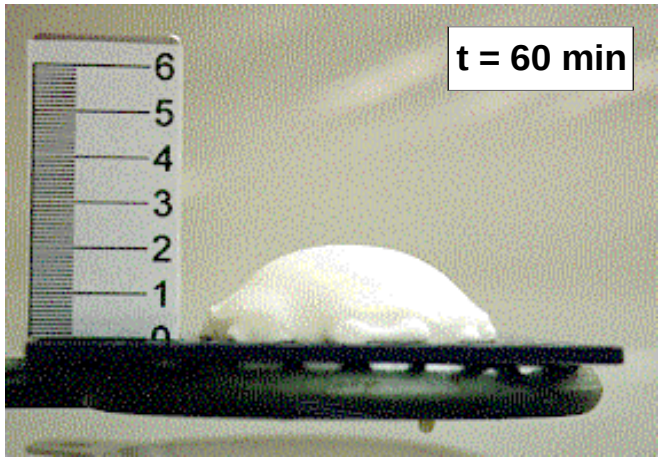


Ice cream: Assessment of the melt behaviour

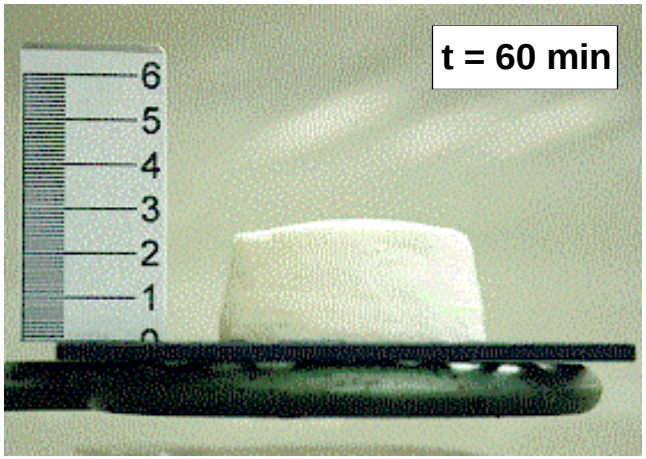


initial state

Ice cream samples with different whey protein supplements: Melt down characteristics at ambient temperature



Addition of whey powder



Addition of whey protein aggregates

(Koxholt 2000)

