# *Mechanisms of Coagulation:*

## *The principles, the science and what they mean to cheesemakers*

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# *What are casein micelles?*
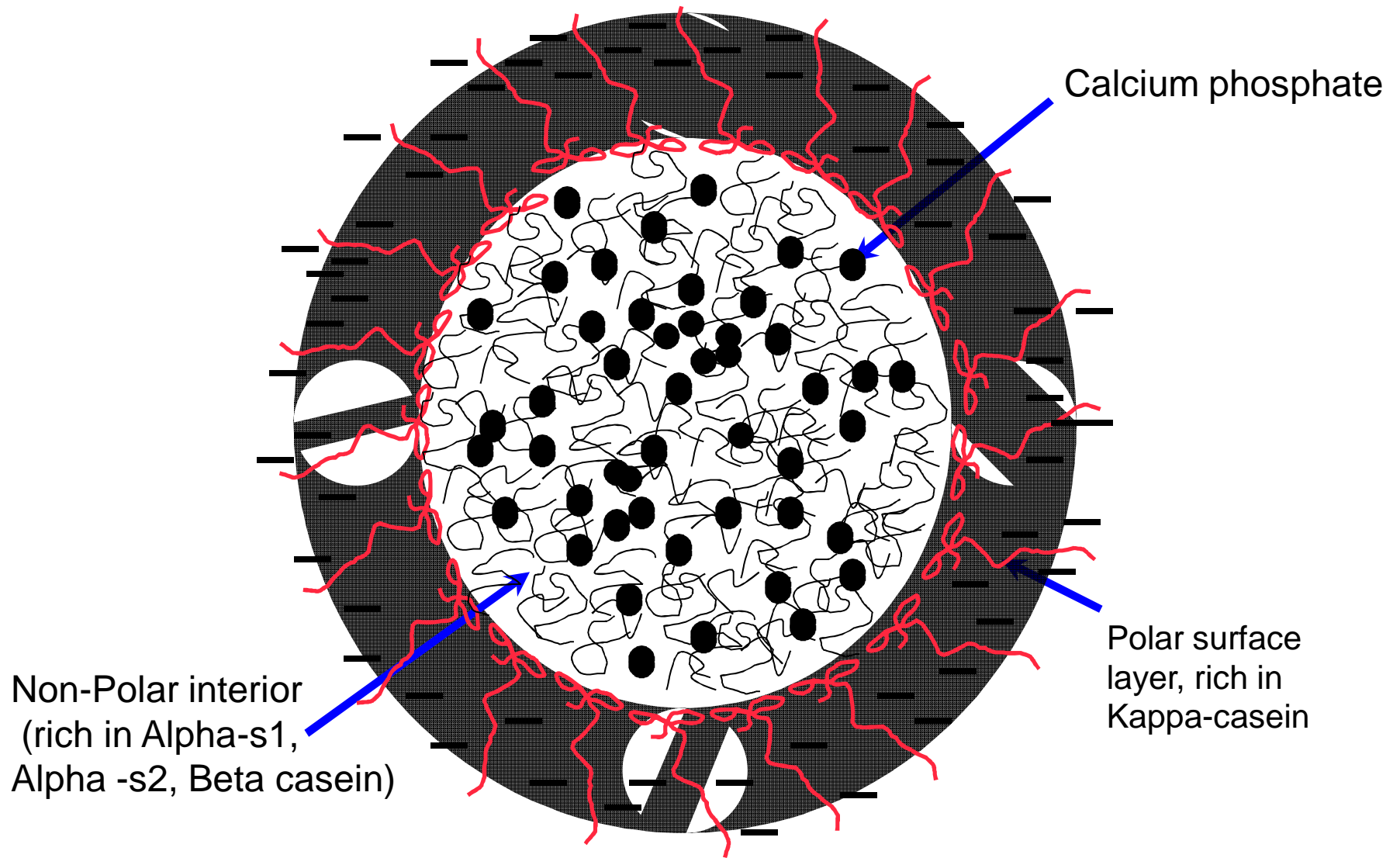


Calcium phosphate

Polar surface layer, rich in Kappa-casein

Non-Polar interior (rich in Alpha-s1, Alpha -s2, Beta casein)
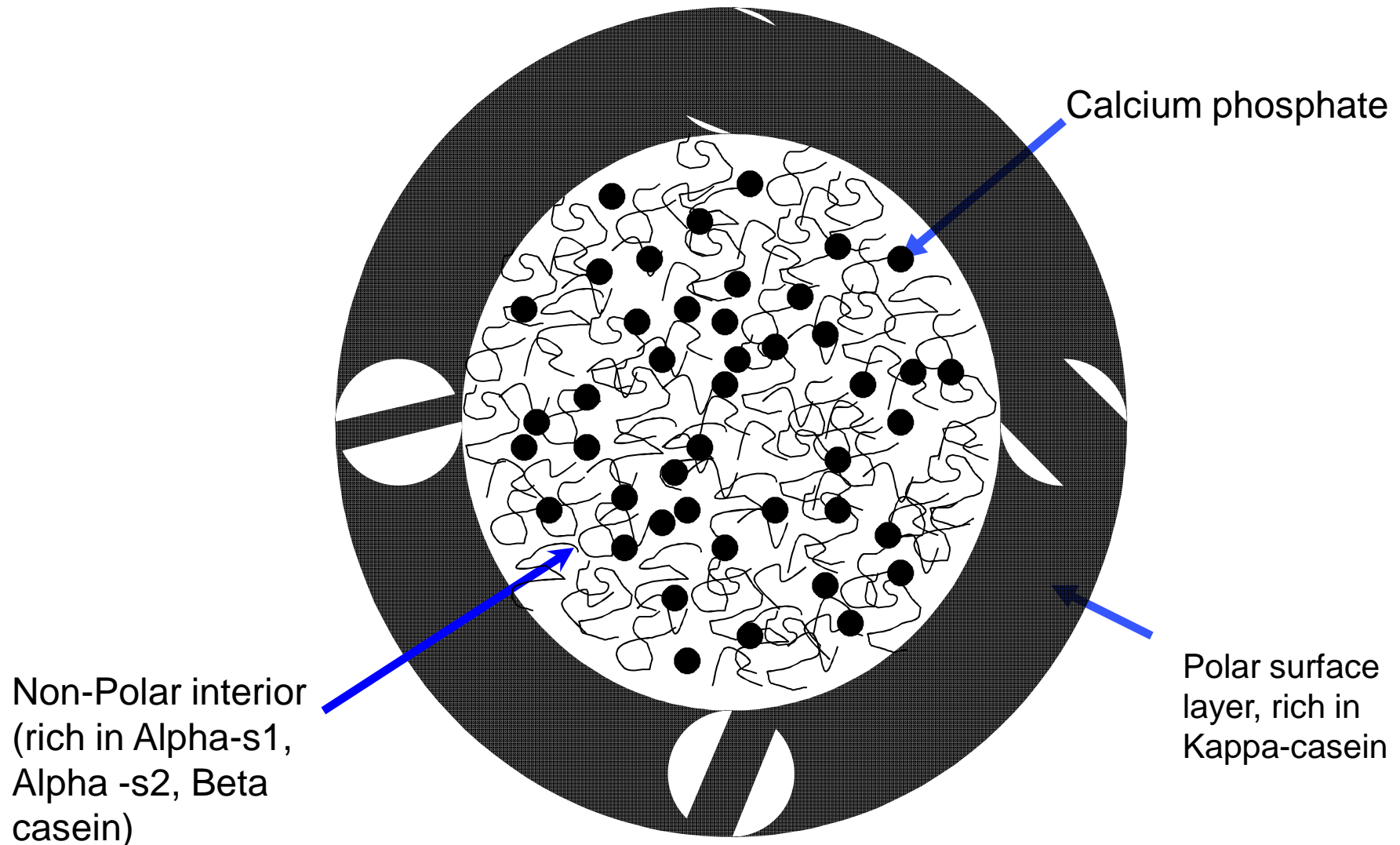
*Adapted from: Horn, D. 1998. International Dairy J. 8:171-177*

# *Kappa casein*

nonpolar

polar

AA 169

# *What are casein micelles?*



Calcium phosphate

Non-Polar interior
(rich in Alpha-s1,
Alpha -s2, Beta casein)

Polar surface
layer, rich in
Kappa-casein
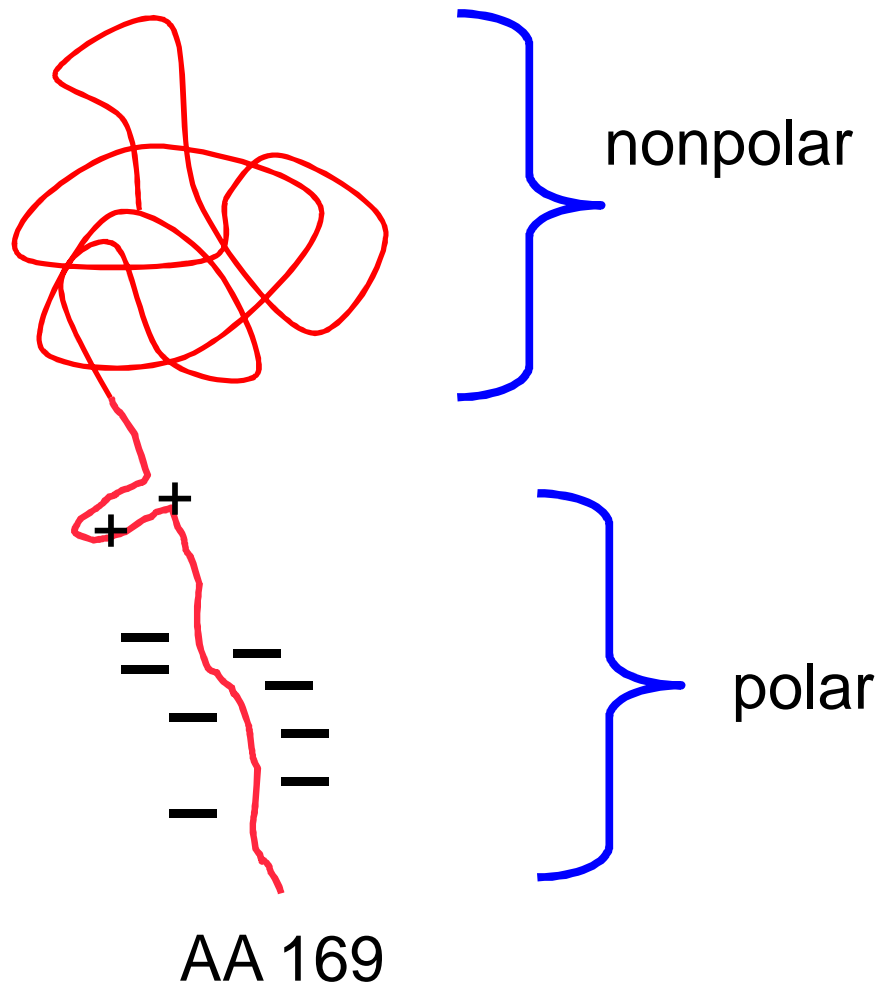
*Adapted from: Horn, D. 1998. International Dairy J. 8:171-177*

# *Mechanisms of coagulation*

˝ Rennet (rapid: 30 - 60 min)

˝ Acid (slow: 5 - 24 hr)

