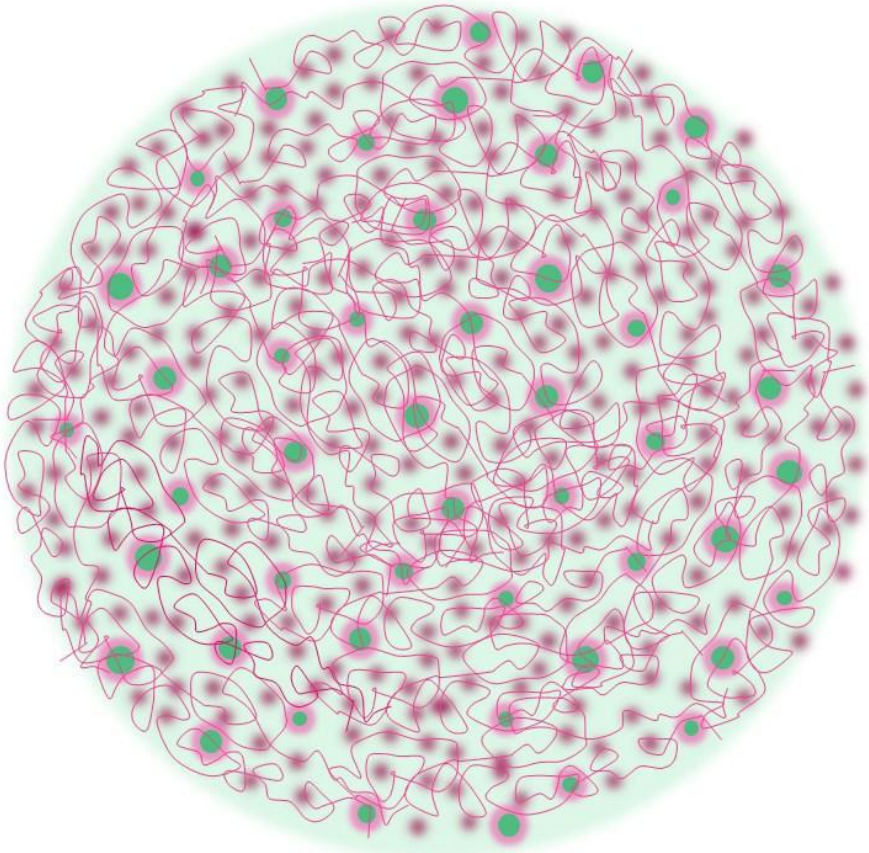
Casein micelles in milk revisited

A cow story

Kees de Kruif



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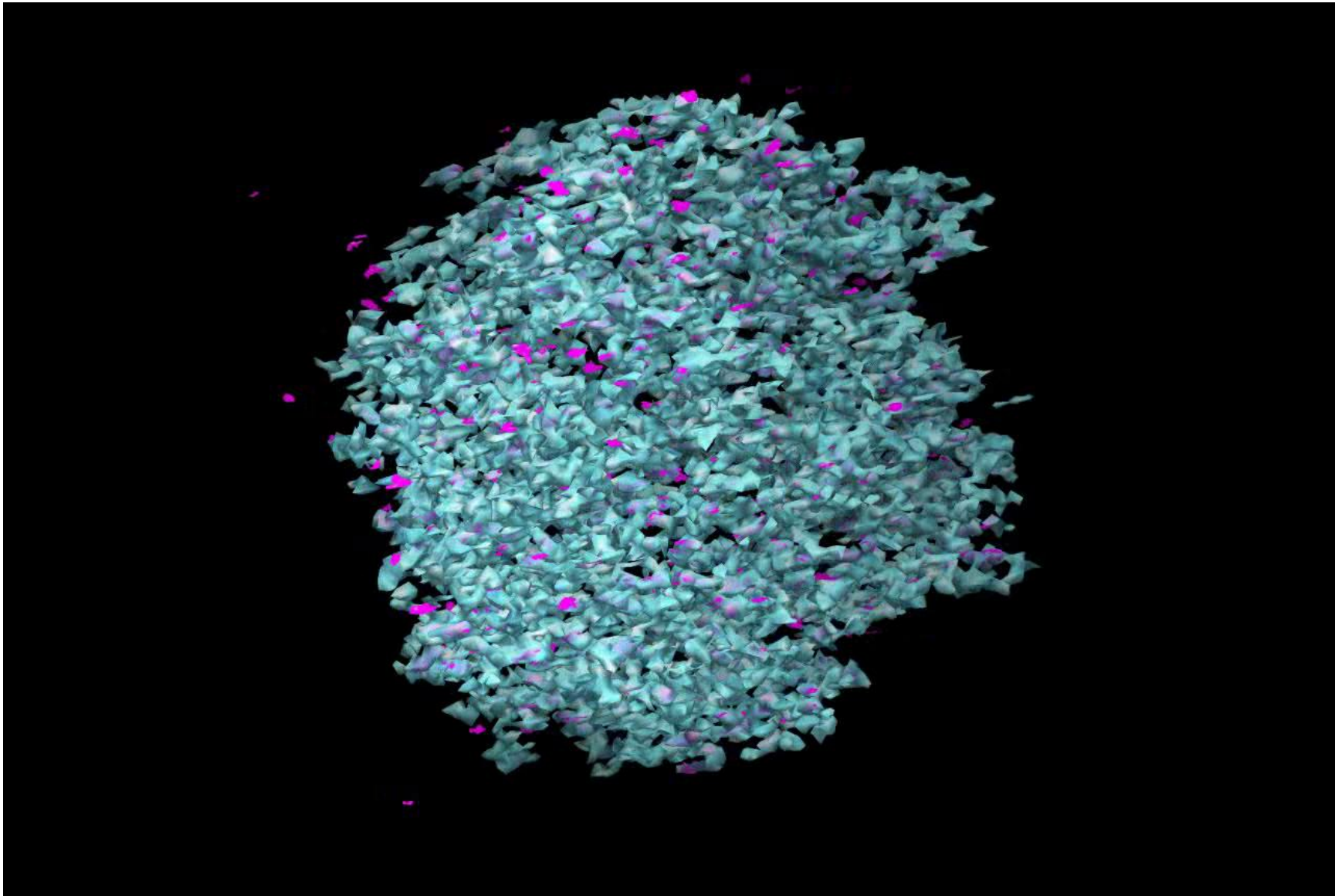
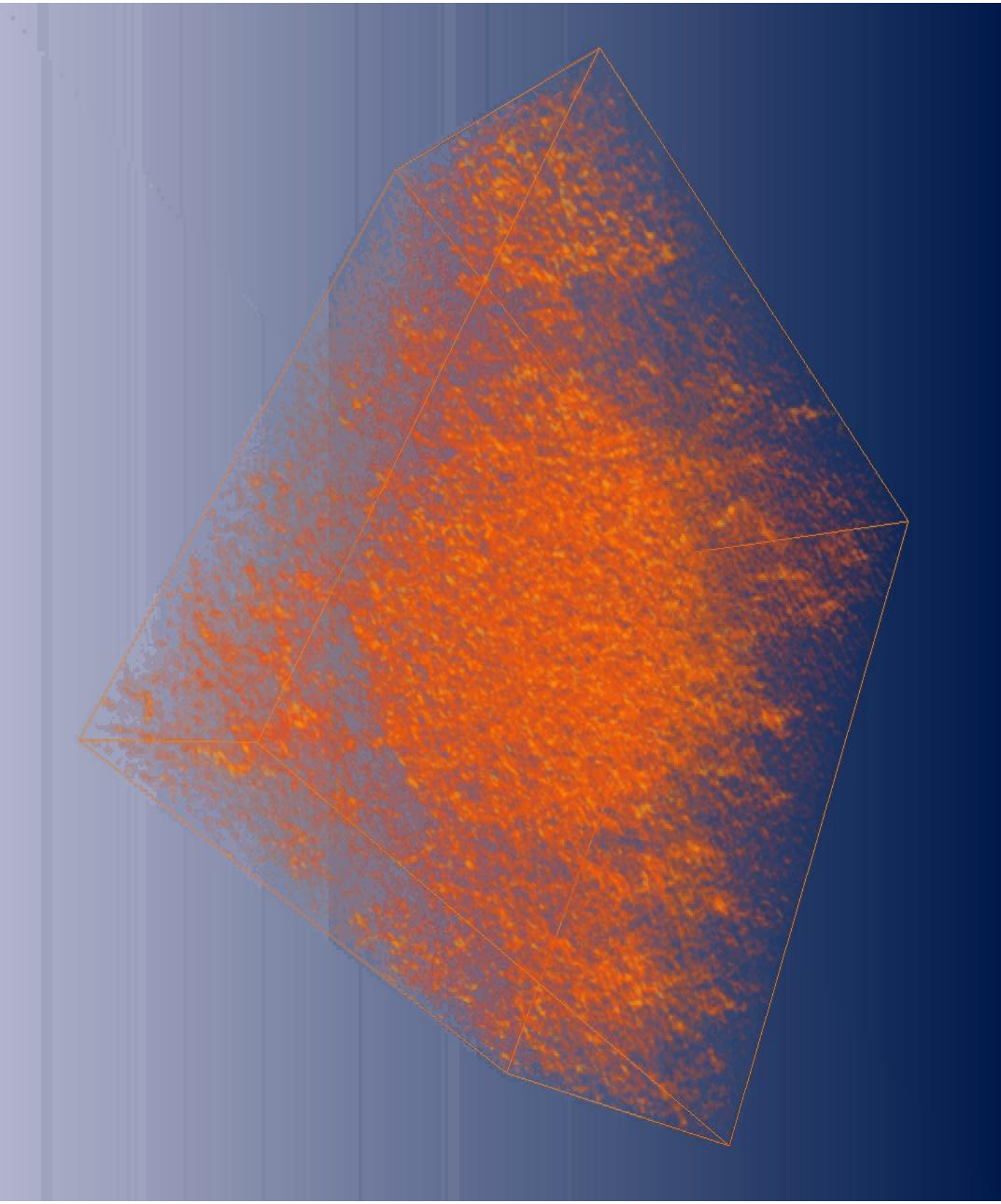
Courtesy Dr. Harte Tennessee JDS 2011



Courtesy ; Arjan Bot@Unilever.NL



Whey protein strands

Casein Micelle

Alice in Wonderland
The Cheshire cat

‘Which model should I believe ,'
said Alice.

‘Which one do you want to believe?'
said the cat.

‘I don’t know ‘
said Alice

‘Well then it does not matter where you go to’
said the cat

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What did we do?!

- Collected milk of individual cows
- Measured size and protein composition
- Choose Martha as favorite cow
- Measured light scattering, SAXS, SANS
- Measured density, viscosity,

- CALCULATED SPECTRA BASED ON MODELS
- No adjustable parameters!!
- No data fitting!!