Manufacture of pure, native α-La in conjunction with ß-Lg aggregates

Whey

UF/Diafiltration
3,5% Protein

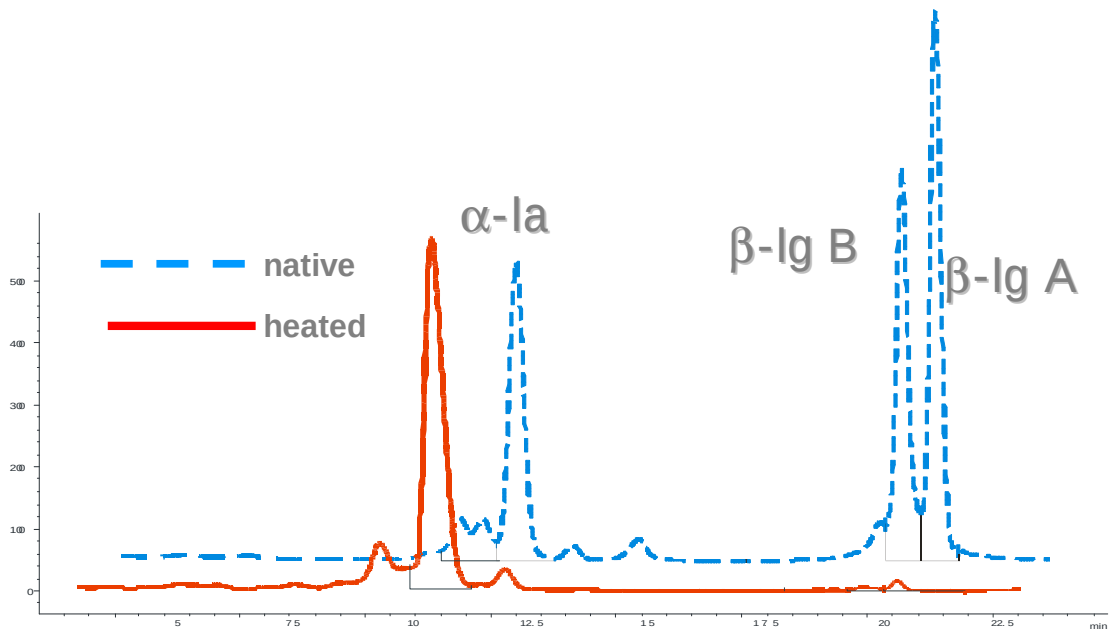
Heat treatment
93°C/20 s; pH 7,5

pH adjustment

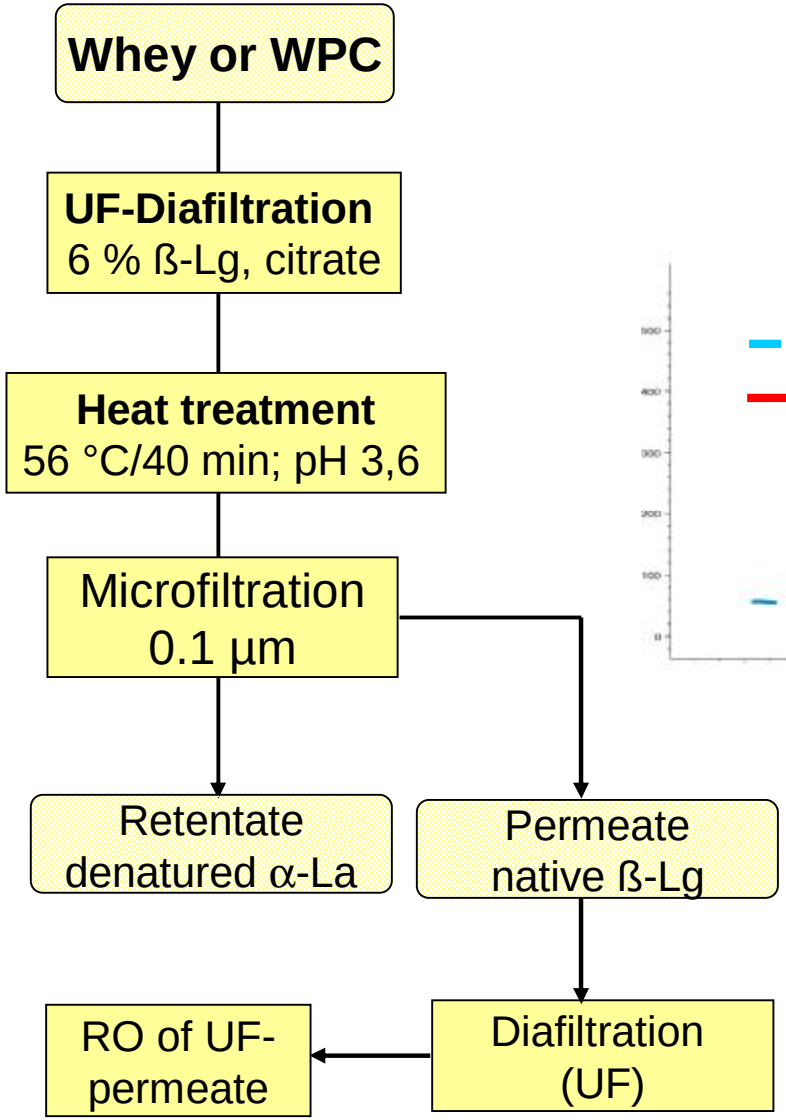
MF
0.1 µm

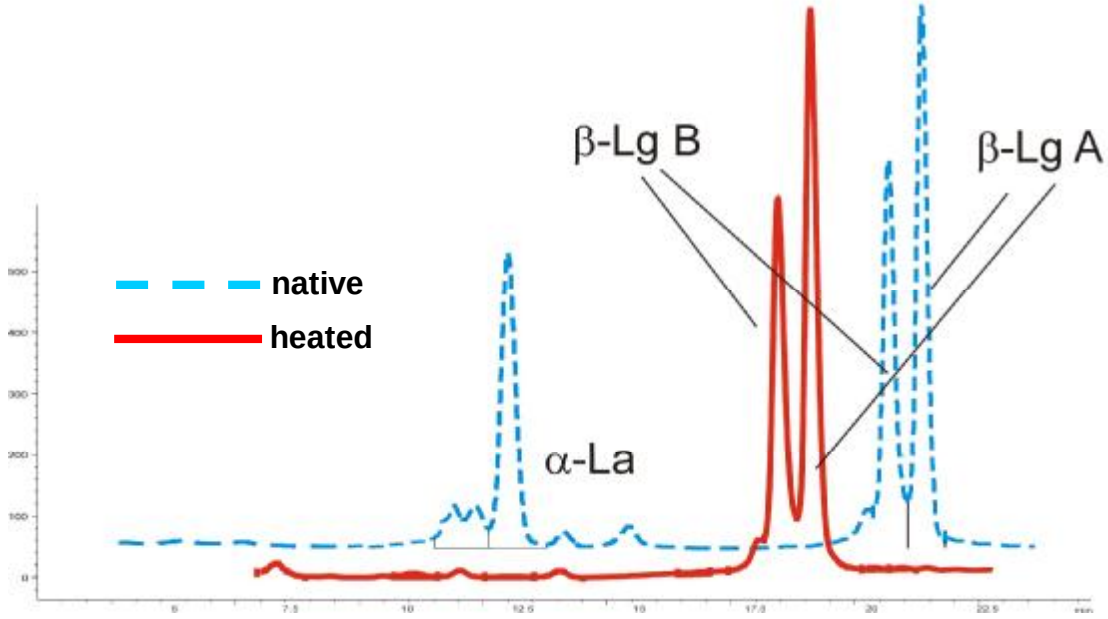
Retentate
denatured β-Lg

Permeate
native α-La

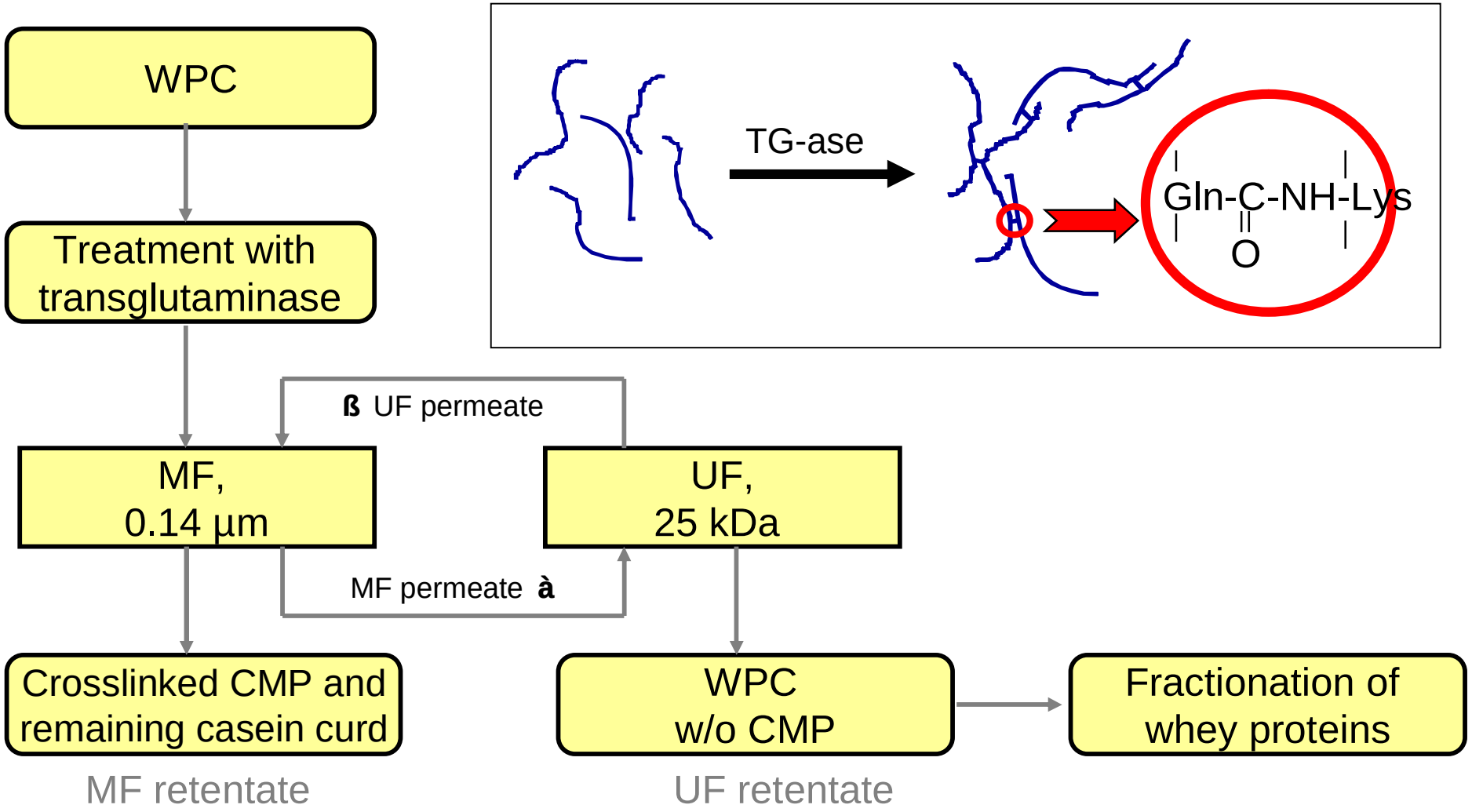