˝ Acid-rennet (slow: 12 -24 hr)

˝ Acid-heat

# *What is rennet?*

- ˝ General term for enzymes used to coagulate milk
- ˝ Technically restricted to enzymes derived from ruminant stomachs
- ˝ All are protein degrading enzymes (proteinase, protease, proteolytic enzyme)
- ˝ All are members of the aspartic proteinase family

# All aspartic proteinases have 2 characteristics in common
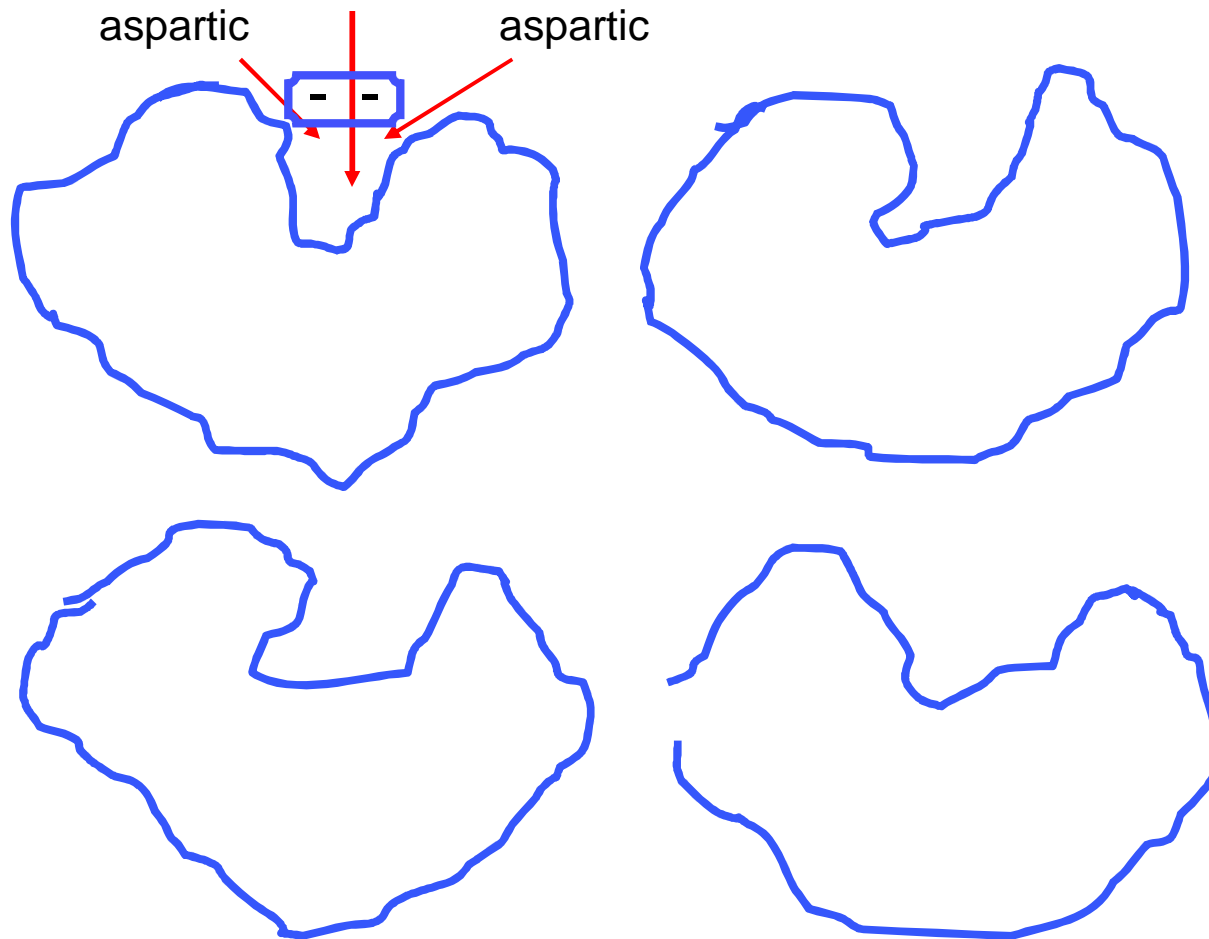


aspartic     aspartic

Source: *after* Crabbe, M.J.C. 2004. Rennets: General and molecular aspects *In* Cheese: Chemistry, Physics and Microbiology, Elsevier Academic Press, London

# *Kappa casein is uniquely vulnerable to the action of aspartic proteases*
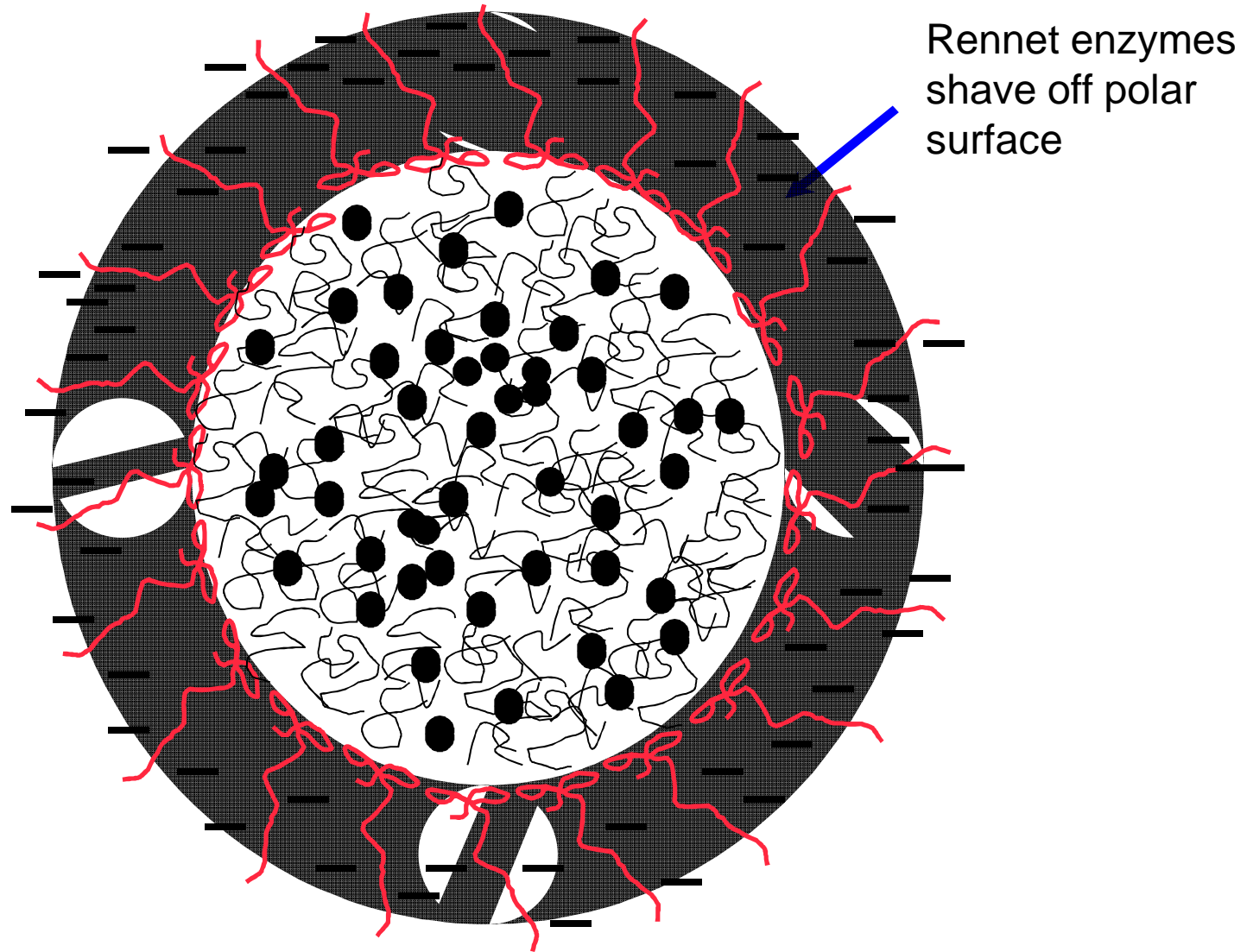
nonpolar

AA 105-106
(very vulnerable
to aspartic
proteinases)

polar

AA 169
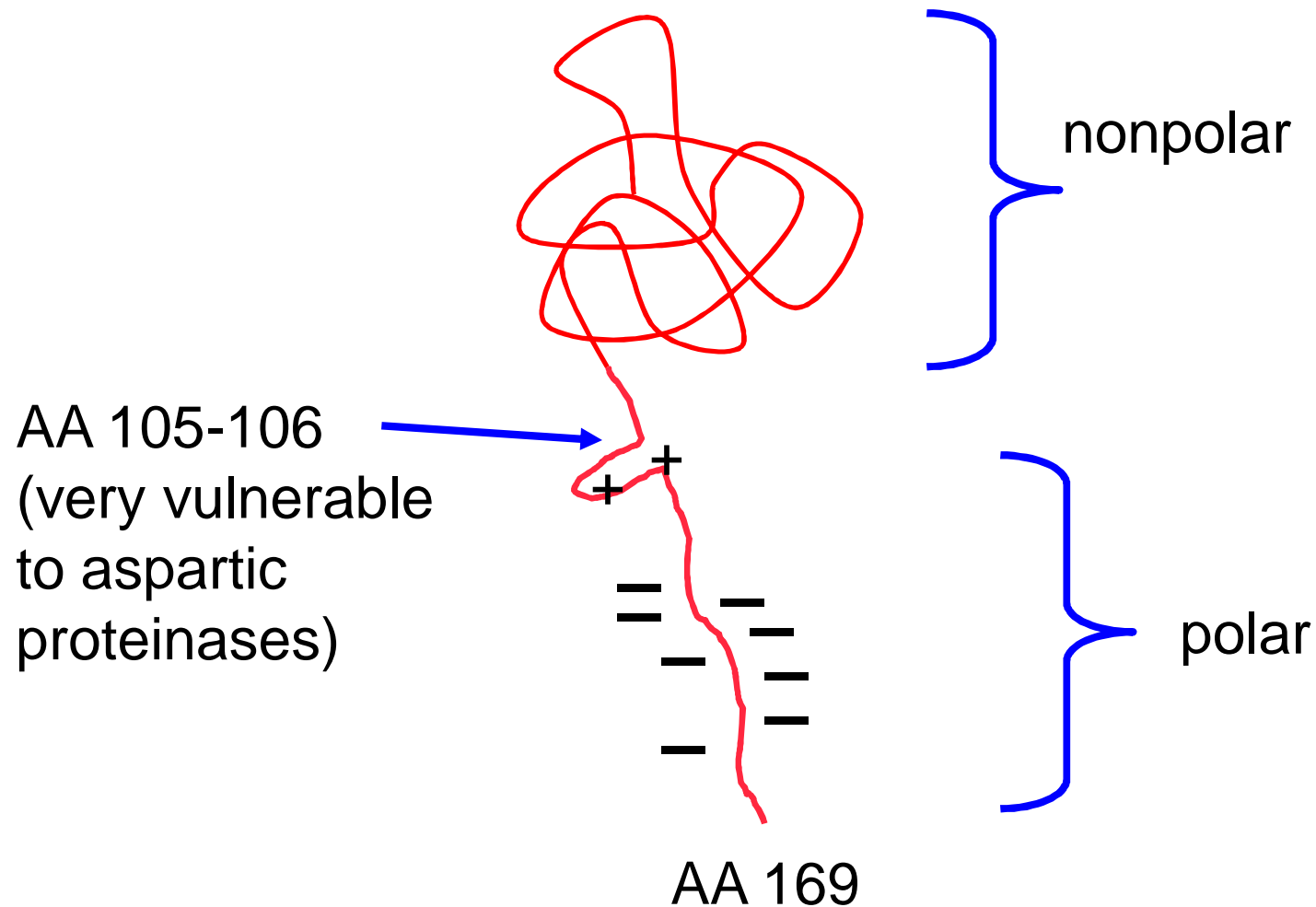
# *Rennet cleavage of kappa casein*