Martha

Casein micelles from individual cows

- 16 Holstein Frisian cows from a herd of 120
- Milk sample from 2 cows during milking
- Milk samples from16 cows `4 times with 6 week intervals
- Milk from one cow Martha 183 (cow 68)
- During 3 year and used for SANS and SAXS
- Particle size analysis and casein composition


NIZO
food research

Cow Nr	Cow name	Age	Lactations	Fat (%, m/m)	Protein (%, m/m)
16	Klara 657	4	3	4.77	3.60
22	Lien 376	2	1	4.04	3.53
23	Lien 374	2	1	4.52	3.42
43	Lien 295	8	6	3.99	3.23
45	Klara 561	5	3	4.86	3.73
48	Klara 580	9	8	4.69	3.80
63	Klara 629	6	4	4.92	3.72
68	Martha 183	6	4	4.91	3.48
75	Klara 675	3	2	4.63	3.44
85	Klara 696	2	1	4.96	3.63
90	Klara 660	4	3	4.95	3.49
93	Klara 555	10	9	4.99	3.43
98	Lien 275	10	9	4.73	3.47
123	Klara 623	6	5	3.66	3.27
130	Gerda 494	5	3	4.71	3.89
139	Lien 349	4	3	5.24	3.40

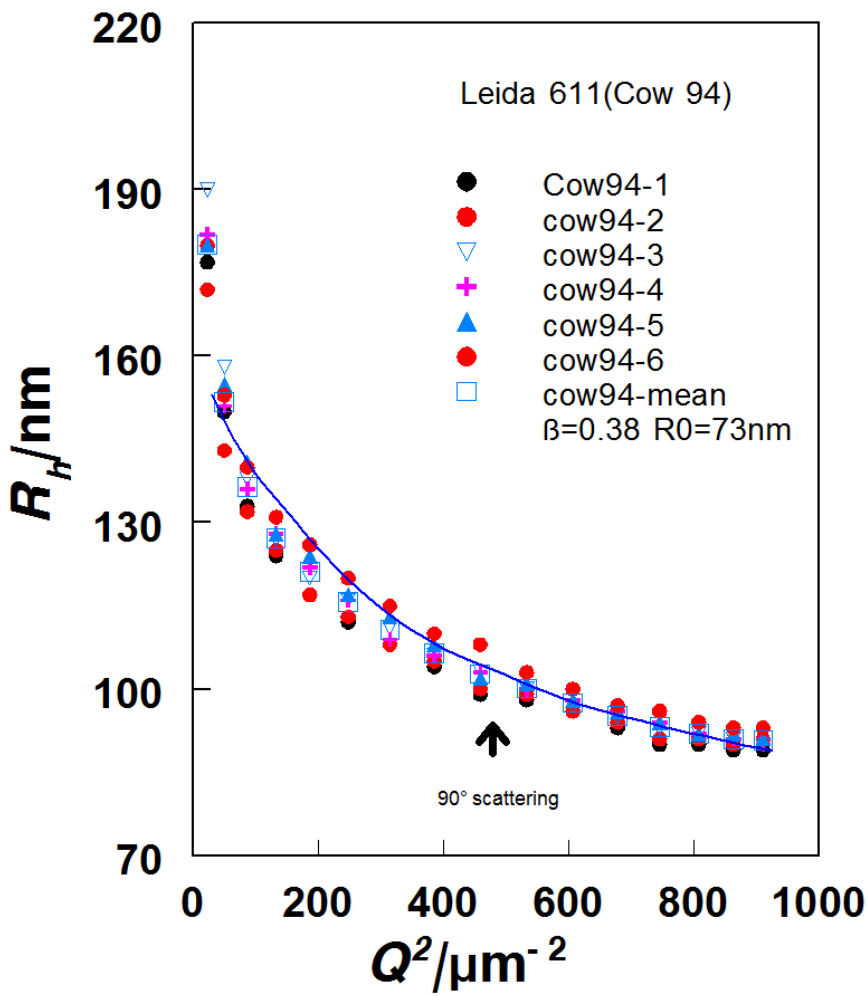
Dynamic Light Scattering (DLS)

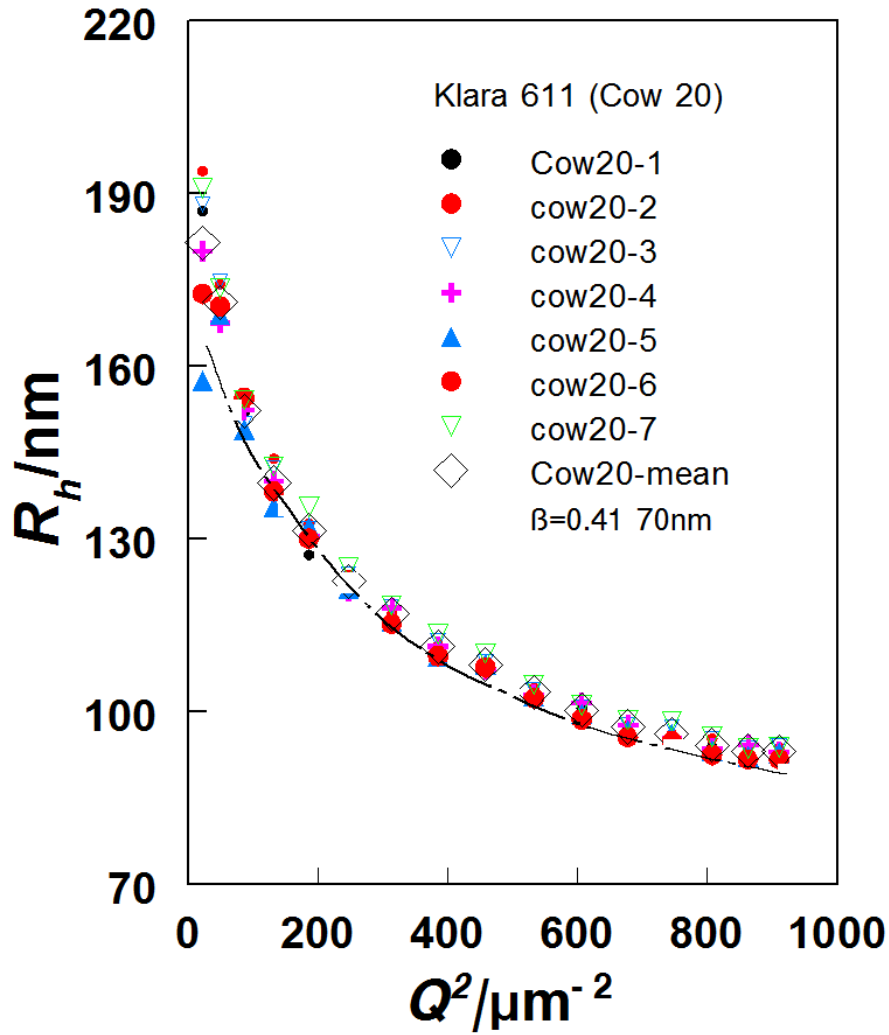
- DLS as function scattering angle (Θ)
- Wave vector $Q=4\pi n/\lambda \sin(\Theta/2)$

- At small Θ (small Q) "see" larger particles
- R_{hydr} decreases with Q (if polydisperse)