Manufacture of pure, native ß-Lg in conjunction with α-La aggregates

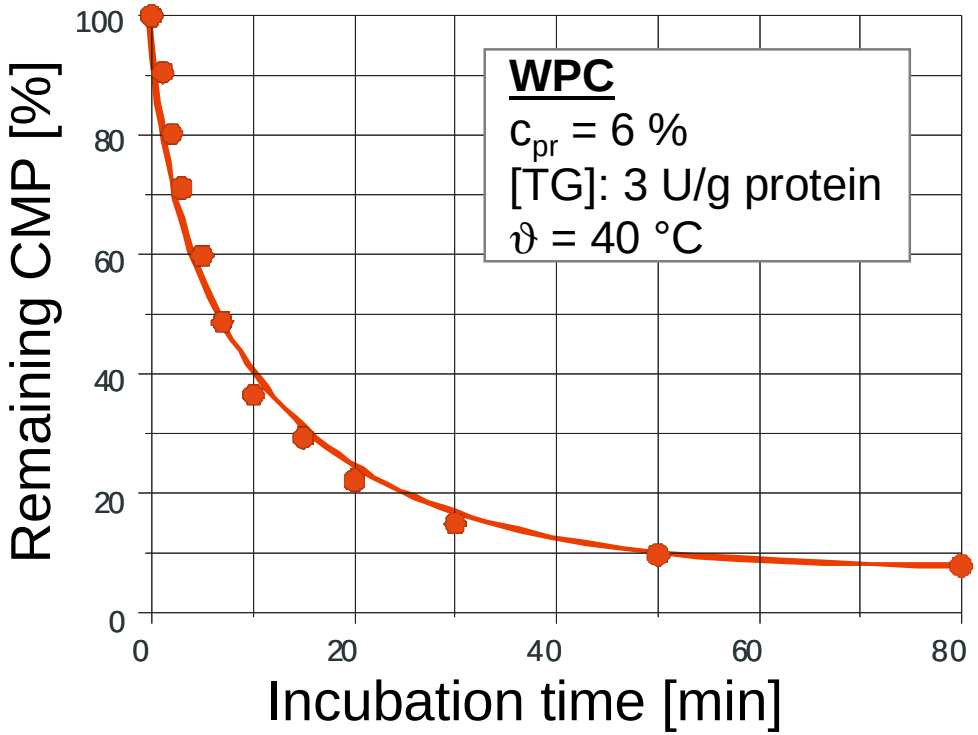


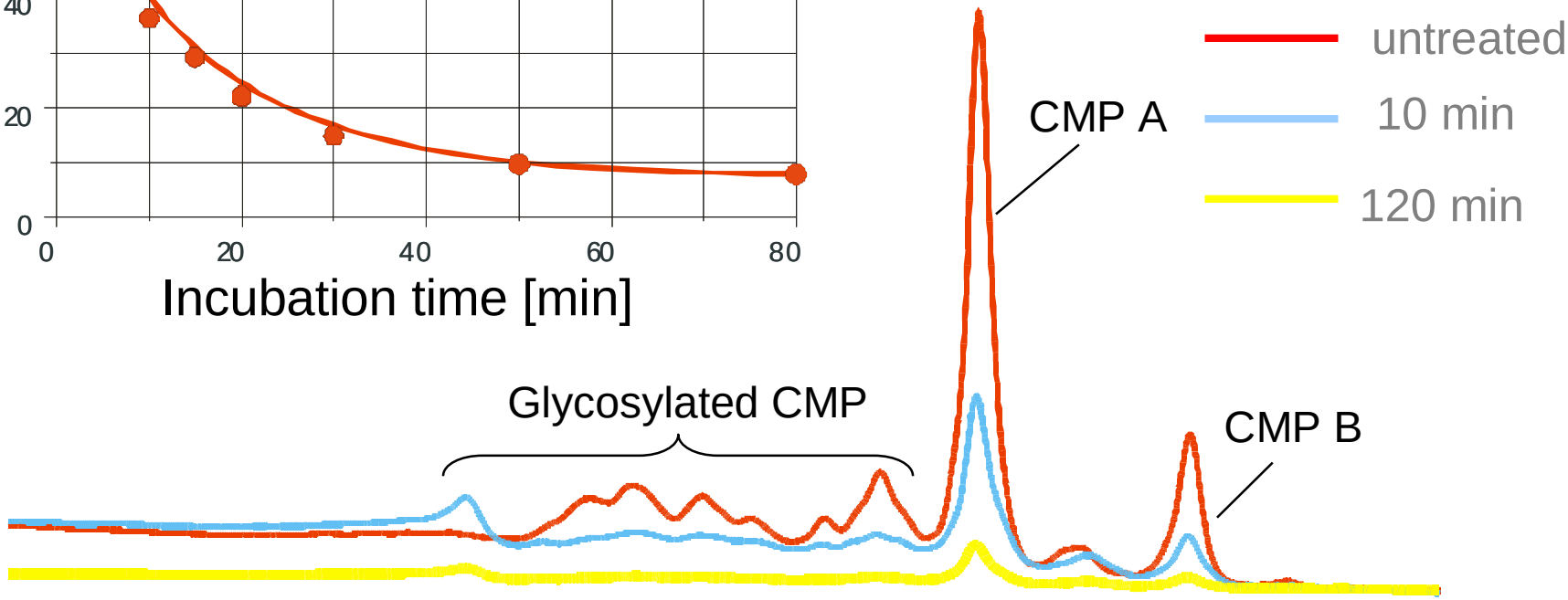


Process to obtain CMP depleted rennet whey

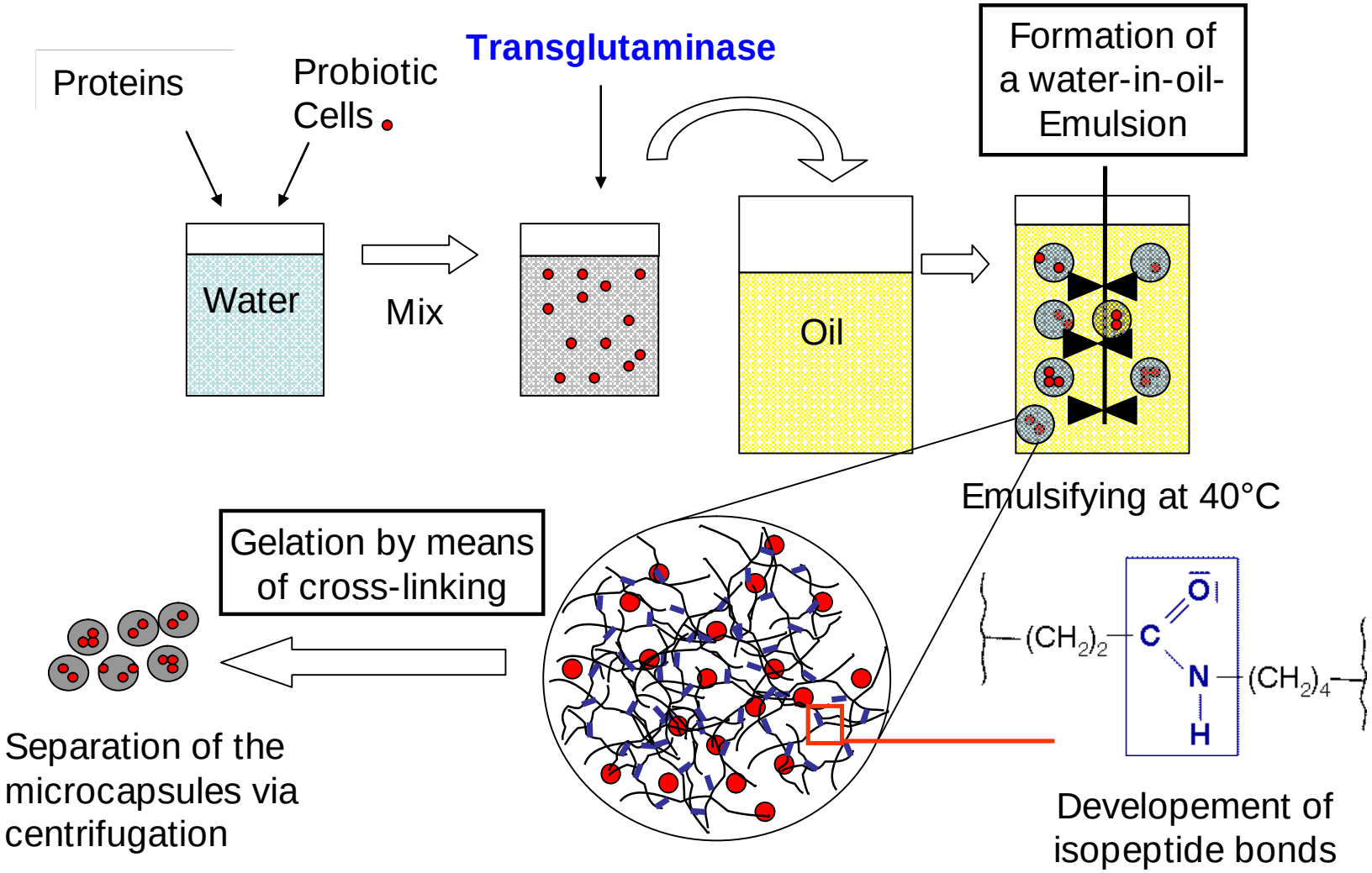


Process to obtain CMP depleted rennet whey by means of enzymatic crosslinking (Transglutaminase)