Kappa casein

chymosin, etc.
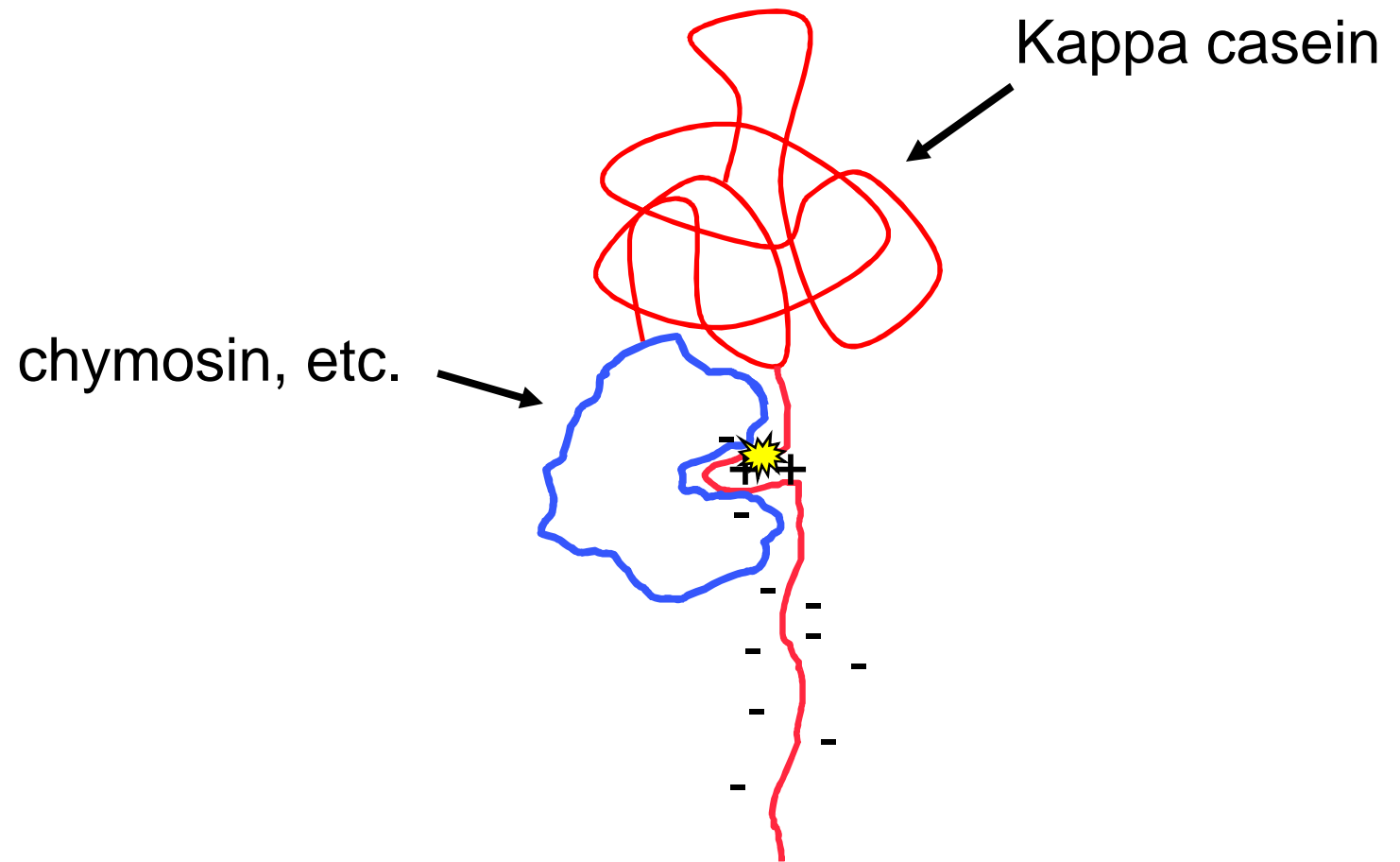
# *Rennet coagulation occurs in two phases*

1.  Enzymatic
2.  Non-enzymatic

# 1. Enzymatic phase:
## cleavage of  -casein

Rennet enzymes
shave off polar
surface

*Adapted from: Horn, D. 1998. International Dairy J. 8:171-177*

# 1. Enzymatic phase:
## cleavage of  -casein



casein micelle

Kappa-Casein with
exposed polar region
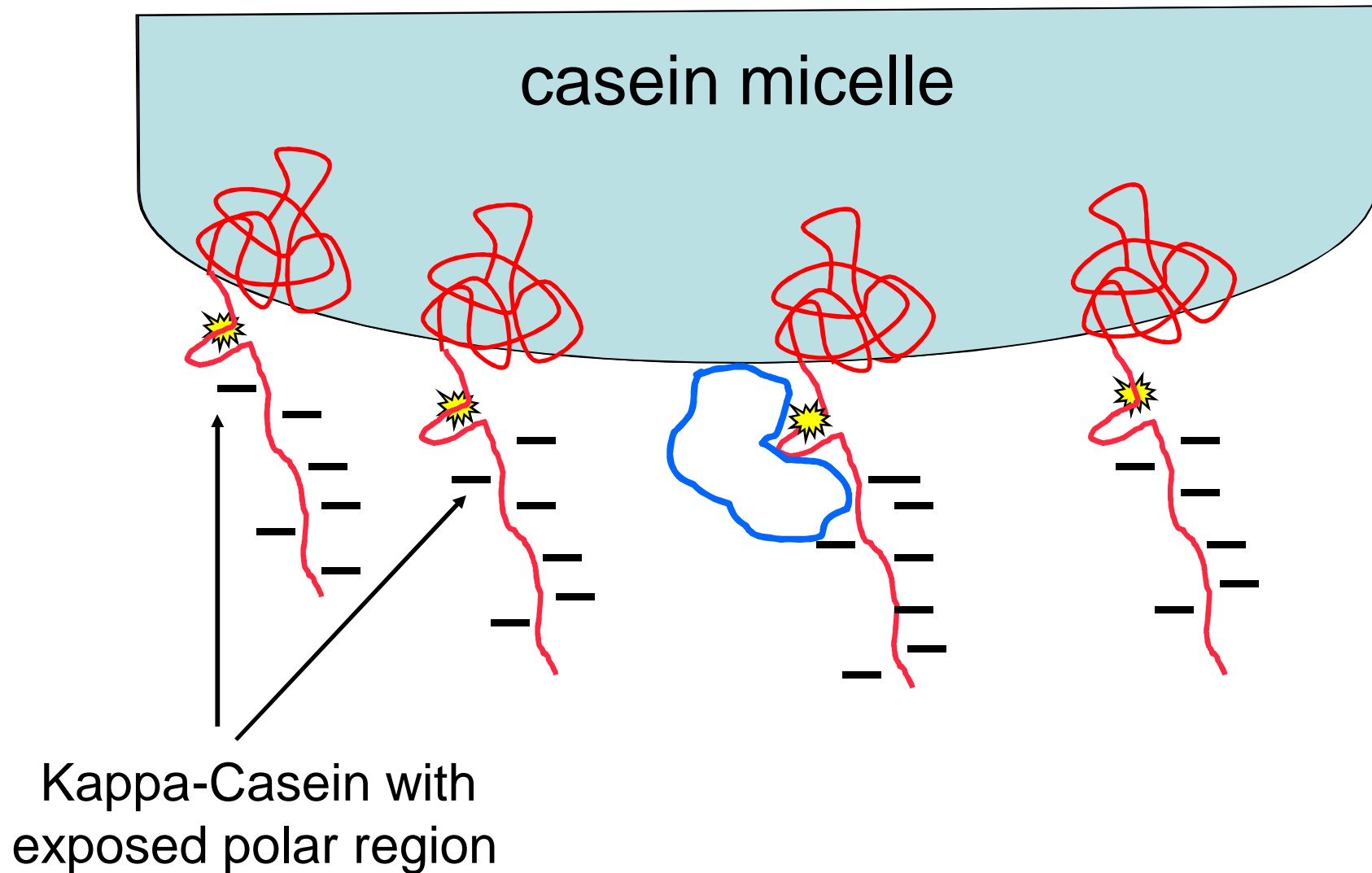
# 2. *Nonenzymatic phase:*
## *Ca++ induced aggregation*