Cow 94 and 20 during milking

NO VARIATION DURING MILKING





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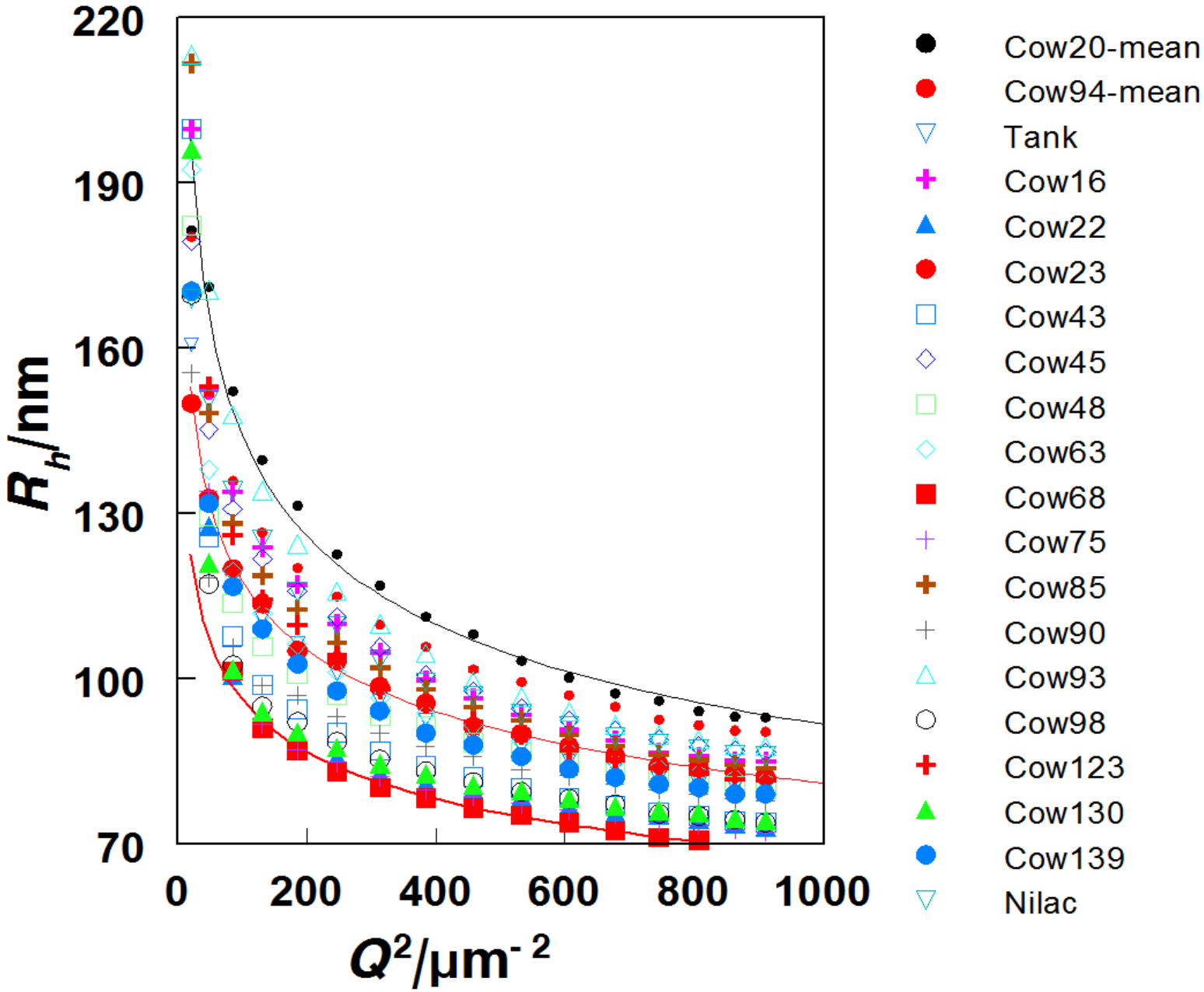
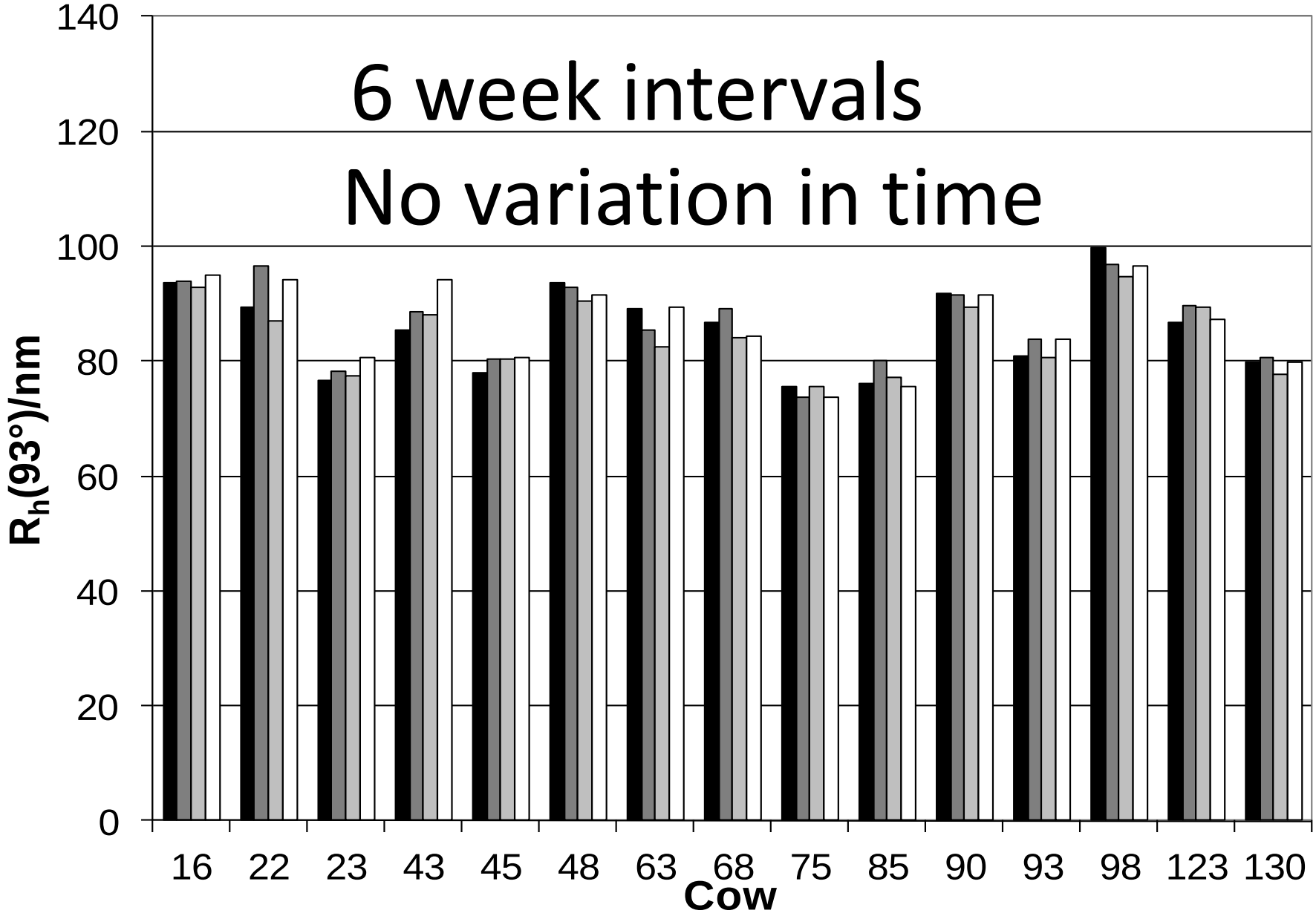
VARIATION FOR COWS





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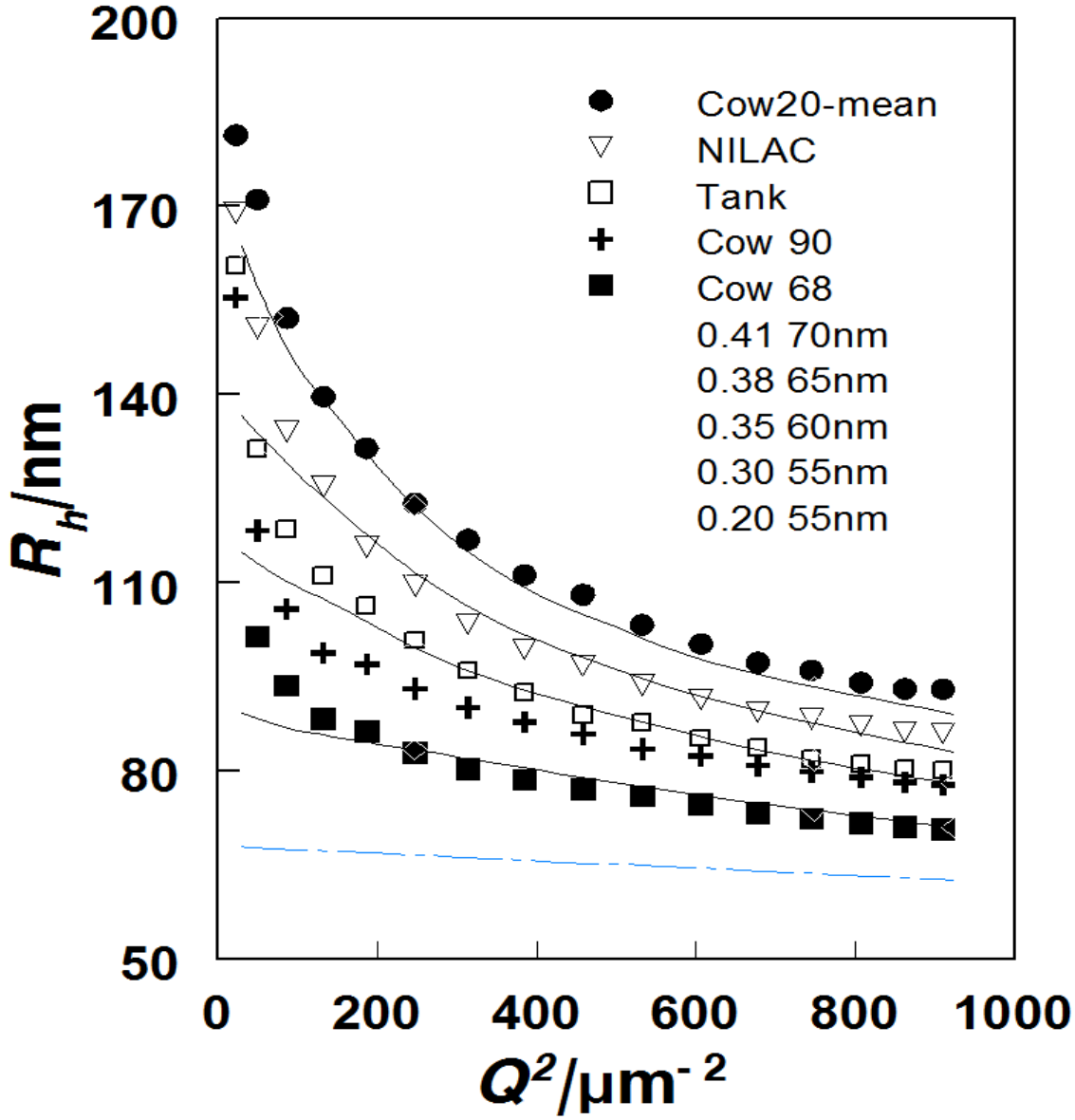
VARIATION FOR COWS



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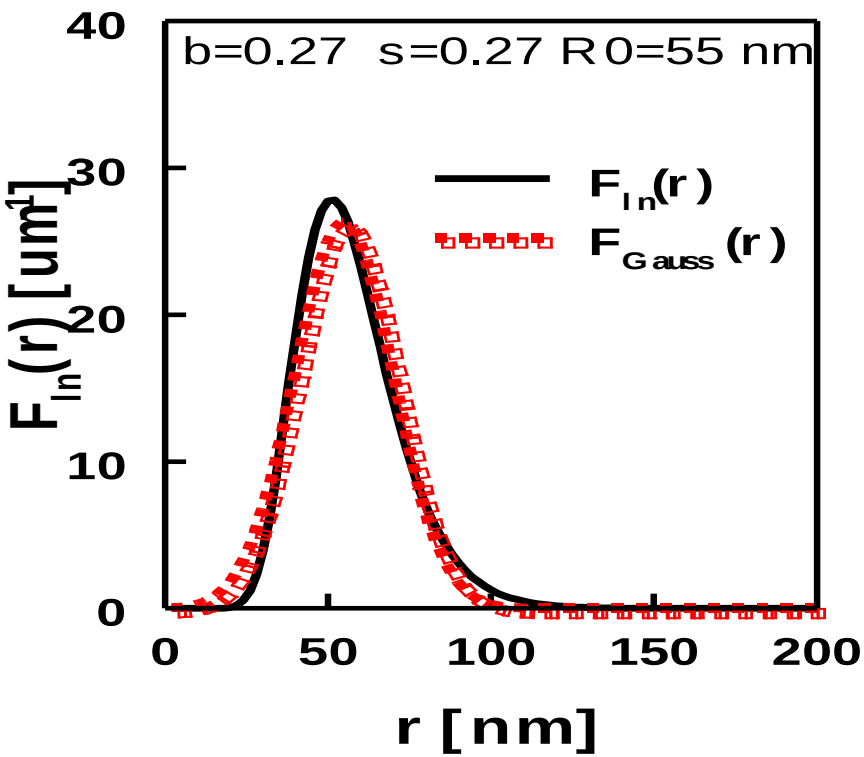
Particle size distribution