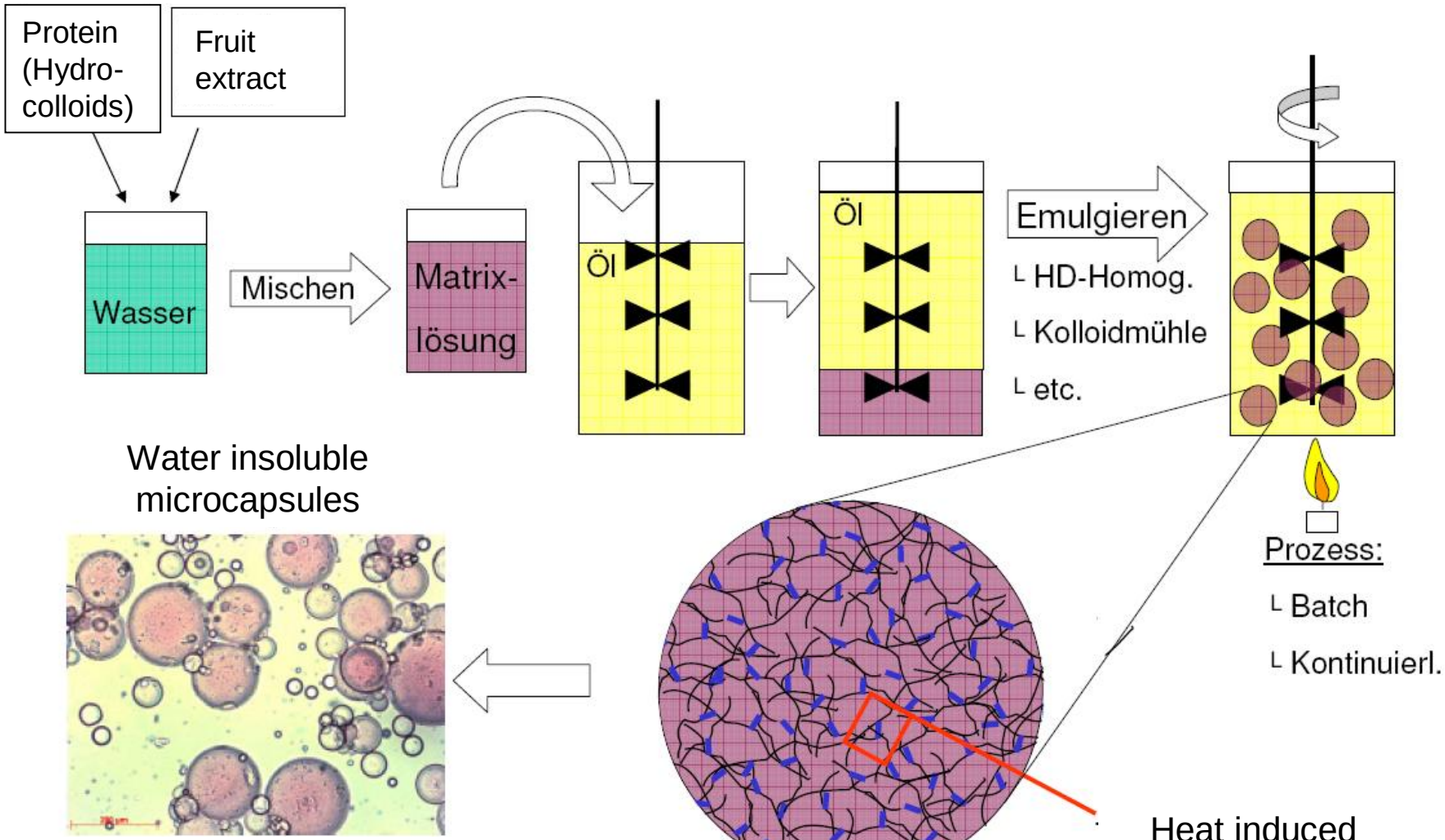




Microcapsulation of sensitive components using casein or whey protein as matrix material



Microcapsulation of sensitive components using whey proteins as matrix material



Water insoluble microcapsules

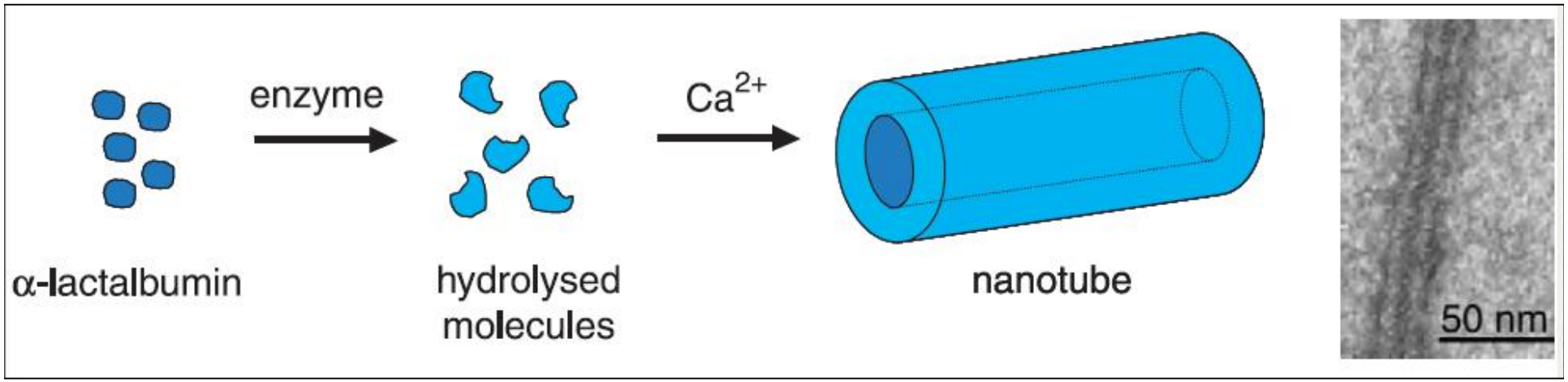
Size impact on sensory and protective effects

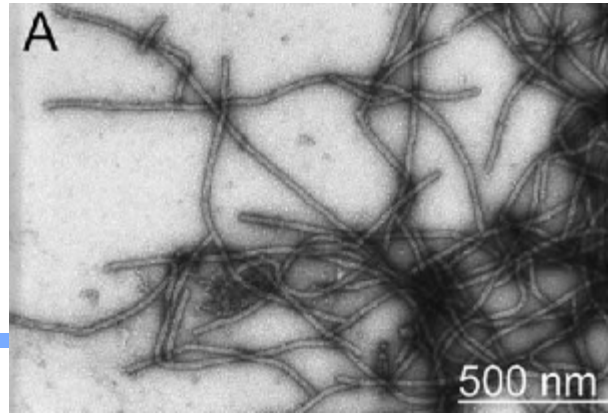
Heat induced gel network

Novel whey protein based nanotubes made from a-lactalbumin

(Left) Schematic presentation of the self-assembly of partially hydrolysed a-lactalbumin into nanotubes in presence of Ca^{2+}.

(Right) Transmission electron micrograph of negatively stained a-lactalbumin nanotubes (negative staining was performed with 3% uranyl acetate for 1 min)





J.F. Graveland-Bikker, C.G. de Kruif
Trends in Food Science Technology 17 (2006)

Approach for production of bioactive peptides

Precursor protein selection
- Single vs. mixed
- Pre-treated or native
- Glyco-conjugated

⇒ **Production** Ü

Enzyme selection:
- single or mixed enzymes
- enzyme cascades

↓

Purification/ Fractionation Ü

Desalting, MAC, HPLC, GPC, UF, NF membranes

↓

Analysis Ü

Elucidate structure-function relation-ships, use of quantitative structure-activity relationships (QSAR)

Techno-functional/ sensory properties
- Interfacial activity,
- Network formation
- Fat-holding
- Bitterness

Bioactivity
- In vitro or In vivo
- Cell cultures
- Toxicological tests
- Human nutrition group

Structure analysis
- MALDI-TOF-MS, ESI
- Peptide synthesis, etc.

Structure-Function-Relationships (?)