casein micelle

Ca++

Ca++

Ca++

Ca++

*VERY* Nonpolar surface
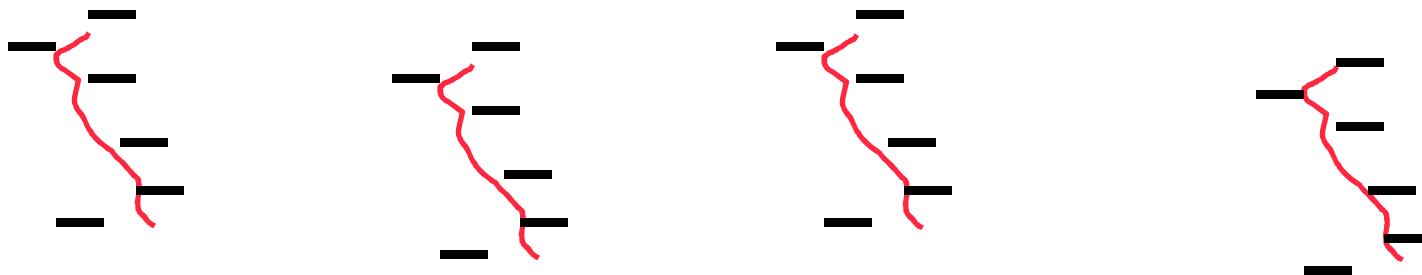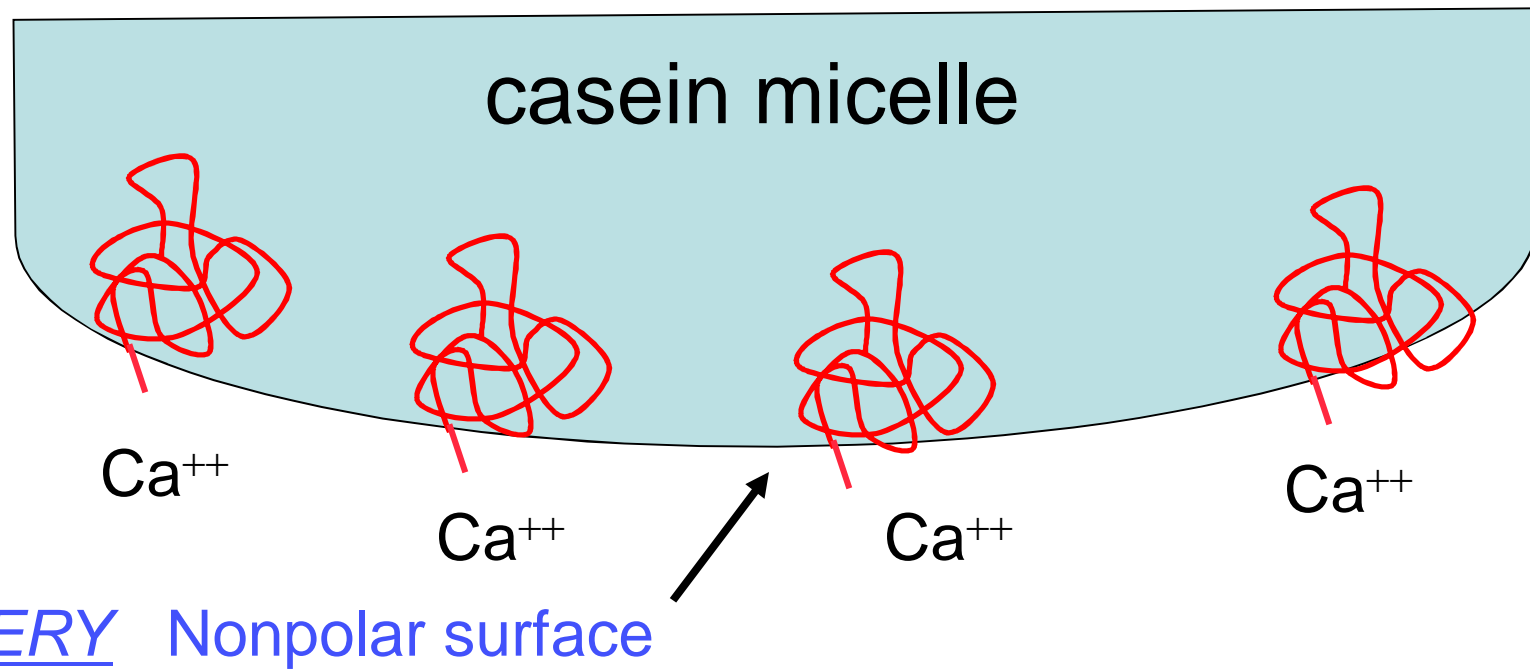
CMP/GMP (AA106-169)

# 2. Nonenzymatic phase:
## aggregation of casein micelles



Ca$^+$

$+$

Nonpolar micelles

Permanent bonding
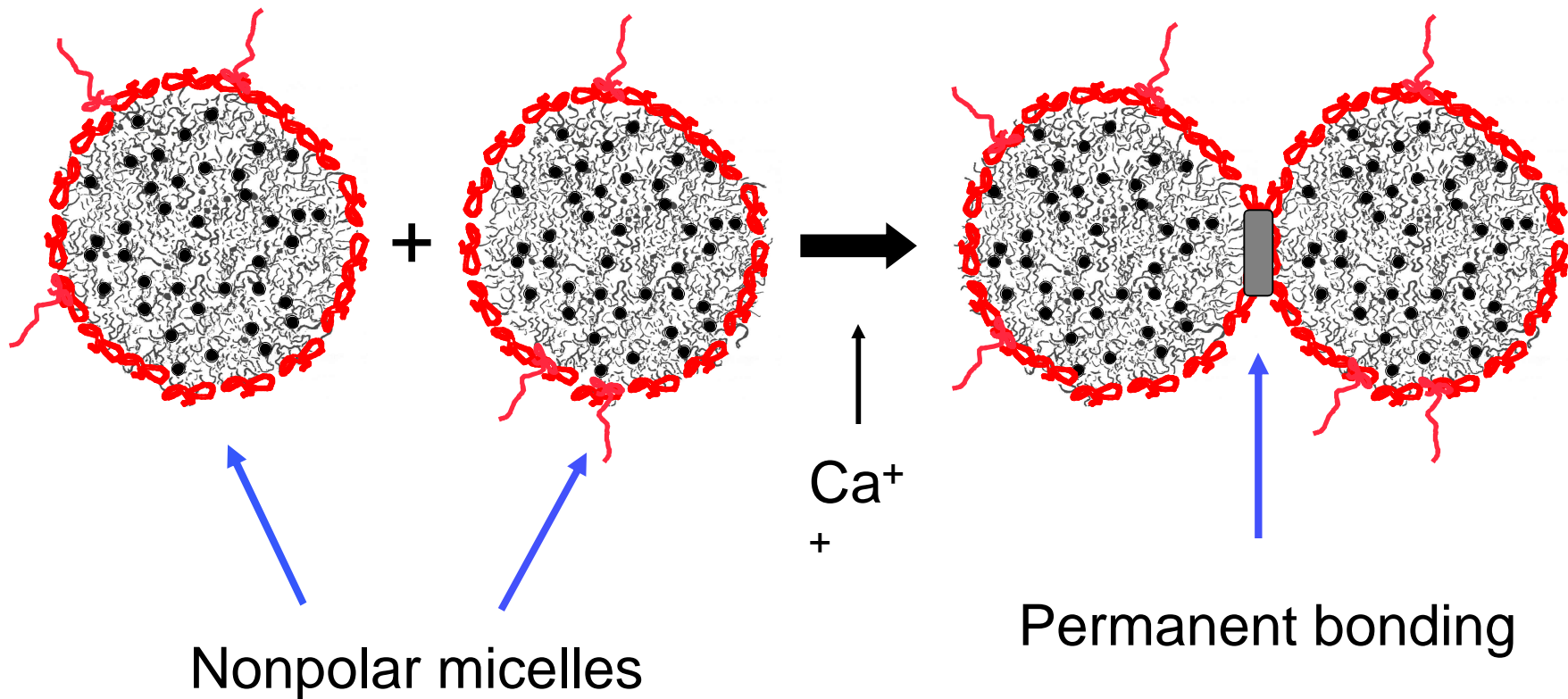
*(commences after 80-90% cleavage of $\kappa$-casein)*