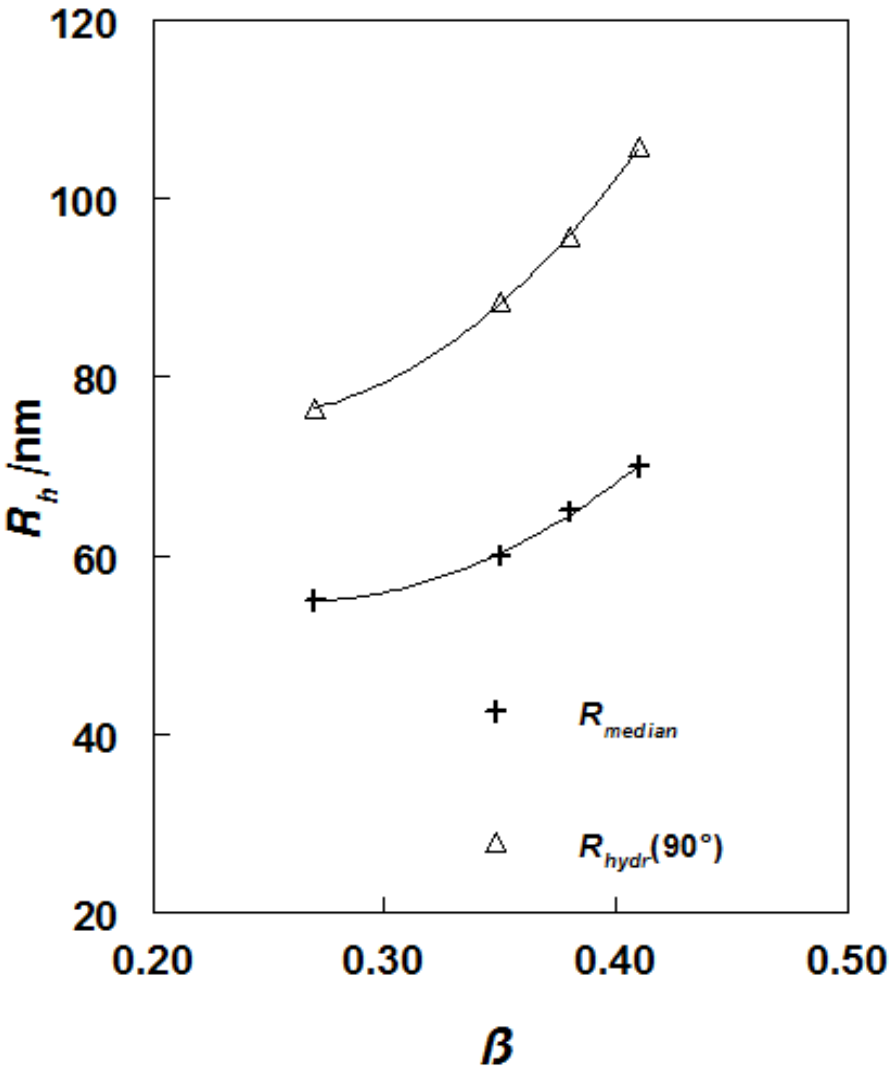


$N_{CM}*4/3\pi\ R^3$=constant. $N_{CM} \infty 1/R^3$
$A \sim N_{CM}*4\pi\ R^2$. $N_{kcas} \infty$R

Polydispersity increases with size!!

: $\beta \infty \sqrt{n}$ ($\infty \sqrt{R}$)

$$R_h \infty \beta^2$$



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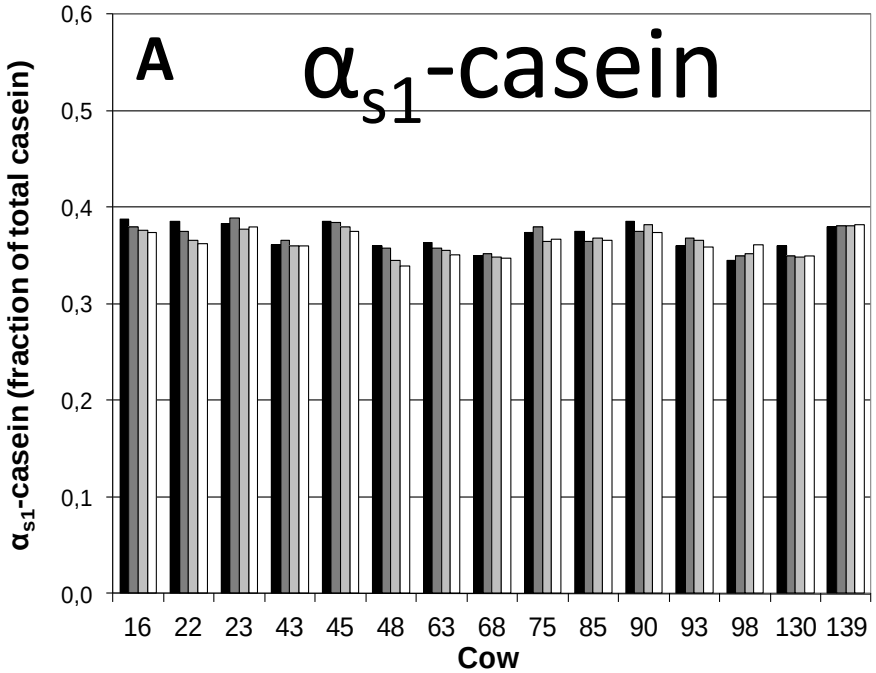
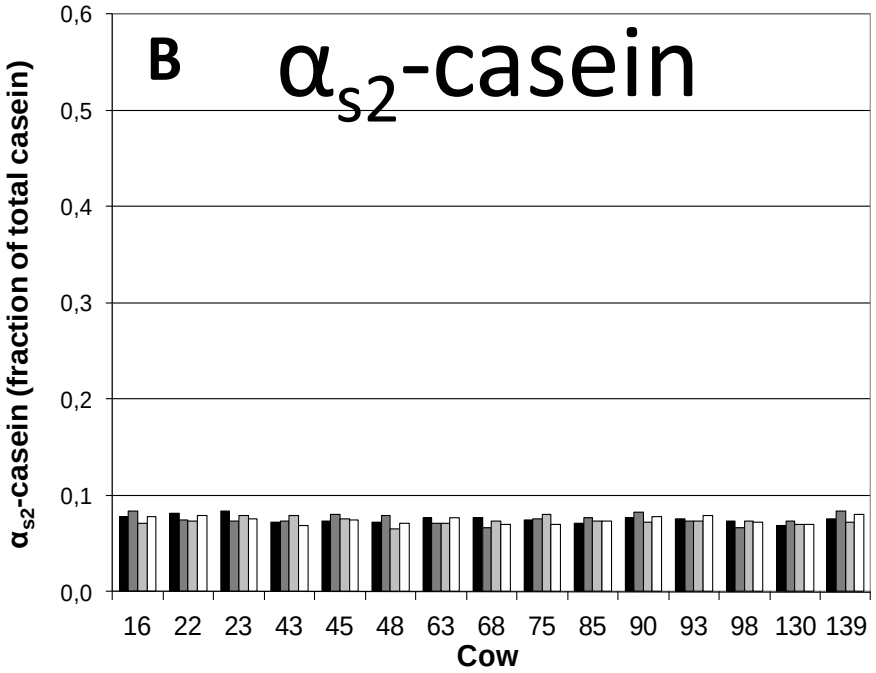
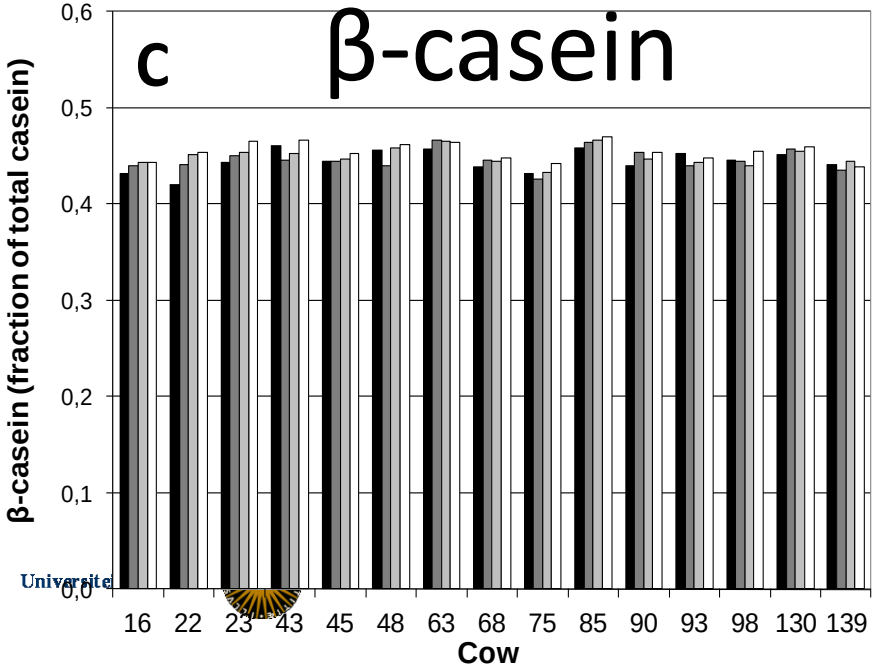

A
α_{s1}-casein
α_{s1}-casein (fraction of total casein)
0,0
0,1
0,2
0,3
0,4
0,5
0,6
16 22 23 43 45 48 63 68 75 85 90 93 98 130 139
Cow


B
α_{s2}-casein
α_{s2}-casein (fraction of total casein)
0,0
0,1
0,2
0,3
0,4
0,5
0,6
16 22 23 43 45 48 63 68 75 85 90 93 98 130 139
Cow


C
β-casein
β-casein (fraction of total casein)
0,0
0,1
0,2
0,3
0,4
0,5
0,6
16 22 23 43 45 48 63 68 75 85 90 93 98 130 139
Cow