TUM campus at Freising-Weihenstephan

Bioactive components in Milk - Target compounds

- Proteins
  - Whey proteins
    - + α-Lactalbumin, ß-Lactoglobulin
    - + Peptides
  - Casein fractions
    - + Peptides (Hormone activity, mineral binding capacity, immune system modulation, etc.)
    - + Glycomacropeptide
- Fat globule membrane components
  - Phospholipoproteins
  - Sphingomyelins
- Minor Components
  - Minerals (Calcium)
  - Lactoferrin
  - Prolactin
  - Lactulose
  - Oligosaccharides

Motivation

- Physiological effects
- Therapeutic effects
- Technological functions

} „Functional“ Products

Ways to produce bioactive peptides

Protein

↓

Release of peptides as fragments of the protein

↓

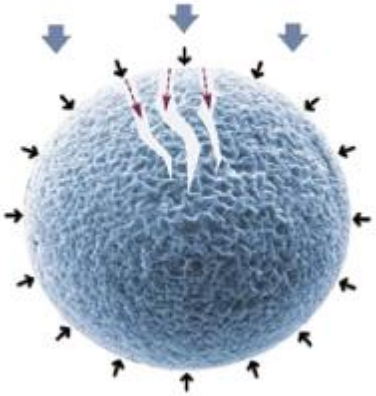
Peptide mix

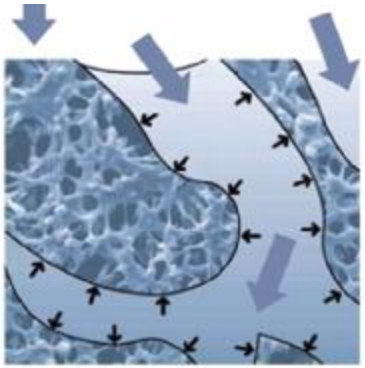
- Hydrolysis by digestive enzymes
- Fermentation by means of proteolytic microbial cultures
  Lactic acid bacteria (*L. lactis; L. helveticus; L.delbrueckii*)
- Proteolysis by means of isolated microbial or plant derived enzymes

à Fractionation and concentration

Solid phase alternatives

Conventional gels ⟷ Membrane Adsorption Chromatography





(Lab scale units)

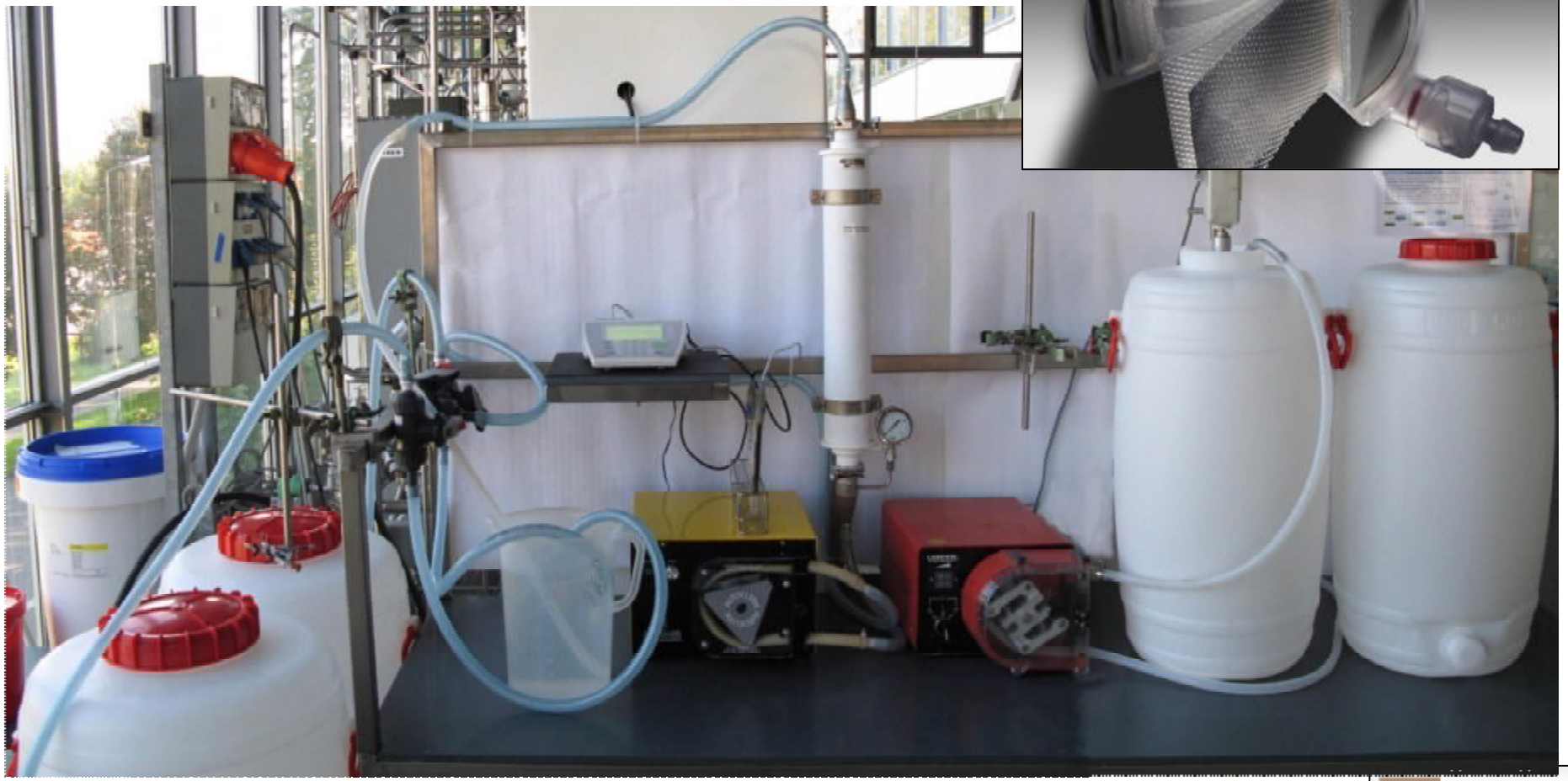
- Polymer beads
- Diffusion is rate limiting
- High backpressure

- Cellulosic membrane
- Convective flow of target component to adsorber surface
- Low backpressure