# 2. Nonenzymatic phase continued:
## chain formation/flocculation
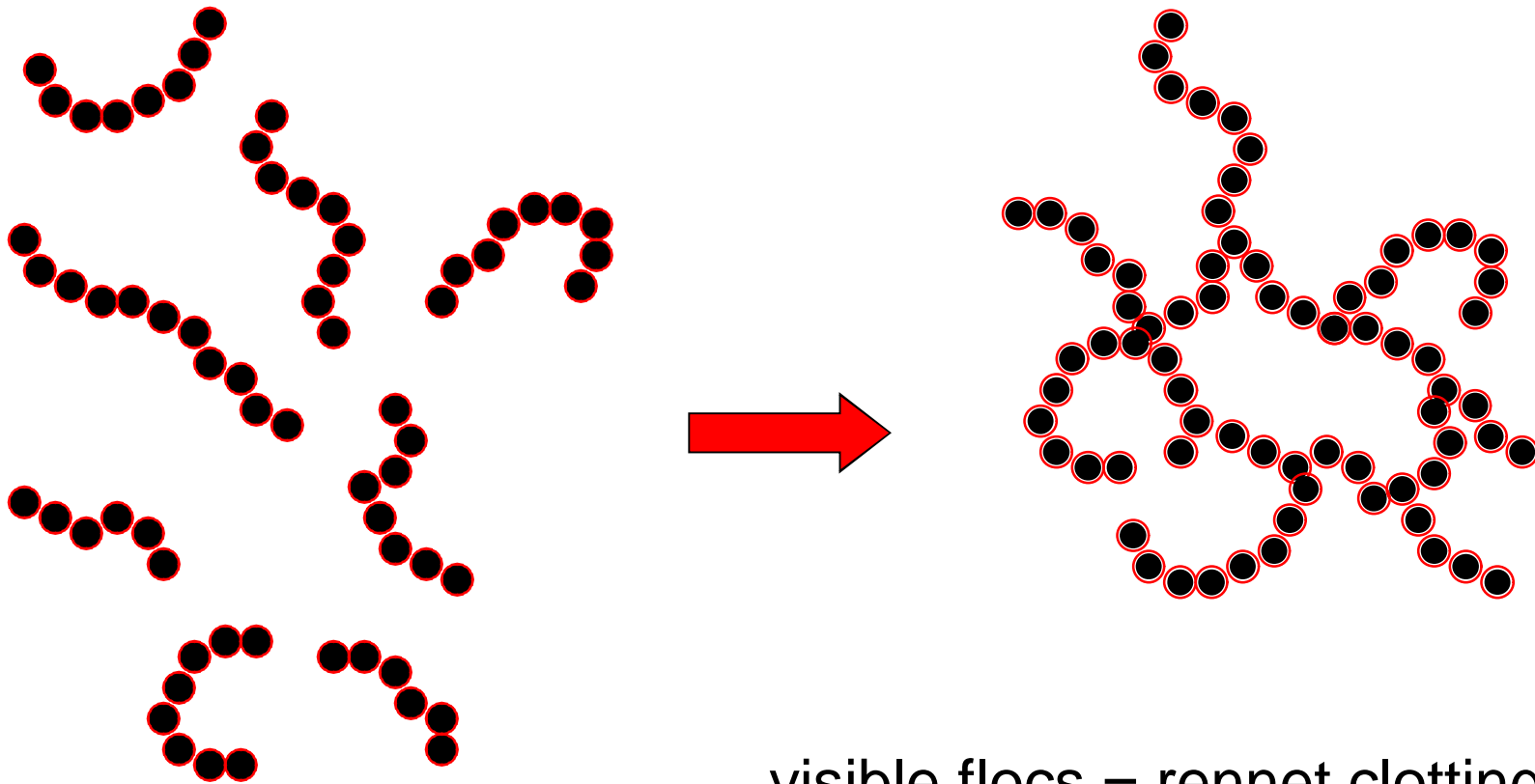


visible flocs = rennet clotting time
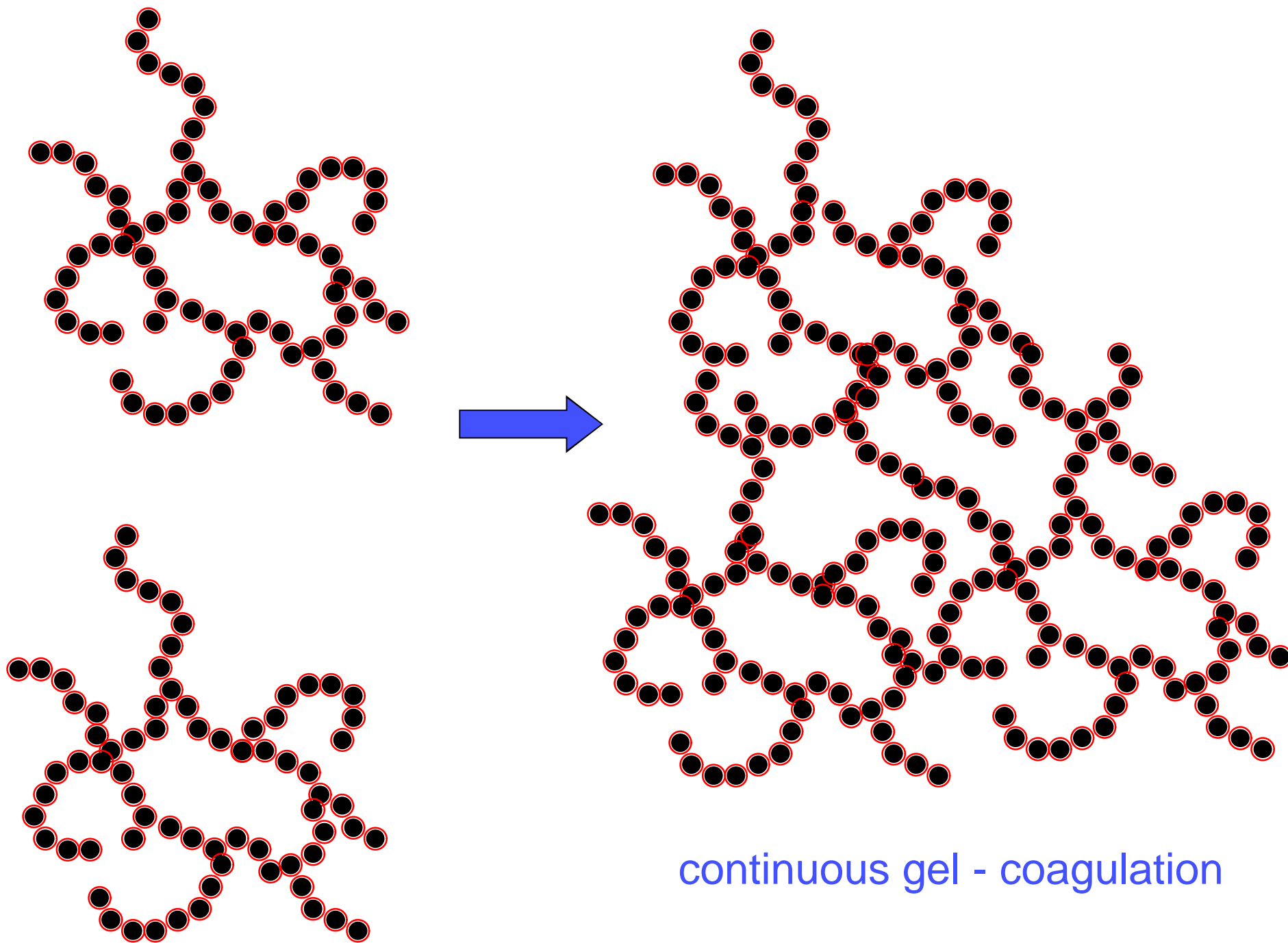
continuous gel - coagulation

# *Matrix rearrangement*

Fine matrix - small
pores,weak gel
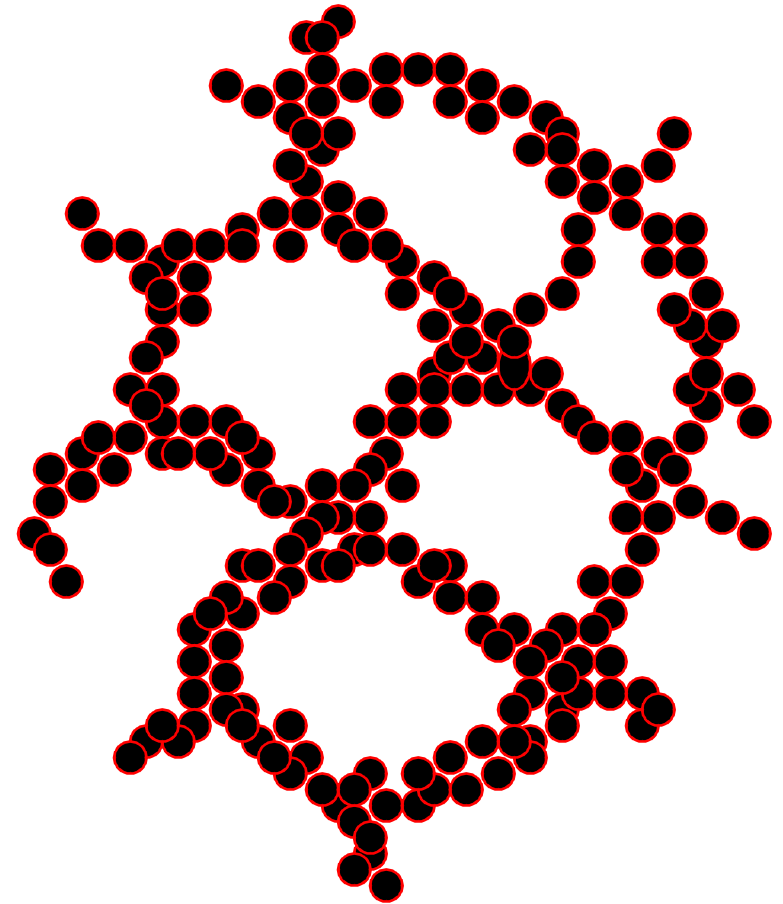
coarse matrix - large
pores, firm gel

# *Repercussions of matrix rearrangement*

˝ Cheese moisture content
˝ Acid development during cheese making
˝ Cheese yield

# *Matrix rearrangement*
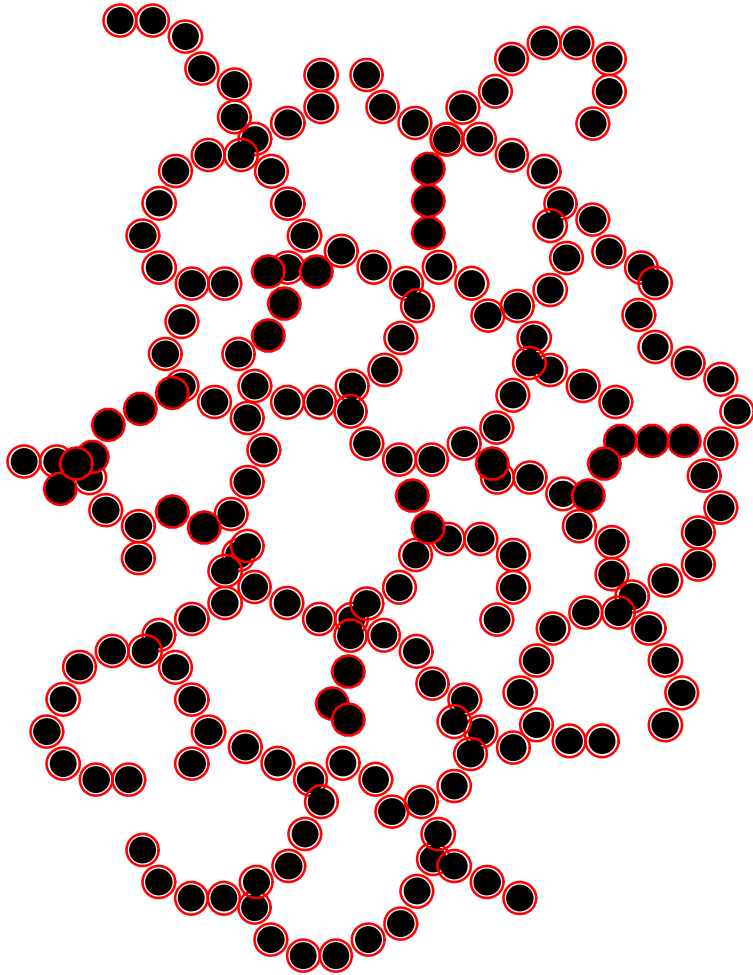


Fine matrix - small pores, weak gel
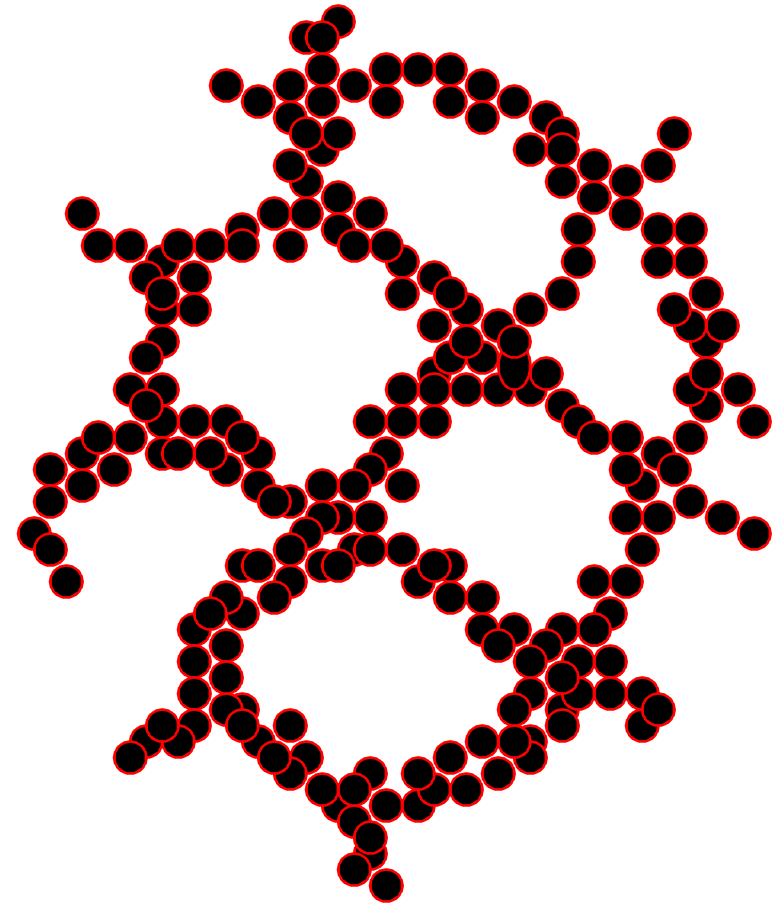
Coarse matrix contracts, syneresis pressure ↑

# *Repercussions of matrix rearrangement*
## *1. Cheese moisture content*

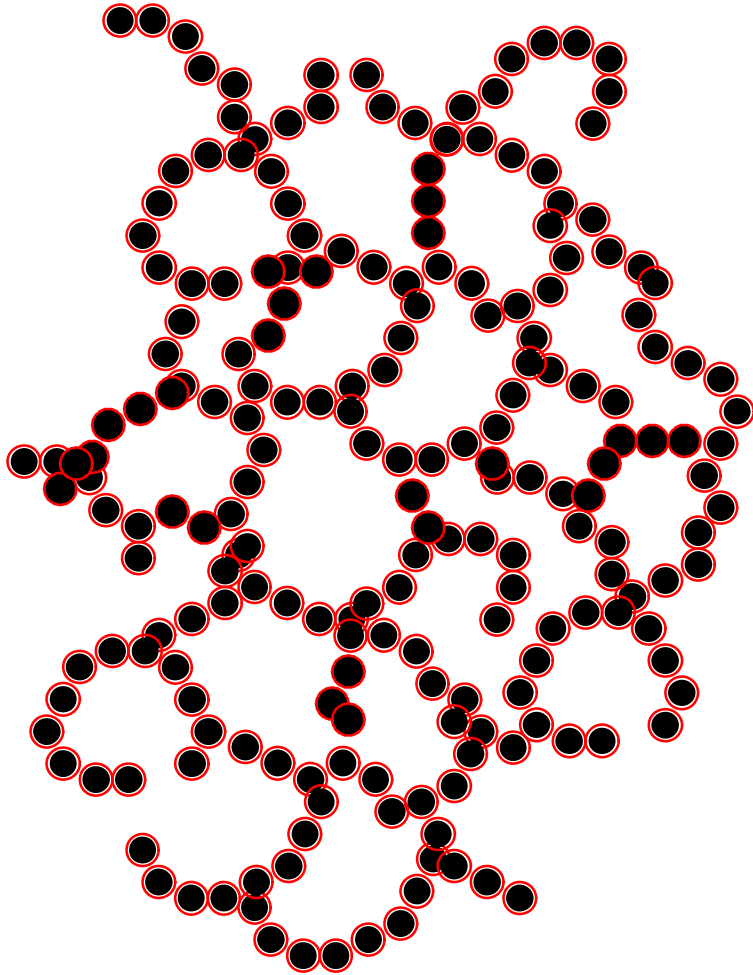˝ Cutting early (weak set) enables much rearrangement to occur after cutting; syneresis ↑, cheese moisture content ↓