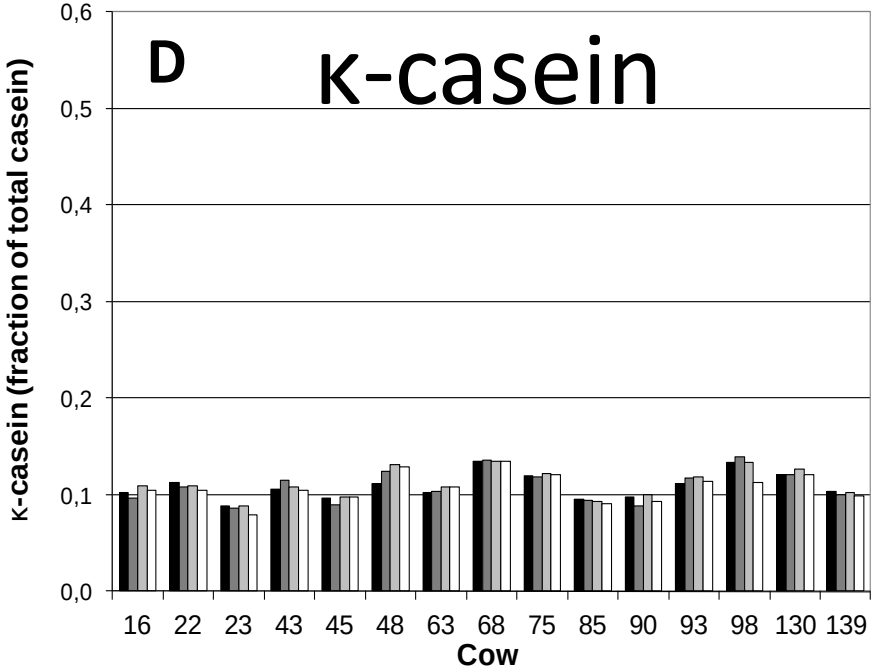
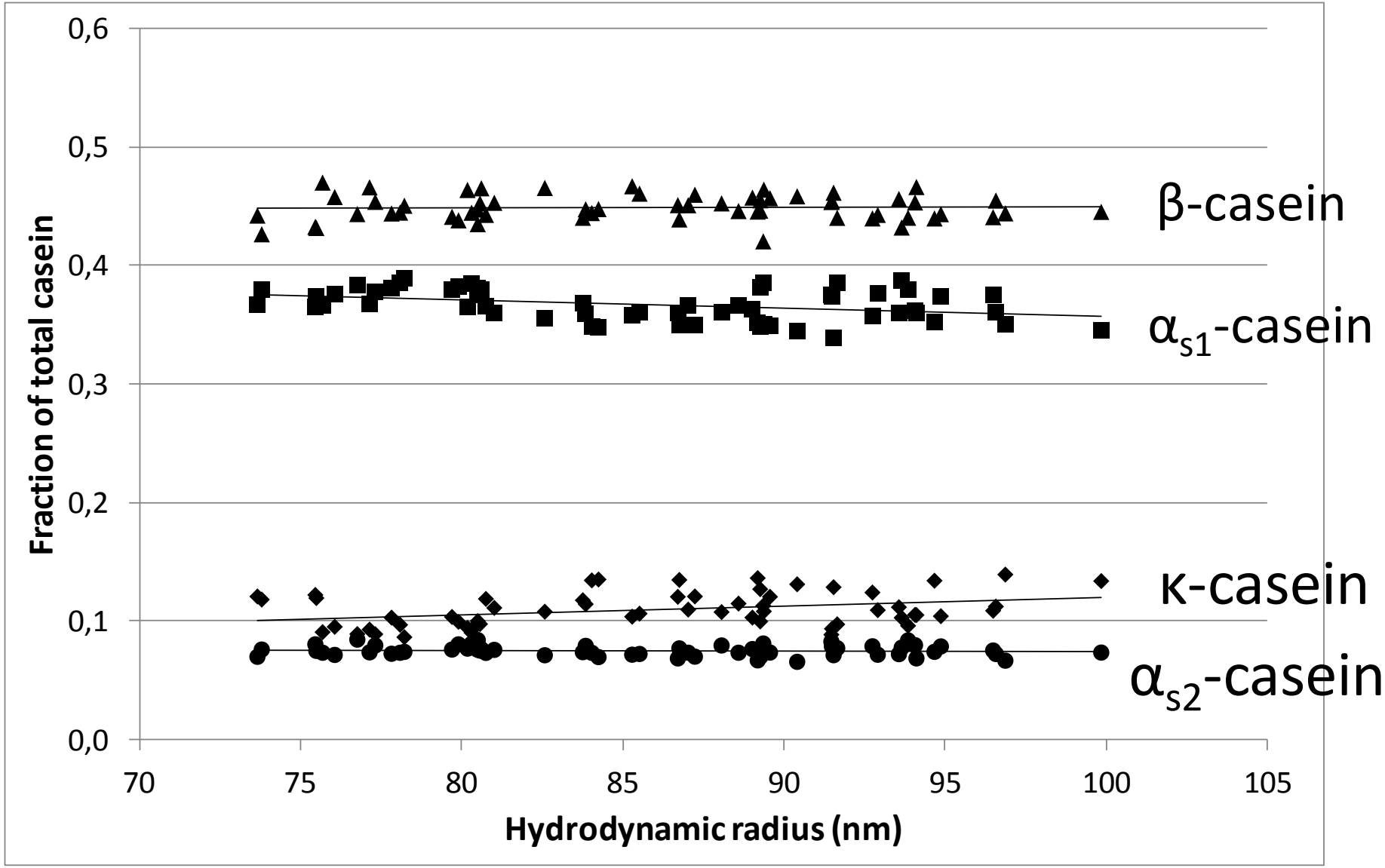

D
κ-casein
κ-casein (fraction of total casein)
0,0
0,1
0,2
0,3
0,4
0,5
0,6
16 22 23 43 45 48 63 68 75 85 90 93 98 130 139
Cow

Figure 9 Proportion of αs1-casein (■), αs2-casein (●), β-casein (▲) and κ-casein (◆) in milk from individual cows expressed as a function of hydrodynamic radius.



New Zealand cows

Jersey (young) Holstein Frisian

Northbrook Milking Shorthorns

David & Johanna Wood
798 Reid Line East
R.D.11
Palmerston North

Phone (06)329 2684

Email dr.jjwood@xtra.co.nz

Gerd ElisabethVegarud
Agricultural university , Aas, NW
59 Norwegian short horns
Int Dairy J. 10 (2000) 313

- **Native micelles: 75-111 m at 90 deg 633 nm**
- **We at Q^2 = 350 μm^{-2} 77- 115nm $\pm$ 4 nm**

NOTE: DLS radii!!!
No correlation!

Same result for

Holstein Frisians

NEUTRON SCATTERING

Highly recommended

WHY??

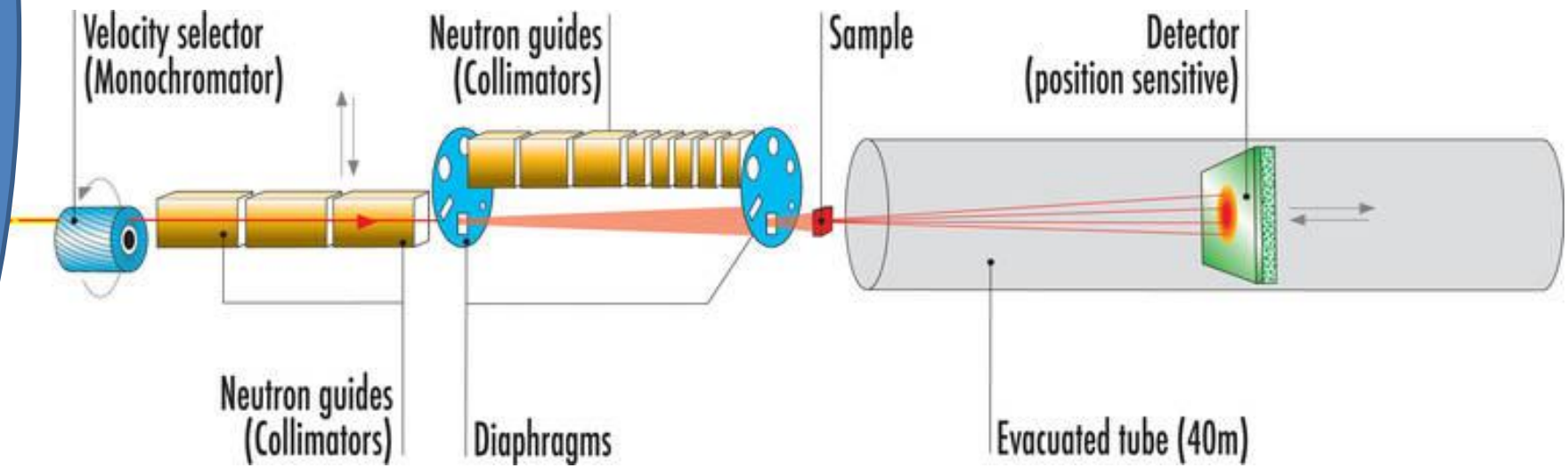
Look at this!!


Oak Ridge TN Melton Hill Lake. Smokey Mountains


Polygone Scientific
Grenoble France
Please note the snow!

REACTOR

Neutron scattering
D11 at the ILL Grenoble France



Volker Urban and CdK

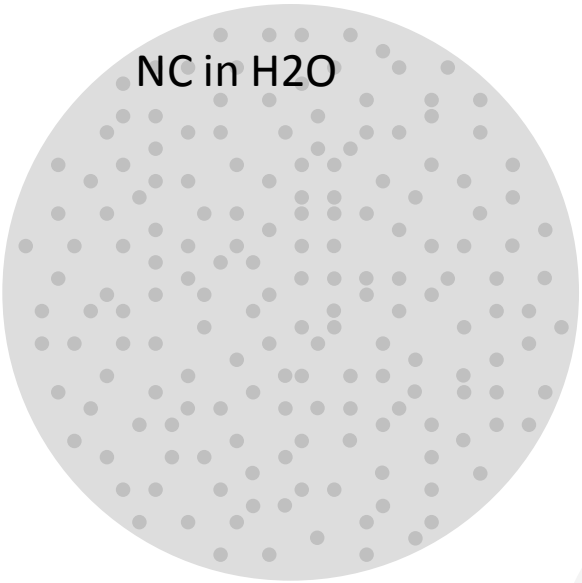
ORNL and ILL

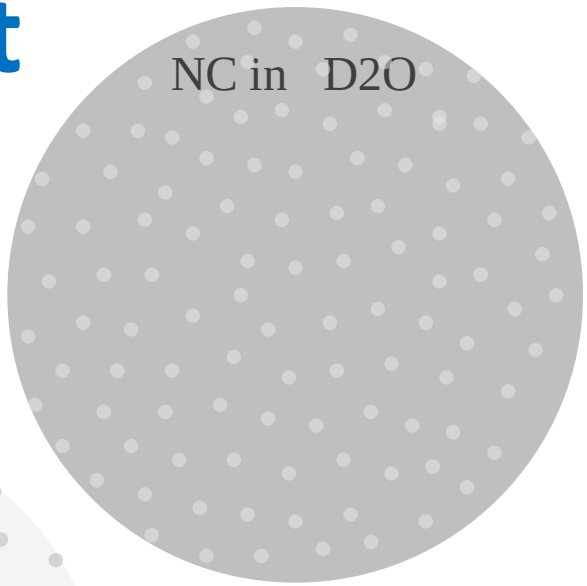
ORNL detector Jurgen Smeenk

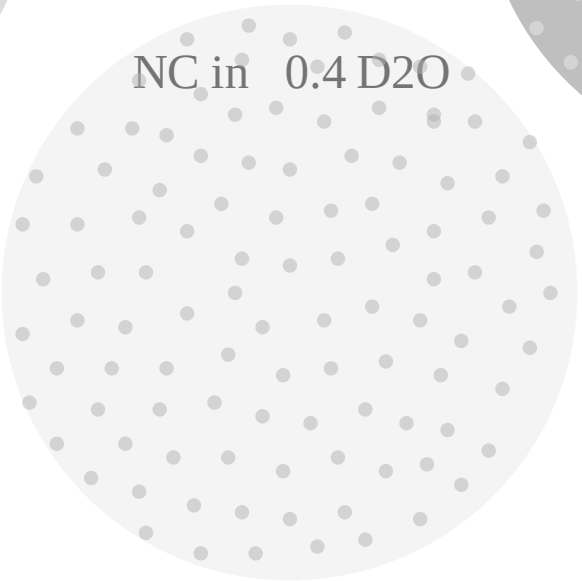
Why use neutrons??