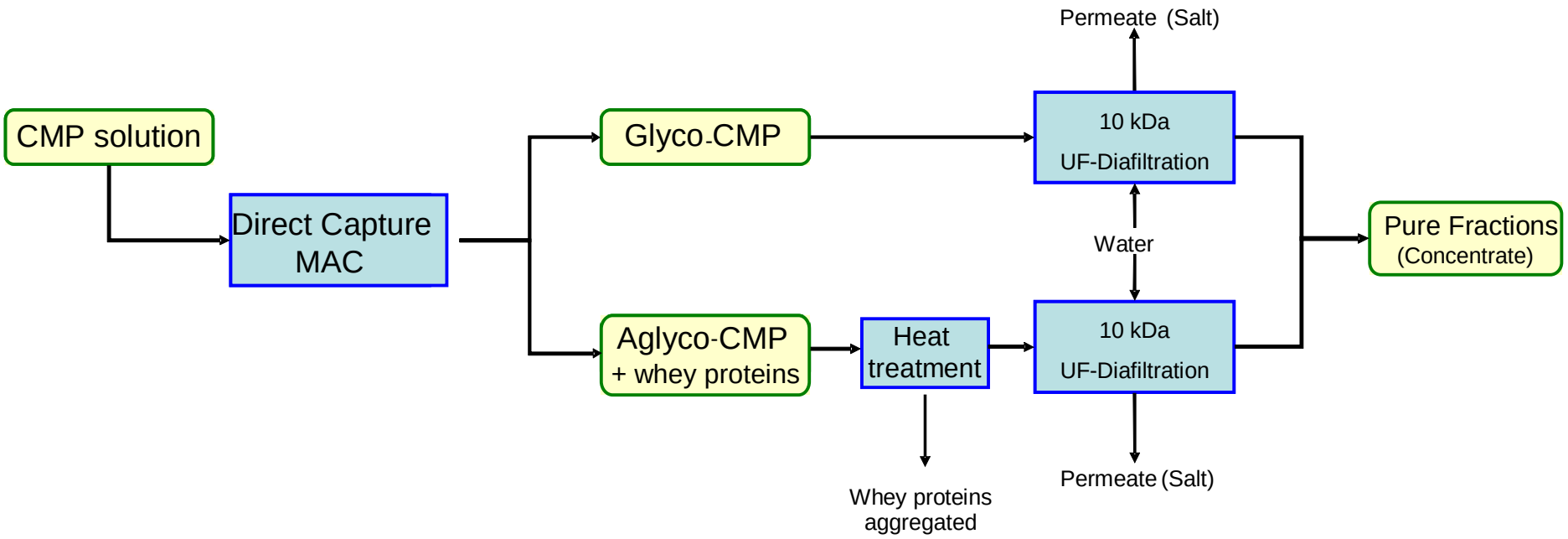
MAC pilot plant unit

Adsorber hold-up volume: 1000 mL

Flow rate: 8 L/min



MAC process based on CMP solution



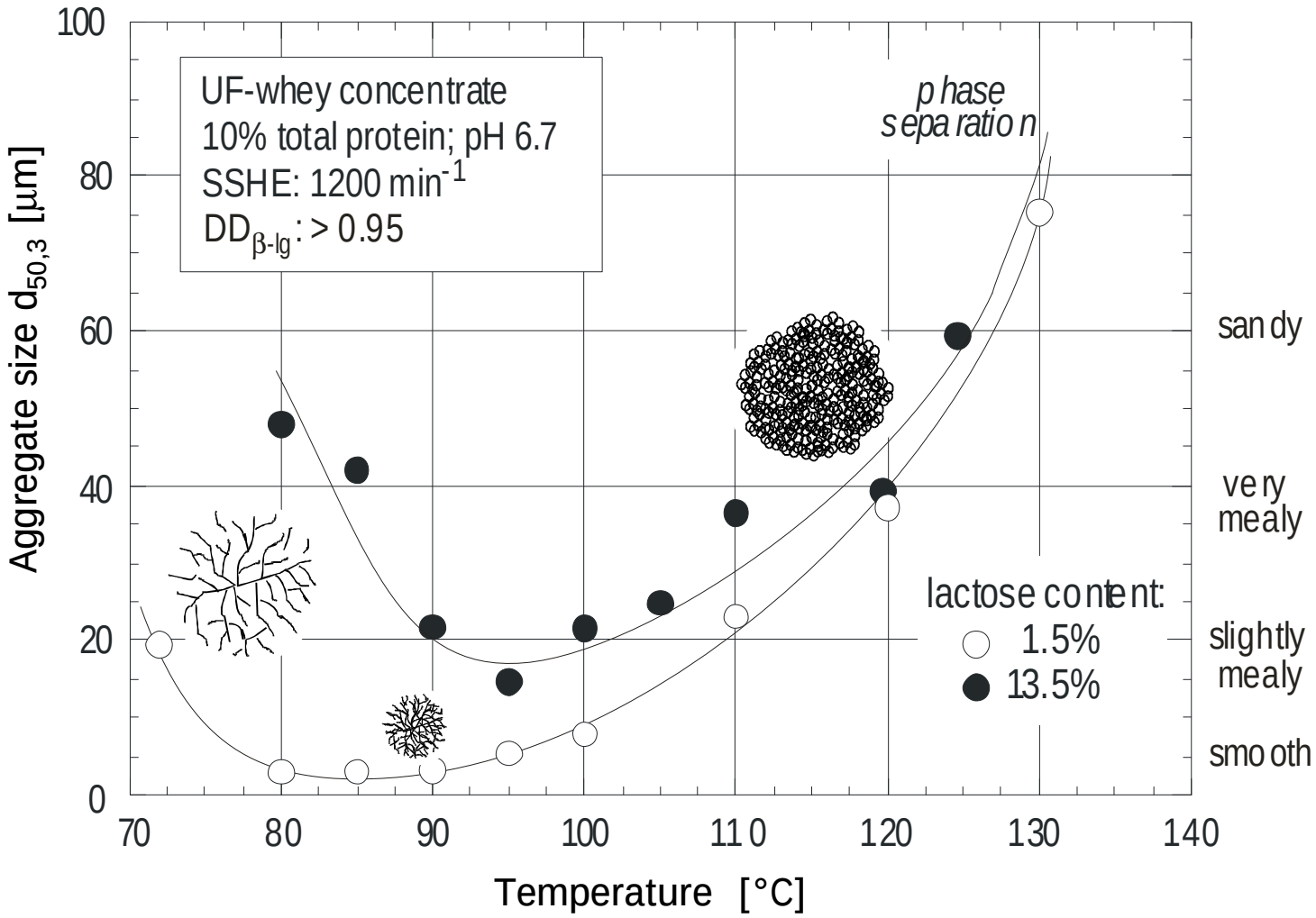
<u>Key data</u>

- Purity eluate: 97 %
- Flow rate: 8 L/min
- Capacity: 10,2 g glyco-CMP/cycle = 0,28 mg/cm²
- No capacity loss after 20 cycles observed

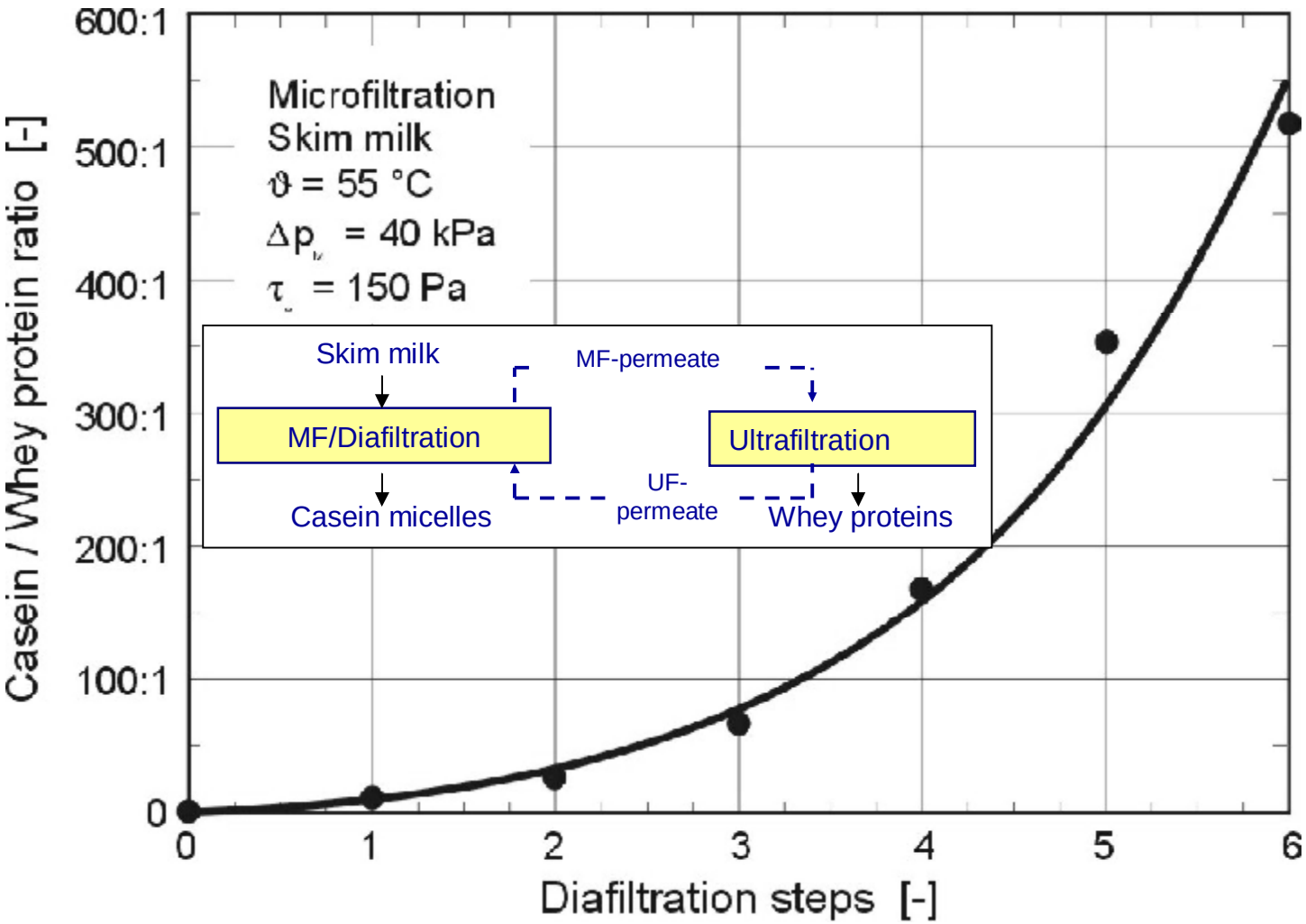
IEC – Analytical method development

- Selection of solid phase
  - Strong, weak exchangers (Q, DEAE)
  - conventional bead gels, membrane adsorbers
- Selection of liquid buffer
  - Citrate, Acetate, Methyl-Piperazin
  - Molarity of buffer (0,01 M to 1 M)
- pH
  - Variation depending on buffer between pH 3 and pH 6
- Dual buffer gradient
- Flow rate
- Protein concentration