   - *ex: alpine cheeses*

˝ Cutting late (firm set) limits rearrangement after cutting; syneresis ↓, cheese moisture content ↑

   - *ex: traditional Brie, Camembert*

É Cutting firmness should be consistent from vat-to-vat to reduce moisture variation

# *Repercussions of matrix rearrangement 2. Acid development*

˝ If the amount of time to the desired cutting firmness varies greatly from day-to-day, the subsequent rate of acidification during the rest of cheese making may be affected:

- . Extended cutting time, ↑ starter culture population, ↑ rate of acidification
- . Reduced cutting time, ↓ starter culture population, ↓ rate of acidification

˝ Therefore, both cutting firmness *and* cutting time should be optimized and held constant from day-to-day

# *Bottom line*

- Cutting should be initiated at a consistent curd firmness that is optimized for the type of cheese being made
- The time required to achieve the target cutting firmness should be consistent from vat-to-vat across season
- In practice, this can be challenging because several factors may influence coagulation and cause curd firmness at cutting and/or cutting time to vary

# *Repercussions of matrix rearrangement*

˝ Cheese moisture content
˝ Acid development during cheese making
˝ Cheese yield

# *Repercussions of matrix rearrangement 3. Cheese yield*

- ˝ Weak curds are fragile and tend to shatter during cutting
  - . fat and casein losses $\uparrow$, cheese yield $\downarrow$
  - . *however*, matrix rearrangement after cutting occurs rapidly, curd particles firm up quickly
- ˝ Firm curds are more forgiving with respect to shattering during cutting
  - . *however*, matrix rearrangement after cutting occurs slowly
  - . curd particles firm up slowly and remain vulnerable to shattering for longer time after cutting

# *Mechanisms of coagulation*

˝ Rennet (rapid: 30 - 60 min)

˝ Acid (slow: 5 - 24 hr)

˝ Acid-rennet (slow: 12 -24 hr)