- Most materials are transparent!
- Contrast variation
- Especially for hydrocarbon and proteins
- Hydrogen and deuterium
- completely different scattering.
- Take H2O/D2O mixtures
- Scattering varies greatly but structure not

Contrast





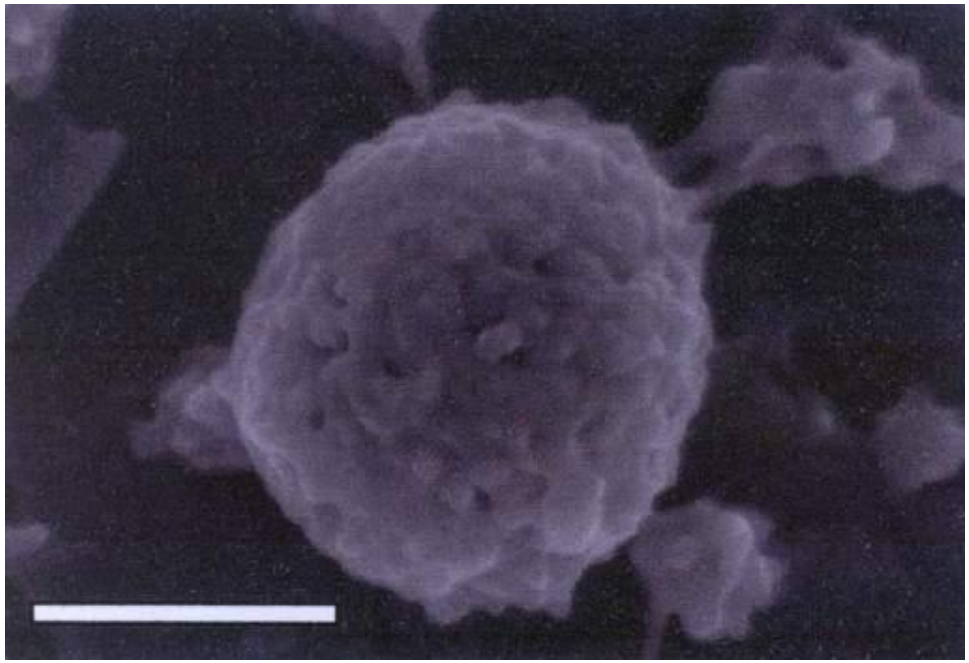


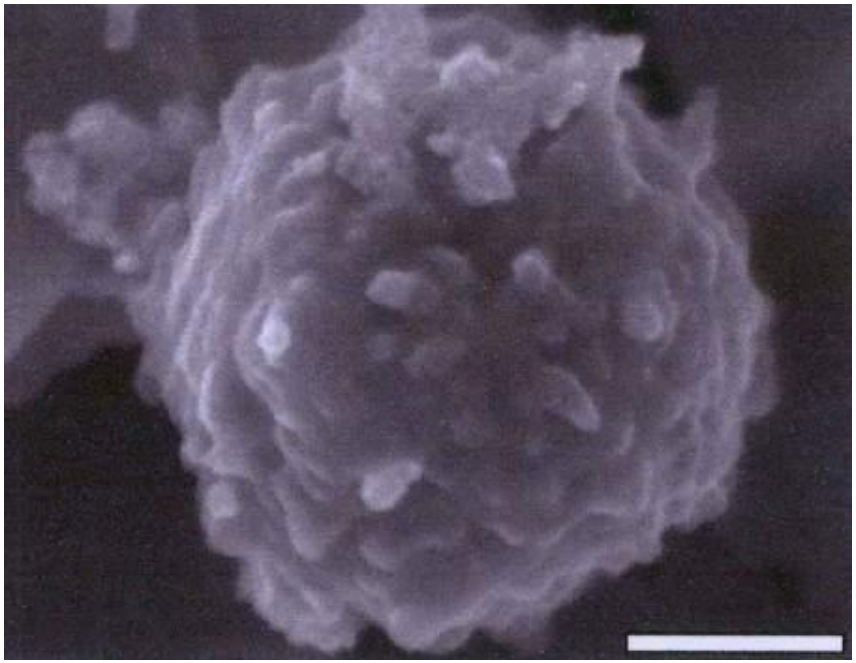
~Equal contrast **Protein matched** **CP matched**

Casein Micelle: Structure????

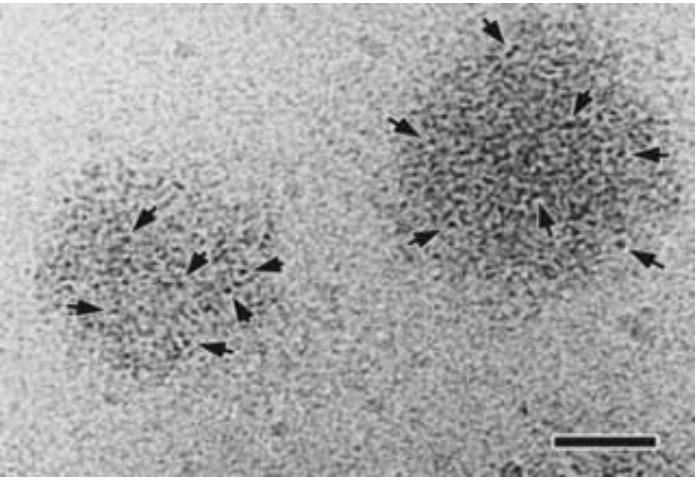
Field Emission Scanning Electron Microscopy

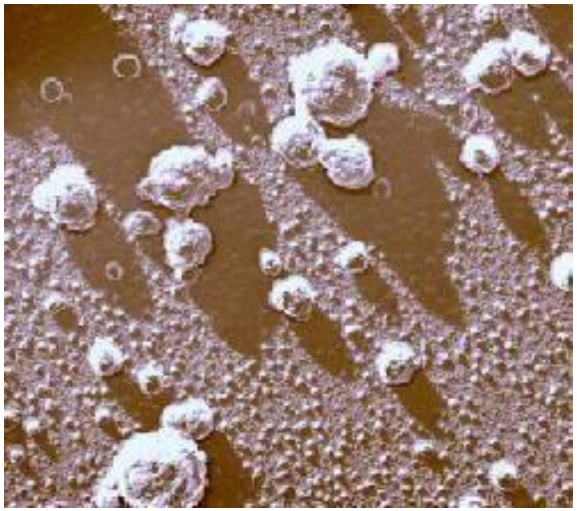
Dalgleish *et al*., (IDJ, 2004)