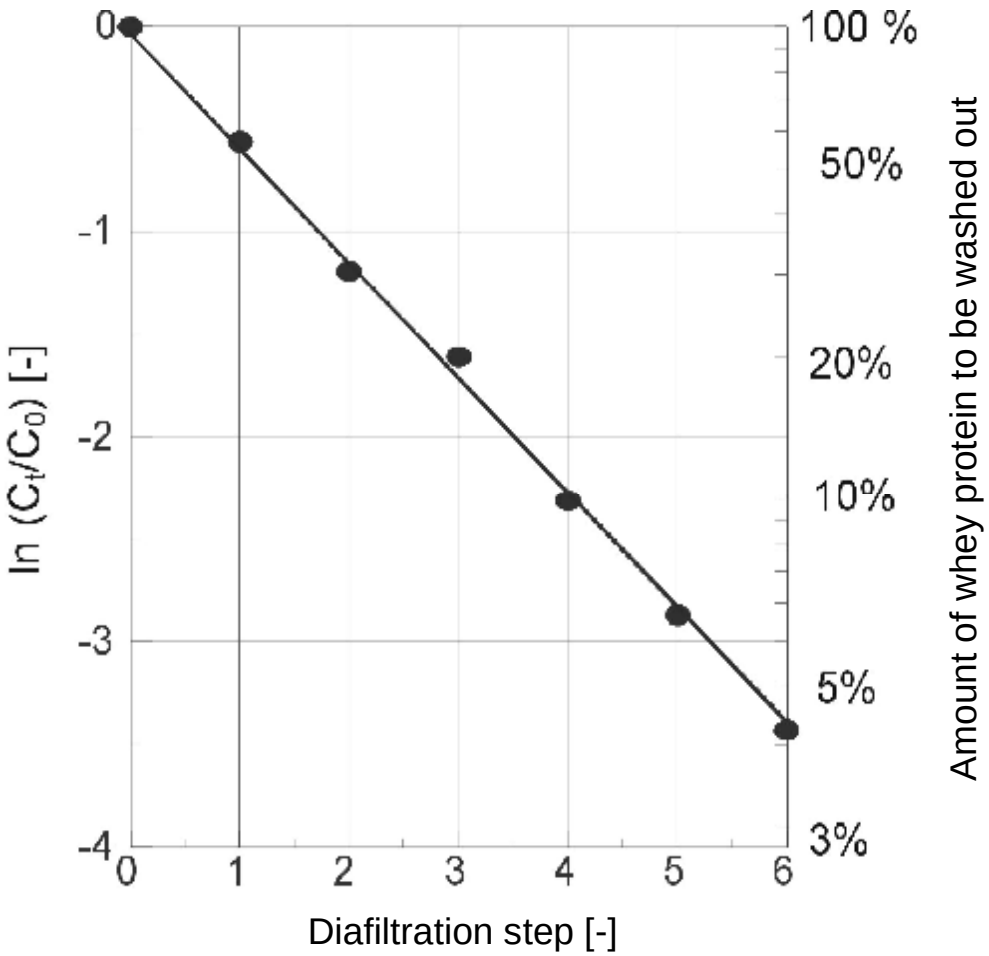
Whey protein micro-aggregate size under various thermal and compositional conditions



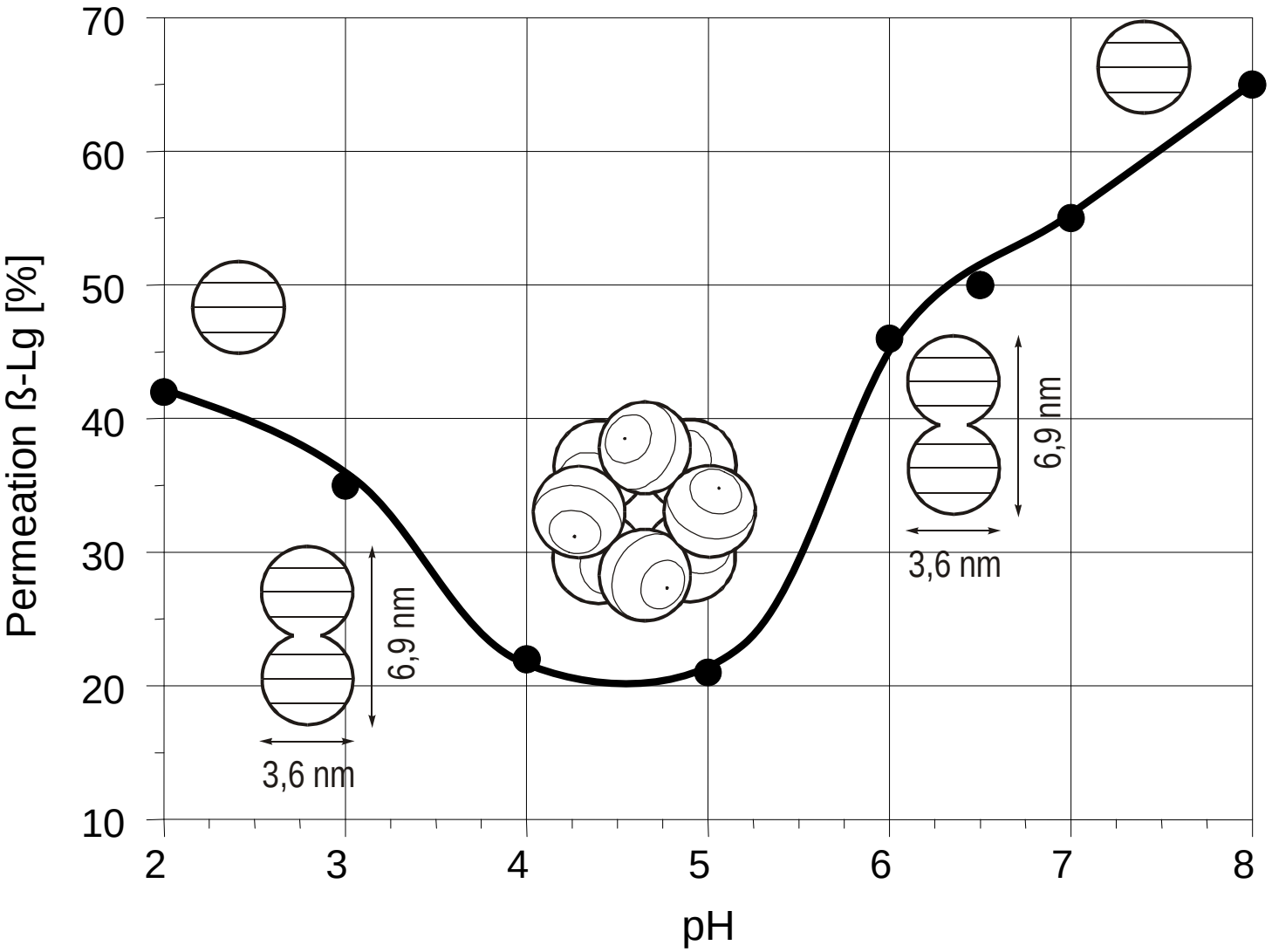
Milk protein fractionation by means of microfiltration (linear scale)