˝ Acid-heat

# *Acid coagulation*



1. Starter culture produces lactic acid, H+ ions accumulate

2. Calcium phosphate dissolves into water phase

3. H+ ions neutralize the negative charges on k-casein

4. Neuralized k-casein collapses

H+ H+ H+ H+ H+ H+ H+ H+

# *Acid coagulation*



H+

pH 4.6
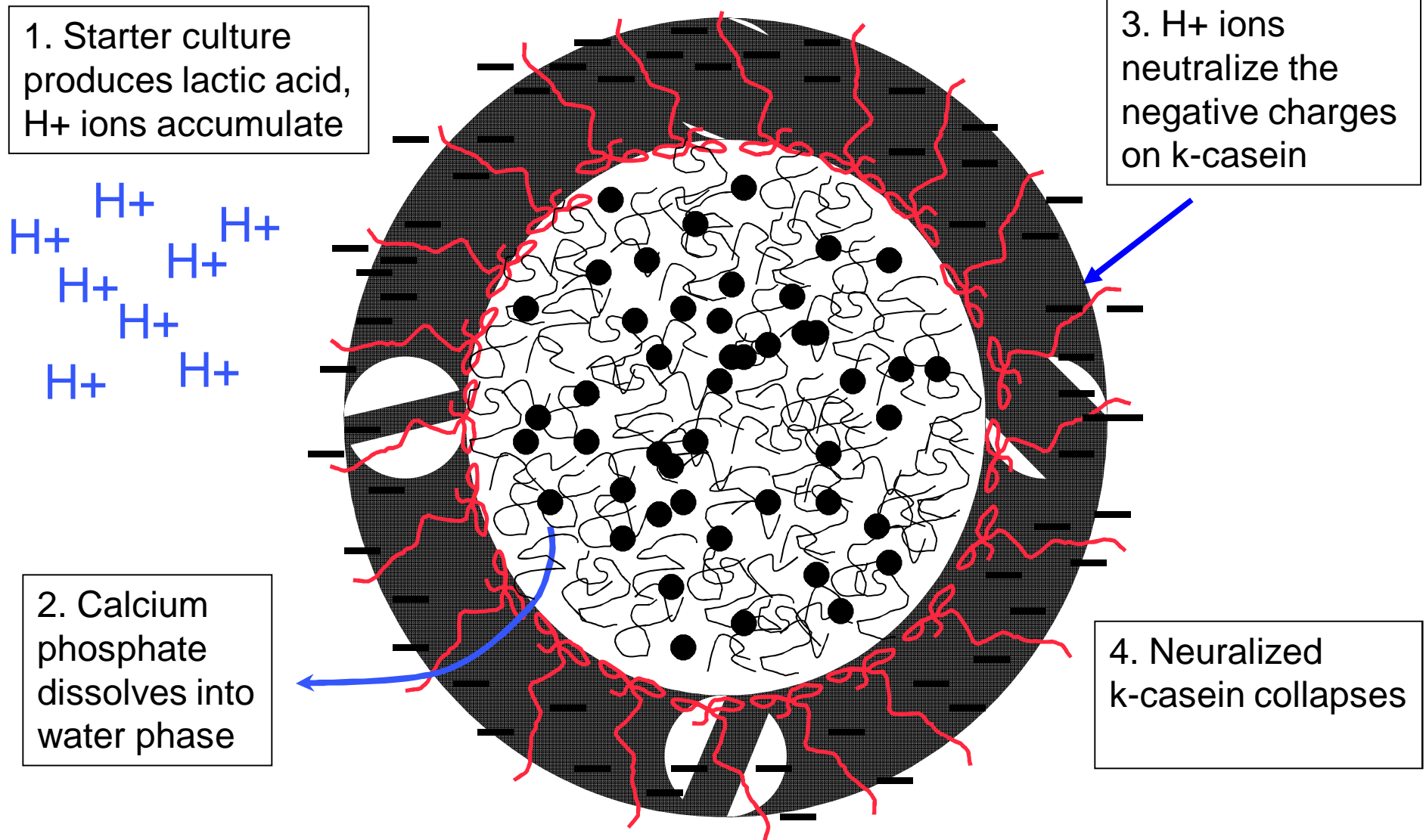
Polar
Surface

Micelle rich in
calcium phosphate

Collapsed neutralized
k-cn nonpolar surface

Micelle depleted of
calcium phosphate

# *Aggregation of casein micelles*
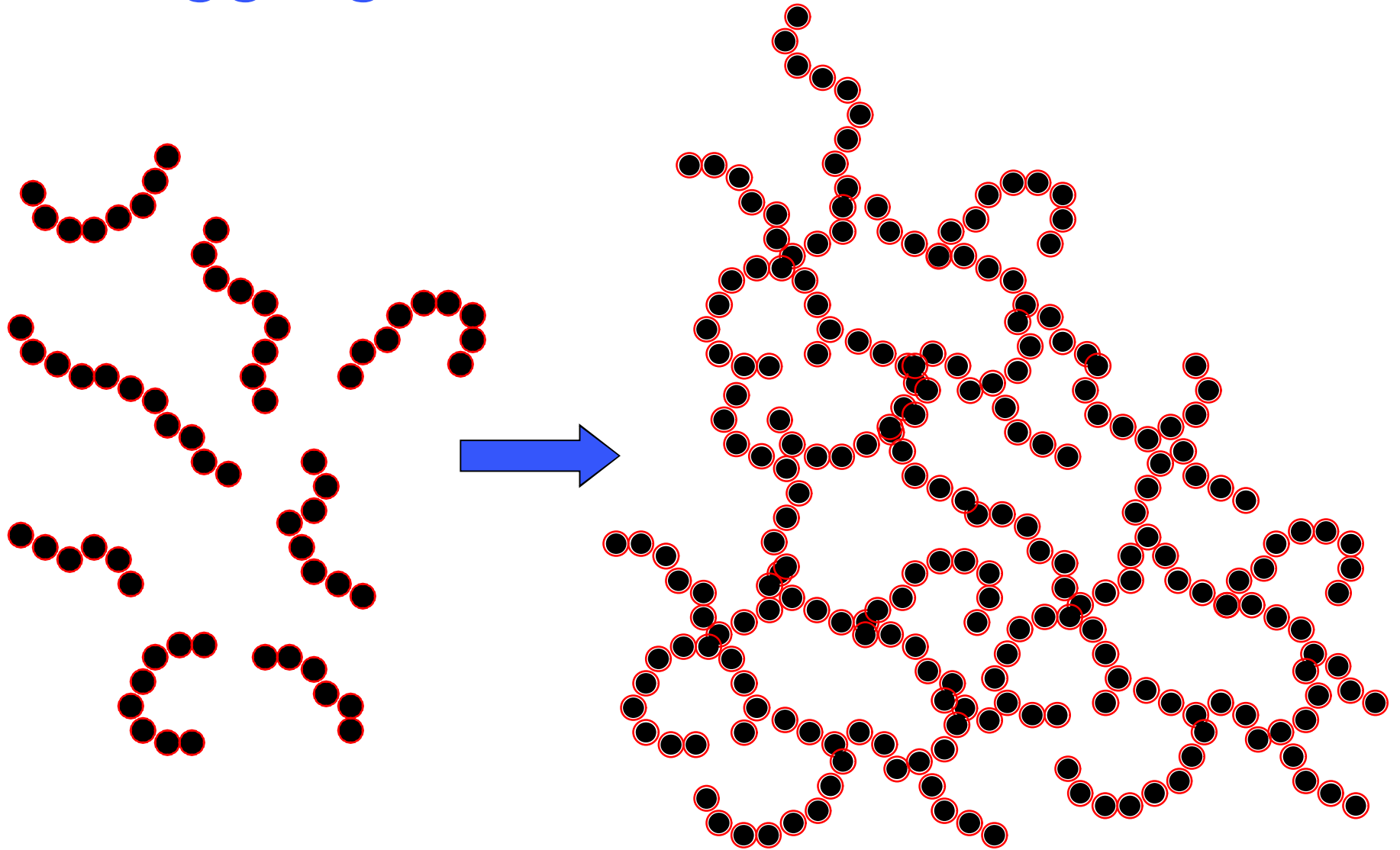
continuous gel - coagulation

# *Acid coagulation*

˝ Acid gels lack the capacity to contract and synerese

˝ Therefore, final cheese moisture content is very high (around 70-80%, depending of the fat content)
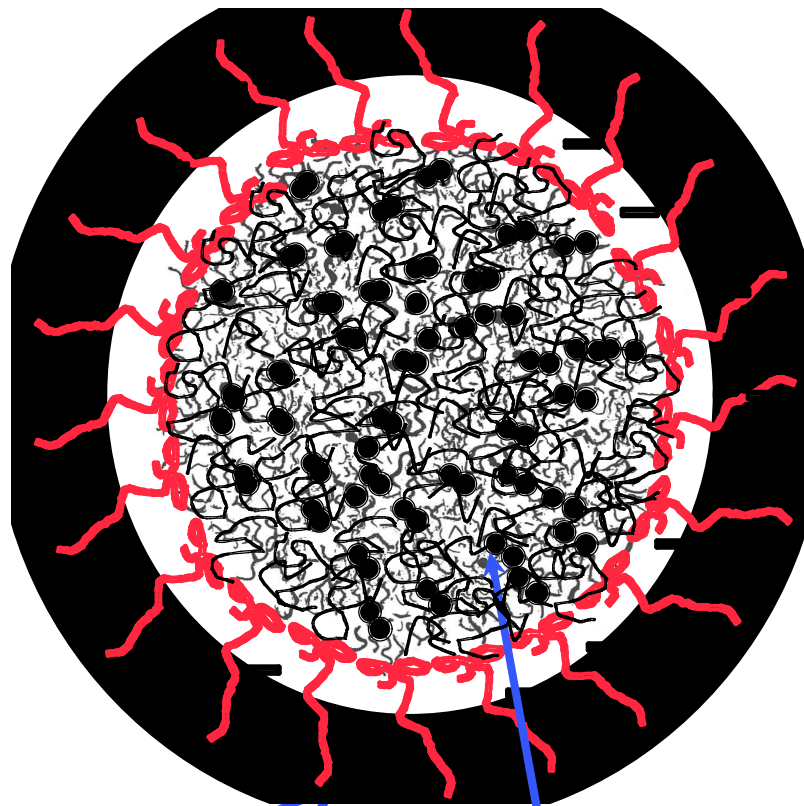
˝ In general, acid coagulated cheeses are eaten fresh, not ripened

# *Mechanisms of coagulation*

˝ Rennet (rapid: 30 - 60 min)

˝ Acid (slow: 5 - 24 hr)

˝ Acid-rennet (slow: 12 -24 hr)

˝ Acid-heat

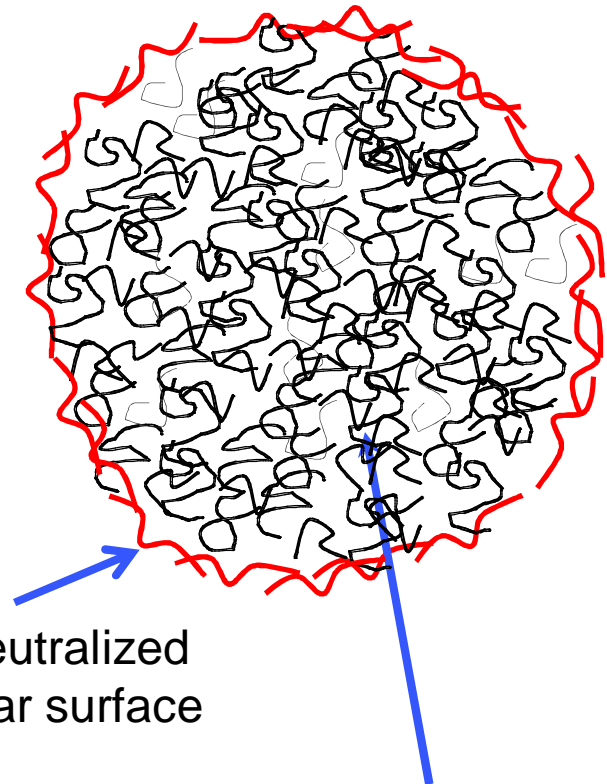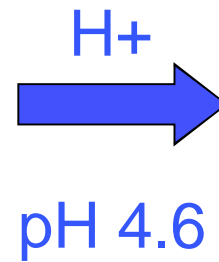# *Key parameters of acid-rennet coagulation*

1. Amount of rennet added to the milk:

   - Anywhere from 1 - 30% of the level used in rennet coagulation

2. Coagulation temperature:

   - Anywhere from 18 - 32°C

# *Example 1: Quark*

- Lactic fermentation at ca. 30°C for 16 hr
- A small amount of rennet (e.g., 1 - 10% of level used in rennet coagulation) added at around pH 6.3
- Rennet proceeds through enzymatic and non-enyzmatic phases as milk pH ↓
- Coagulation occurs at pH 4.8 or 4.9 instead of ph 4.6
- The resulting curd develops hybrid characteristics that fall somewhere between those of rennet curd and acid curd

# *Advantages of acid-rennet coagulated Quark*

. Better draining results in a lower moisture content

.  Lower moisture content along with a firmer coagulation result in a firmer cheese body, improved texture

. Higher cutting pH (e.g. from pH 4.6 to 4.8 or 4.9) results in a less acidic flavor

# *Example 2: soft ripened goat's milk cheese*

- The milk undergoes lactic acidification at around 20°C for 24 hr

- Rennet (about 1/3 the level used in rennet coagulation) is added, often around pH 6.3.

- The rennet proceeds through enzymatic phase but the non-enyzmatic phase is strongly impeded at 20°C,

- Coagulation occurs in 24 hr when the pH reaches around pH 5.3

- The resulting curd develops hybrid characteristics that fall somewhere between those of rennet curd and acid curd

# *Advantages of acid-rennet coagulation for goat's cheeses*

- ˝ Syneresis is improved, resulting in a final cheese with ca. 60-70% moisture.
- ˝ This moisture range is low enough to support controlled ripening
- ˝ The end result is a group of soft ripened goat's milk cheeses with a unique lactic acid dominated texture

# *Factors that affect rennet coagulation*

- Temperature history of milk
- pH of milk during coagulation
- Temperature of milk during coagulation
- $Ca^{++}$ ion content of milk
- Casein content of milk

# *Temperature history of milk:*
## 1. Cooling (< 10ºC)



$H^+$

$Ca^{++}$
$HPO_4 =$

ß- casein

0.2 - 0.3 pH ↑
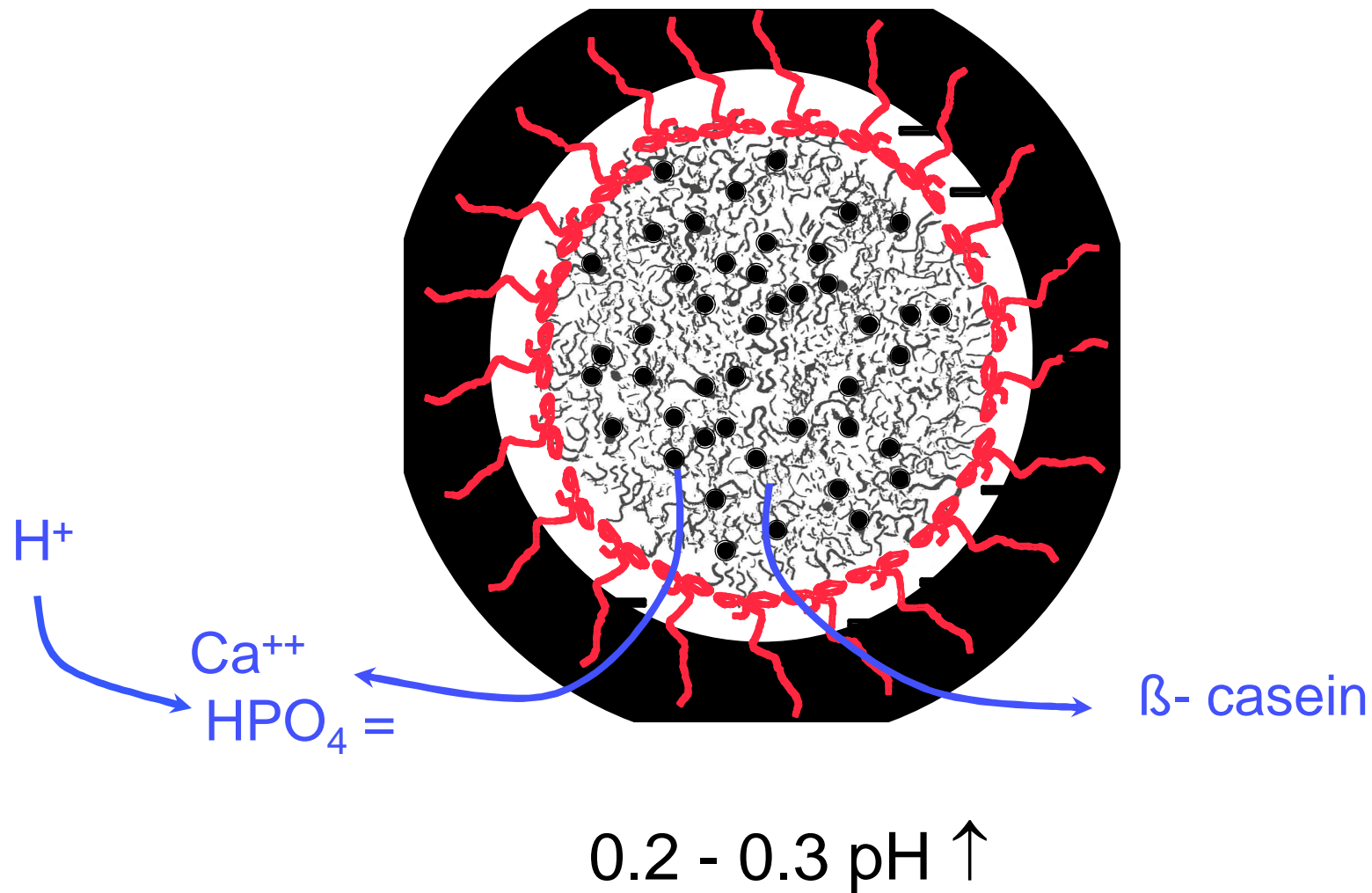
# Repercussions of cooling milk to 4⁰C

˝ ↑ milk pH

. Slower enzymatic phase

. ↓ attraction between rennet enzymes and casein micelles, ↑ cleavage of k-casein needed to induce coagulation

. Therefore, longer time needed to attain target cutting firmness

˝ Altered casein micelle structure

. Curd matrix less able to undergo structural rearrangement, contraction

. Therefore, weaker set, slower syneresis, higher moisture content in cheese (especially in high moisture types, e.g., bloomy rind)

˝ May cause problems when switching from warm milk fresh from the animal to cold stored milk

# Compensating for cooling milk to 4⁰C

˝  The changes can be largely reversed by normal pasteurization before cheese making

˝  These changes can be partly overcome by adding calcium chloride.