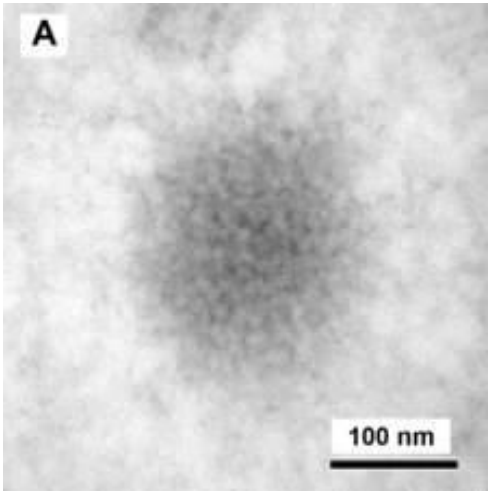


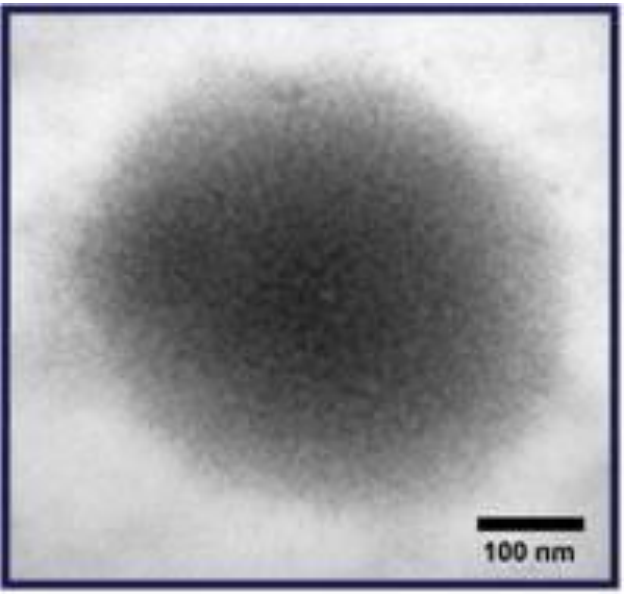


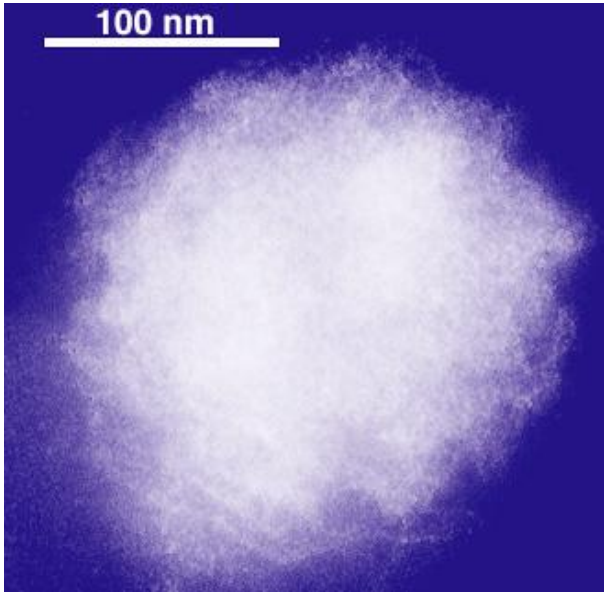
EM pictures

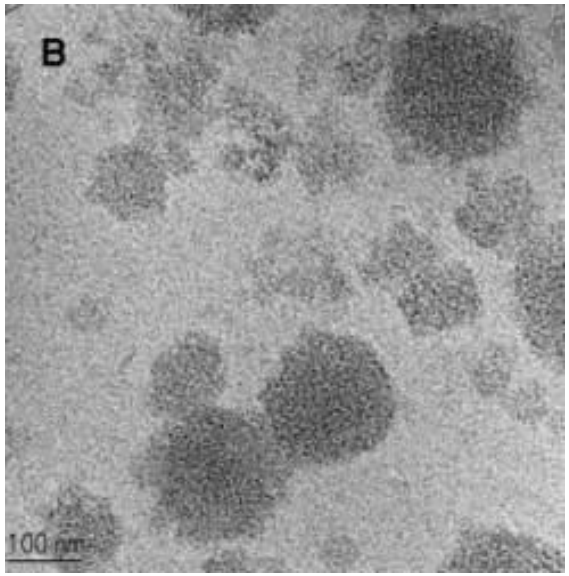




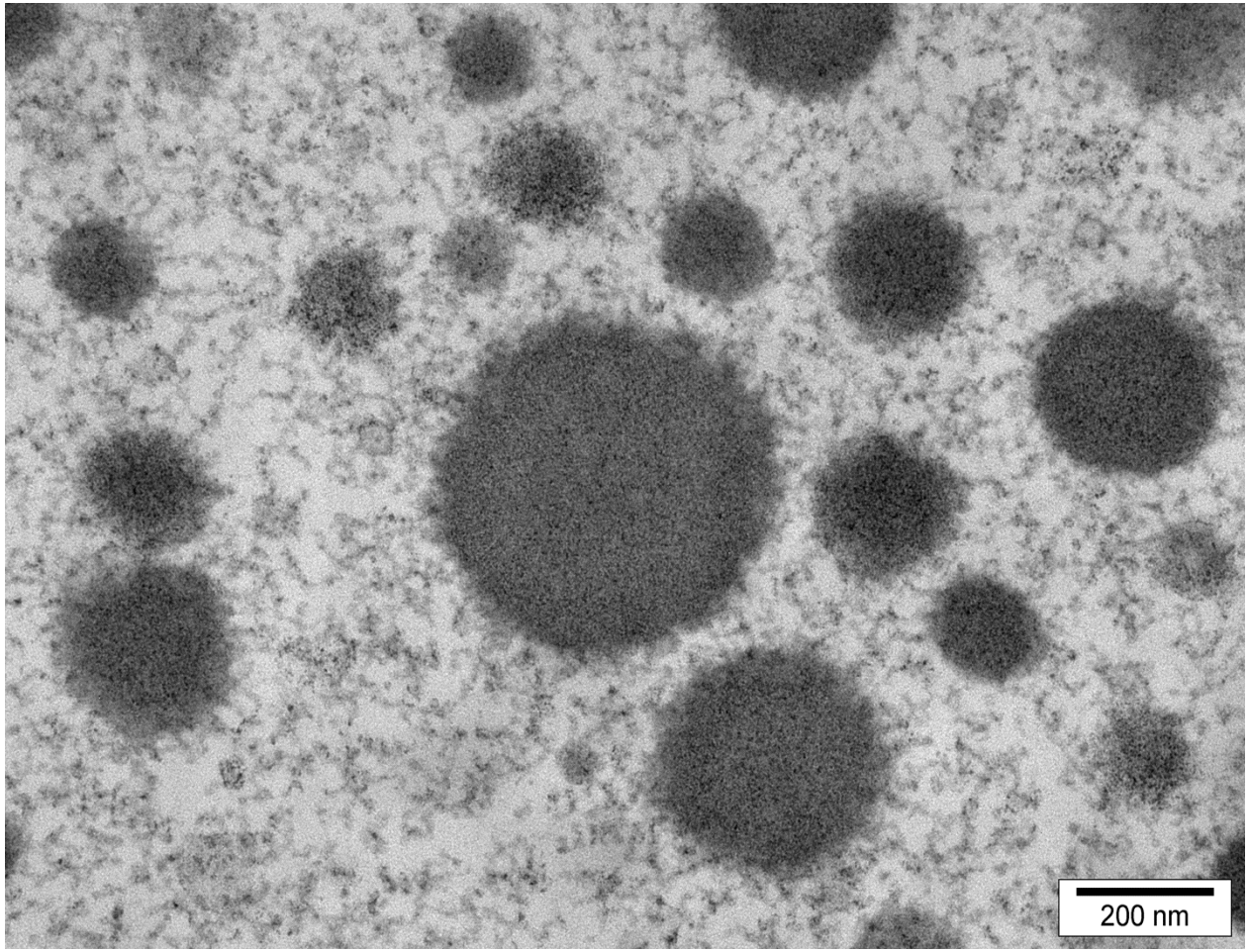








TEM - Retentate at low pH
Christina Coker, Fonterra



Micelles swelling and beginning to dissociate

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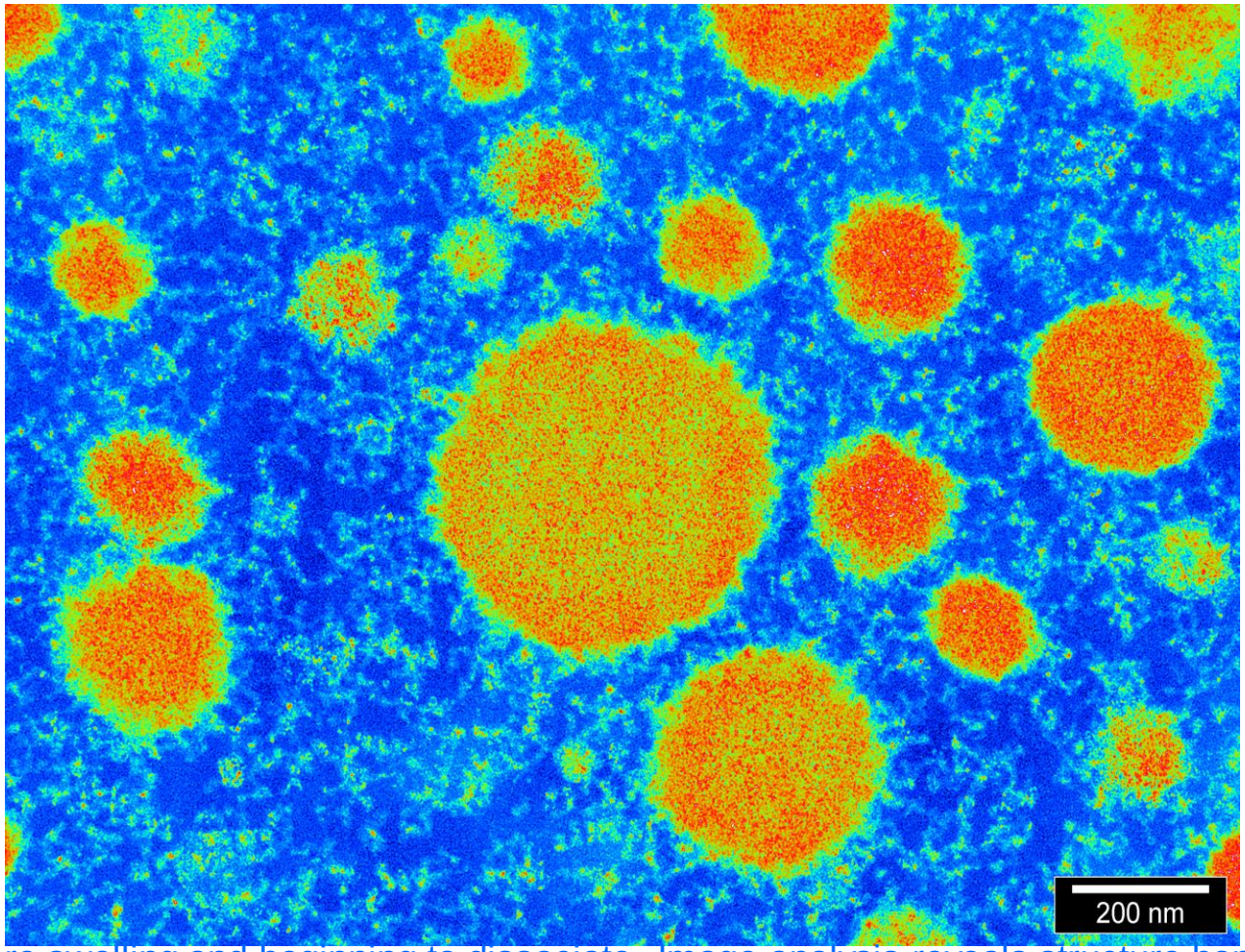
TEM - Retentate at low pH – Christina Coker, Fonterra



Micelles are swelling and beginning to dissociate – Image analysis reveals structure homogeneity and dense particles (orange/red) that may be calcium phosphate nano-clusters. (Blue is lowest density – background)

Fig. 3a. Model of casein micelles proposed by Waugh 1958 [44]

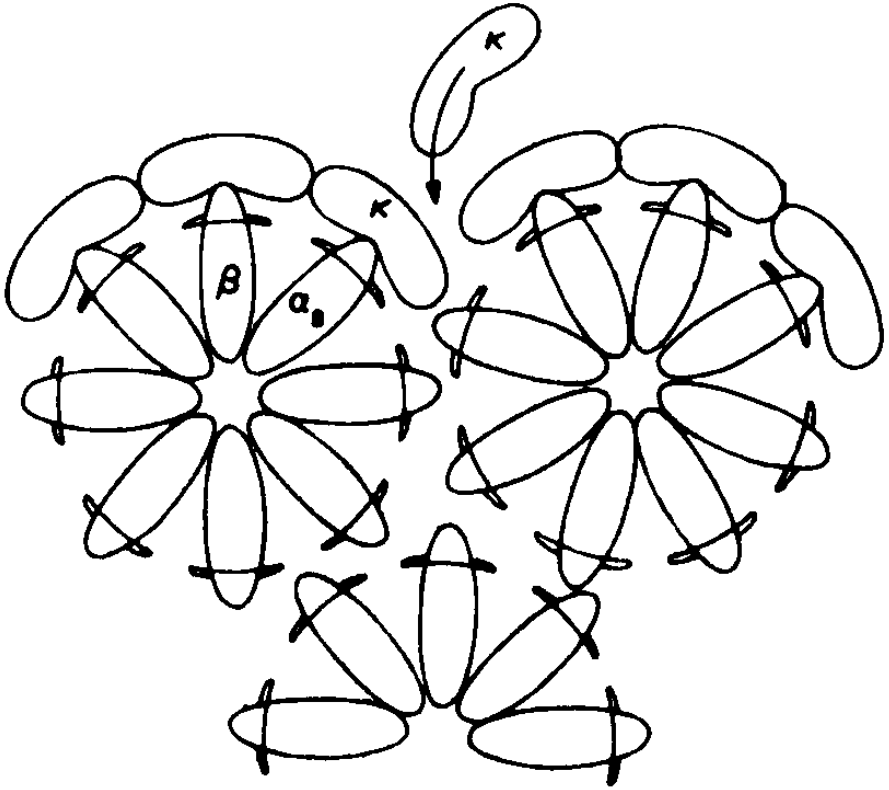


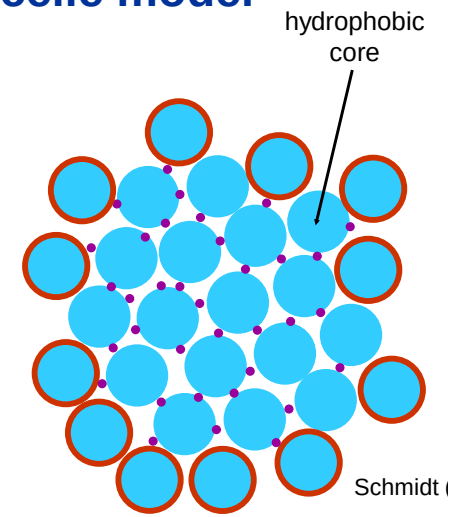
Fig. 3b. Representations of the model of casein micelles proposed by Schmidt [45]