Milk protein fractionation by means of microfiltration (log scale)

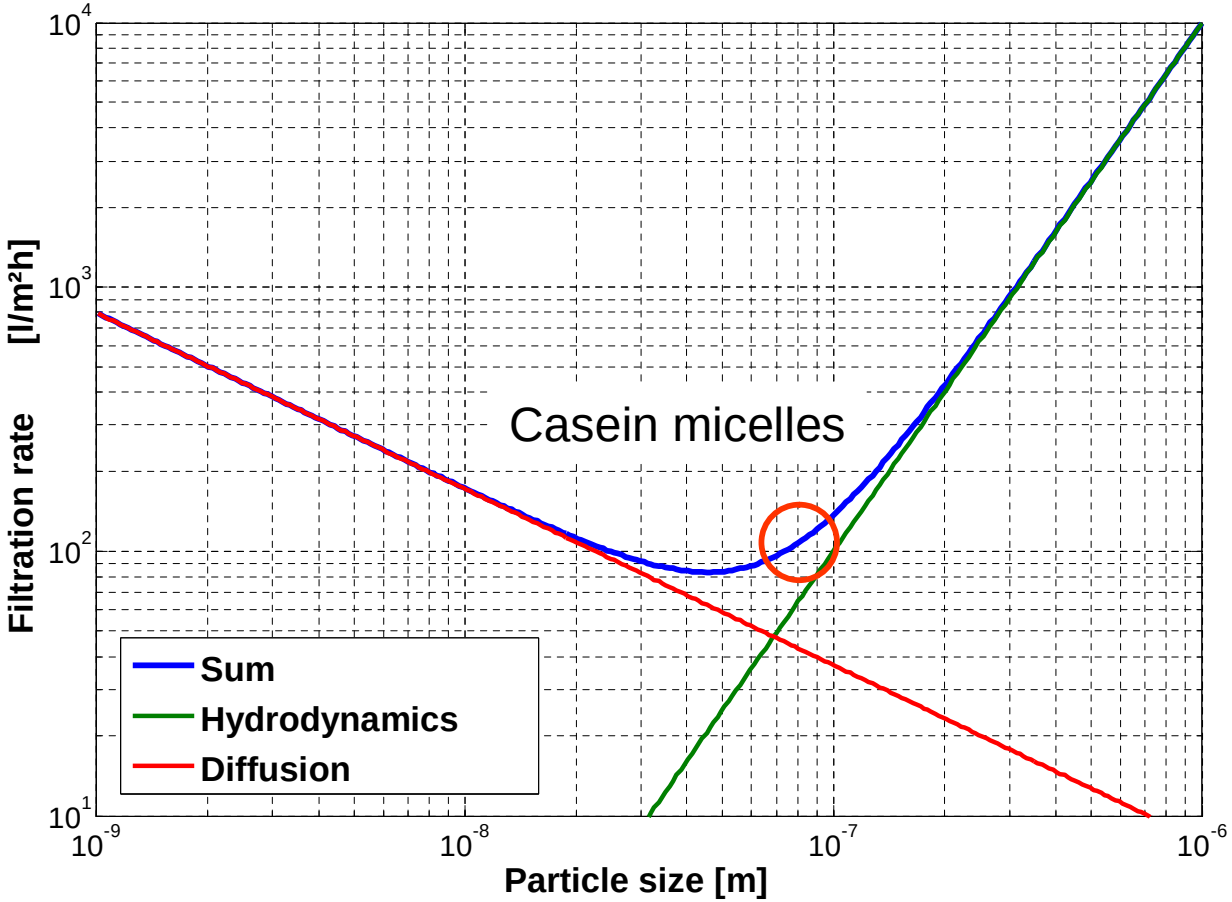


Influence of pH on size and permeation of ß-Lg



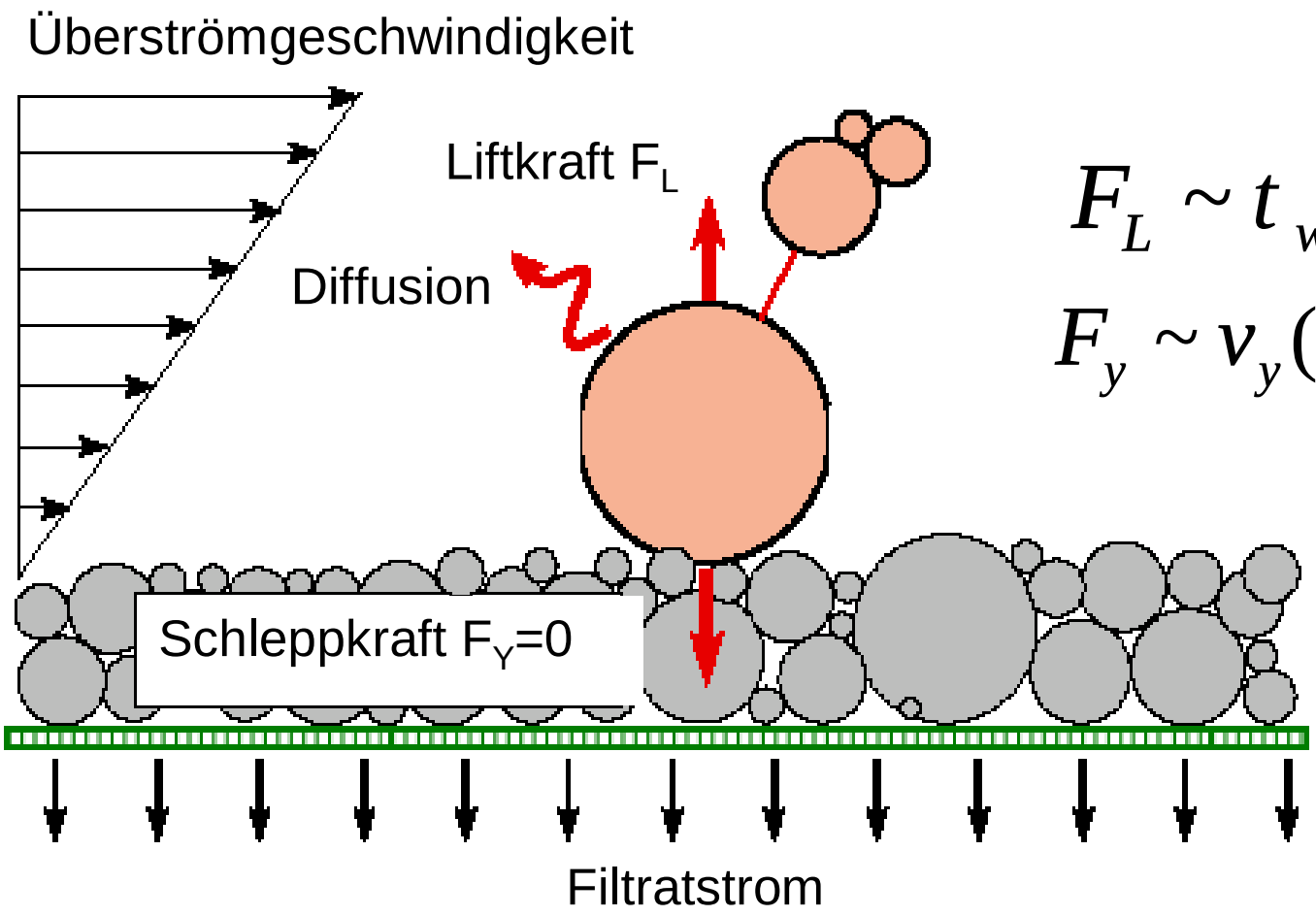
Critical filtration rate as a function of particle size forming membrane deposits

$$w_{krit} = w_{Diff} + w_{hydro}$$



Grein; MVT Kaiserslautern 2006

Deckschichtbildung: Grundlagen



$$F_L \sim t_w^{1,5}$$

$$F_y \sim v_y(TMP)$$

Altmann 2000