˝  If necessary, increase cutting time to restore the target curd firmness

˝  If necessary, adjust starter usage to restore target acifidification schedule

˝  If necessary, take action during cheese making to enhance syneresis/draining to reduce cheese moisture content

# *Factors that affect rennet coagulation*

˝ Temperature history of milk

˝ pH of milk during coagulation

˝ Temperature of milk during coagulation

˝ $Ca^{++}$ ion content of milk

˝ Casein content of milk
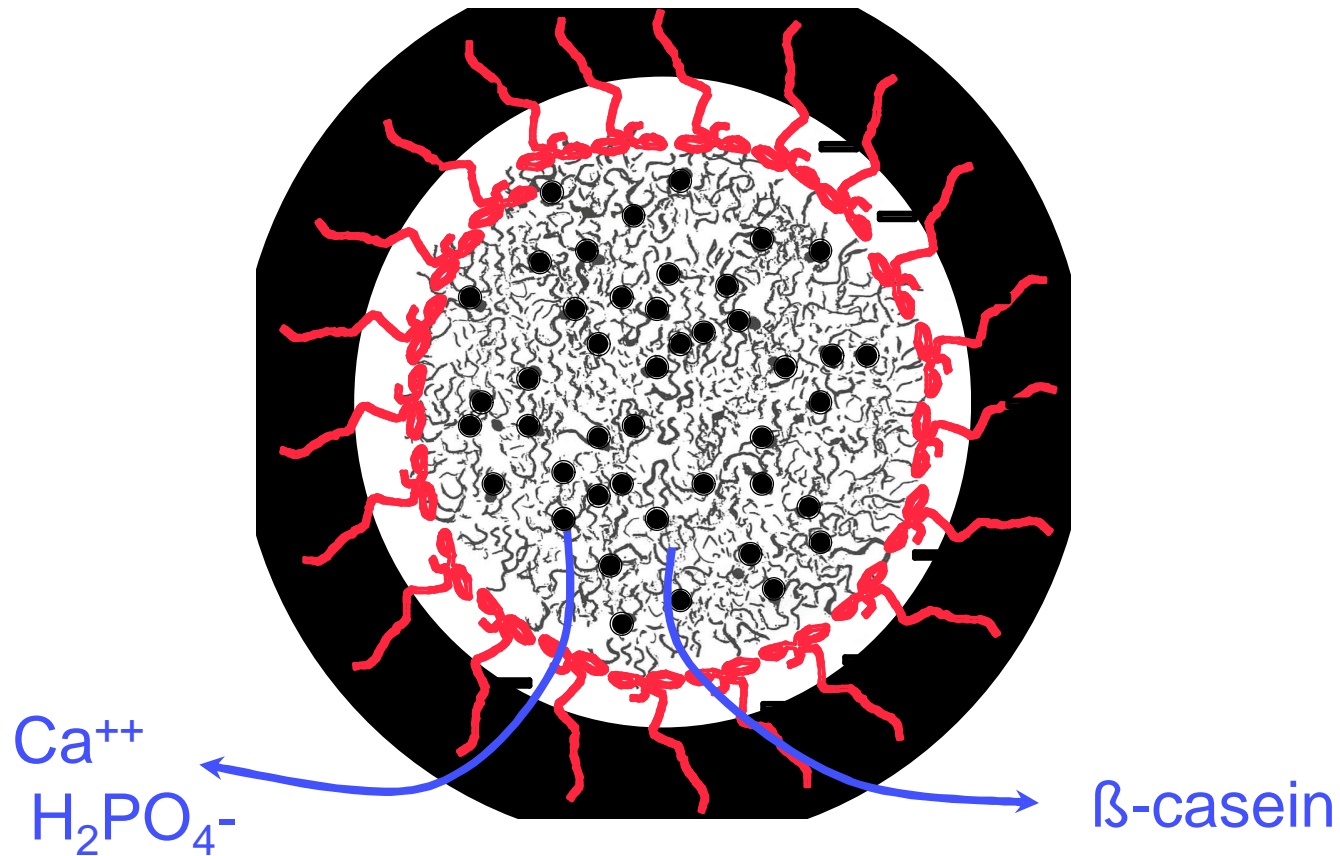
# *Repercussions of milk pH*

˝ As milk pH ↓
- ˝ Rennet enzyme activity increases
- ˝ Rennet enzymes are more strongly attracted to casein micelles
- ˝ Therefore, the enzymatic phase occurs more rapidly

˝ Furthermore:
- ˝ Rennet enzymes adsorb more tightly onto the casein micelle surface, resulting in %patches+of k-casein cleavage
- ˝ Therefore, the amount of k-casein cleavage needed to induce micelle aggregation ↓
- ˝ Also, higher $Ca^{++}$ ion concentration speeds up the aggregation of casein micelles
- ˝ Therefore, Non-enzymatic phase occurs more rapidly

˝ Consequently, rennet clotting time ↓, cutting time ↓, and curd firmness ↑

# *Factors that affect rennet coagulation*

˝ Temperature history of milk
˝ pH of milk during coagulation
˝ Temperature of milk during coagulation
˝ $Ca^{++}$ ion content of milk
˝ Casein content of milk

# As coagulation temperature ↑ from ca. 25° - 40°C

˝ Enzymatic phase occurs more rapidly:
  - ˝ Rennet enzyme activity increases with ↑ temperature
˝ Non-enzymatic phase occurs more rapidly
  - ˝ Rennet enzymes adsorb more tightly onto the casein micelle surface
  - ˝ Therefore, the amount of k-casein cleavage needed to induce micelle aggregation ↓
˝ Consequently, rennet clotting time and cutting time ↓, and curd firmness ↑

# *As coagulation temperature ↓ from ca. 25° - 15°C*

˝ Enzymatic phase gradually slows down but the non-enzymatic phase fails catastrophically

   . At 20°C, the non-enzymatic phase is severely impeded and casein aggregation/curd formation occurs very slowly (many hours)

   . This principle is exploited in the production of acid-rennet coagulated cheeses

   . At 15°C, the nonenzymatic phase is completely prevented; casein aggregation cannot occur even when k-casein has been completely cleaved from the micelle surface

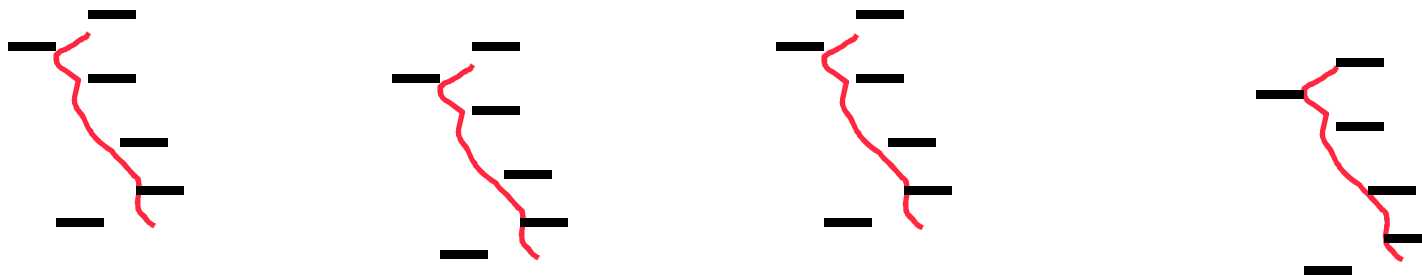˝ Bottom line: coagulation temperature should be tightly controlled

# *Factors that affect rennet coagulation*

˝ Temperature history of milk

˝ pH of milk during coagulation

˝ Temperature of milk during coagulation

˝ $Ca^{++}$ ion content of milk

˝ Casein content of milk

# Ca⁺⁺ ion content of the milk



casein micelle

Ca$^{++}$

Ca$^{++}$

Ca$^{++}$

Ca$^{++}$
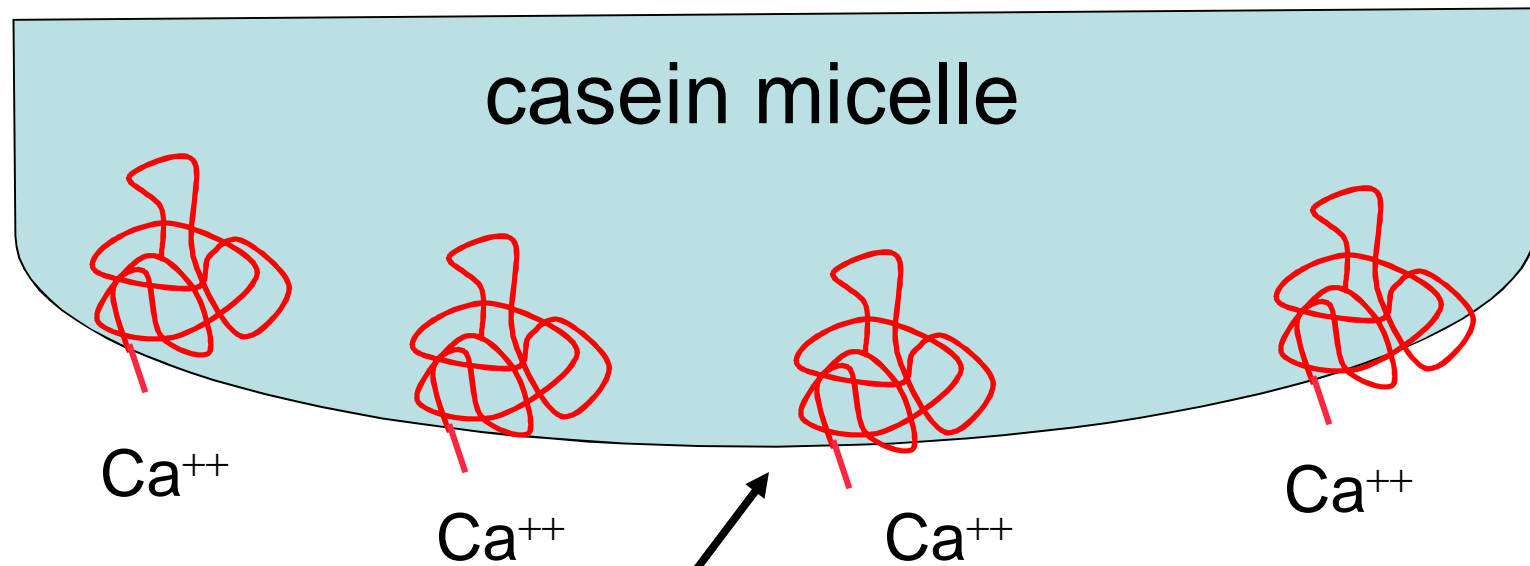
*VERY* Nonpolar surface

CMP/GMP (AA106-169)

# *Calcium chloride addition*

˝ Supplies $Ca^{++}$ ions, enables nonezymatic phase (aggregation of casein micelles) to proceed

˝ Decreases the milk pH, thereby stimulating the enzymatic phase

˝ End result: rennet clotting time ↓, cutting time ↓, and curd firmness ↑

# Factors that affect rennet coagulation

˝ Temperature history of milk

˝ pH of milk during coagulation

˝ Temperature of milk during coagulation

˝ Ca$^{++}$ ion content of milk

˝ Casein content of milk

# *As casein content ↑:*

˝ The frequency of collisions between casein micelles increases dramatically

˝ This causes micelles to aggregate at much lower levels of k-casein cleavage

˝ End result: the nonezymatic phase is greatly accelerated: rennet clotting time ↓, cutting time ↓, and curd firmness ↑

# *General rules of thumb*

- ″ Cut curd at a consistent firmness (for moisture control)
- ″ Cut curd at a consistent time from rennet addition (for maintaining acidification schedule)
- ″ If necessary:
  - . Add calcium chloride (up to 0.02% maximum)
  - . adjust cutting time to hold cutting firmness constant
  - . adjust starter usage to maintain target acidification schedule

# Thank You!