sub-micelle model

low in κ–casein

rich in κ–casein

CaP cluster



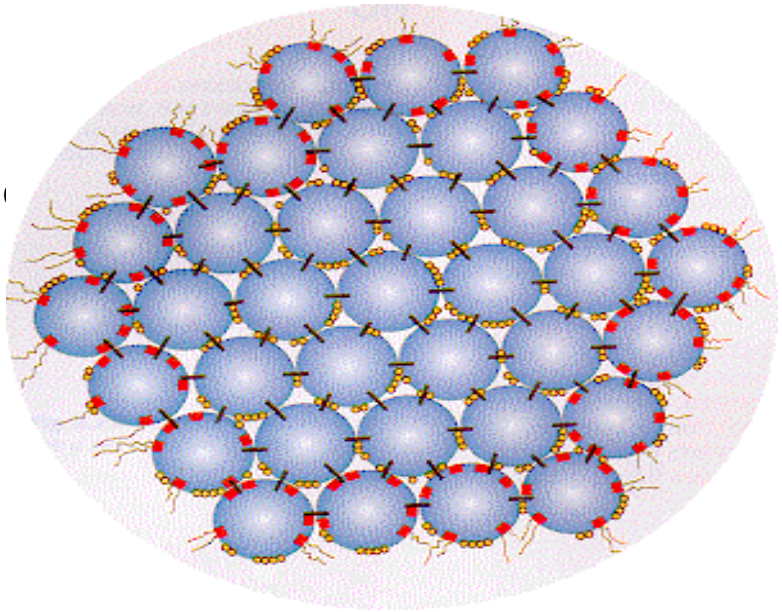
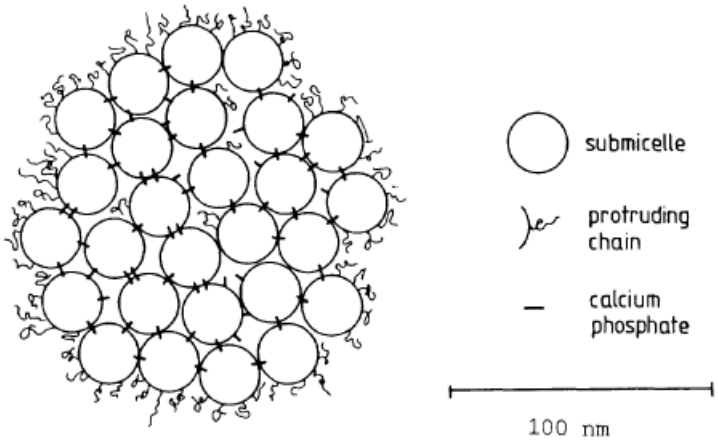


Fig. 3c. Representations of the model of casein micelles proposed by Walstra 1990 [46] and 1999[47]. Note the change in scale and position of the CCP



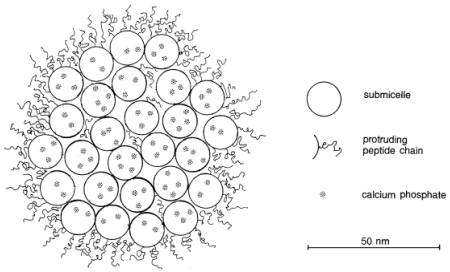


Fig. 3d. Representation of the dual binding model proposed by Horne 2003 [48], and the interpretation of Schmidt's model in a review 2005 [15]

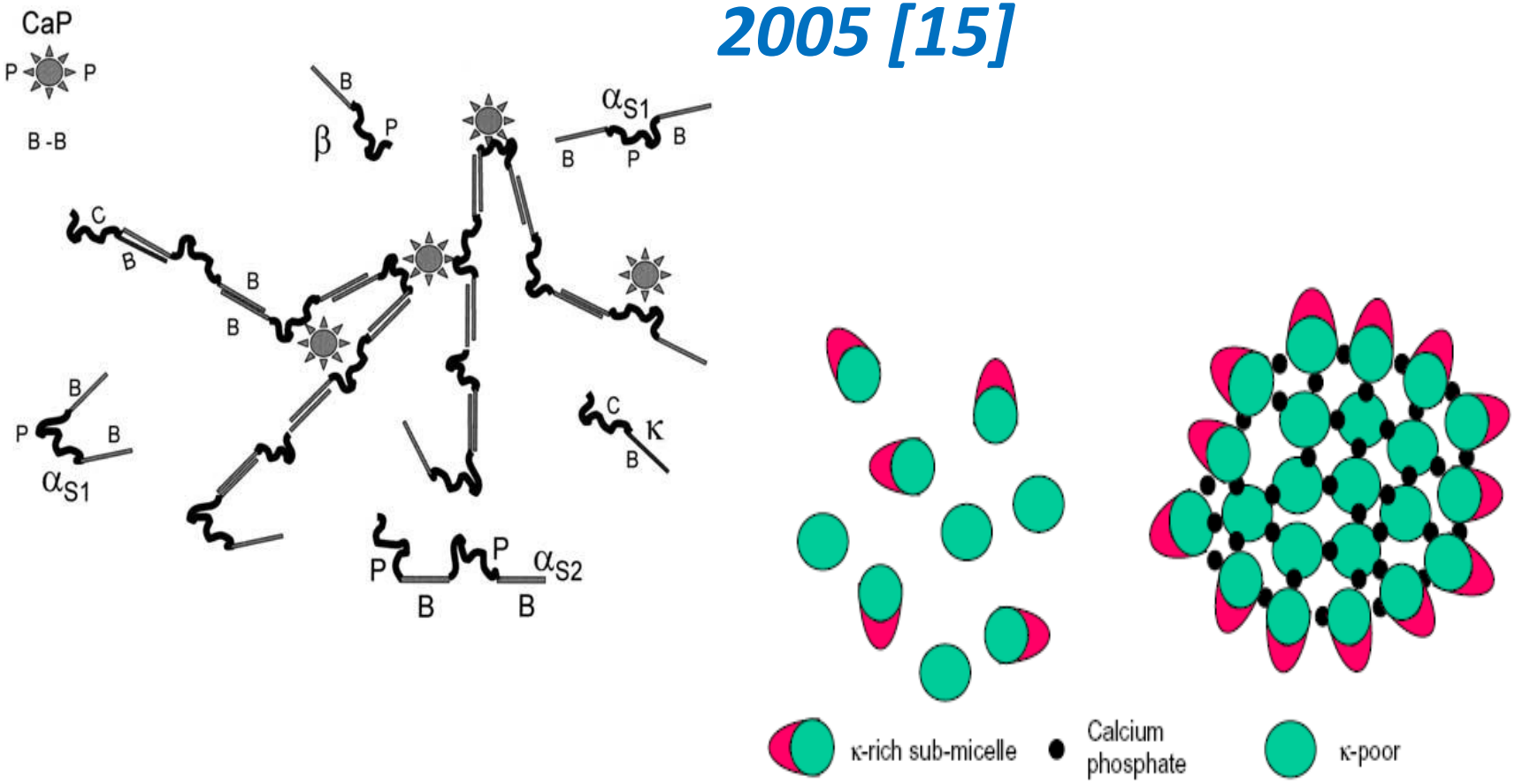
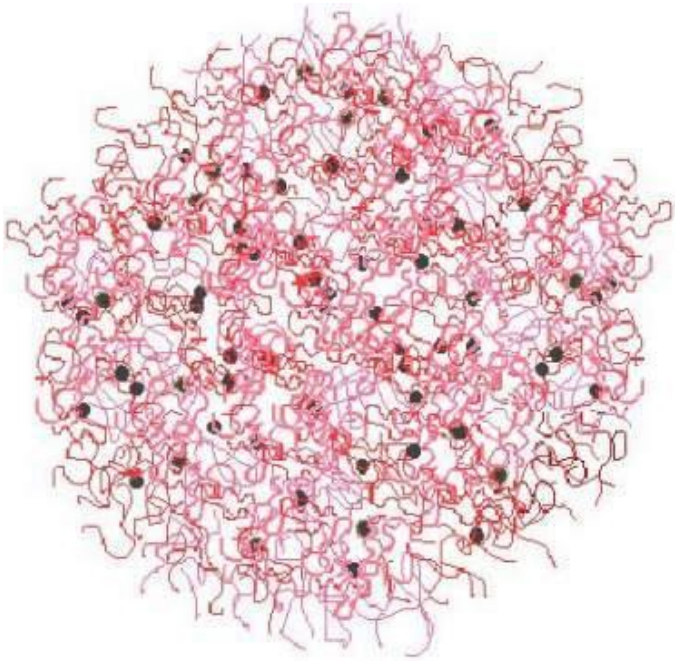
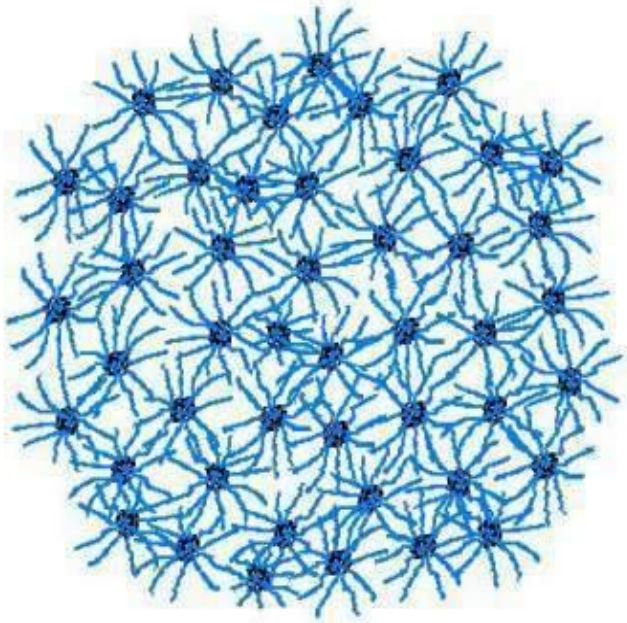


Fig. 3e. Representations of the model of casein micelles proposed by Holt, [50,12]





Alice in Wonderland
The Cheshire cat

‘**Which casein model should I believe** ,'
said Alice.

‘**Which one do you want to believe?'**
said the cat.

‘I don’t know ‘
said Alice

‘**Well then it does not matter where you go to**’
said the cat

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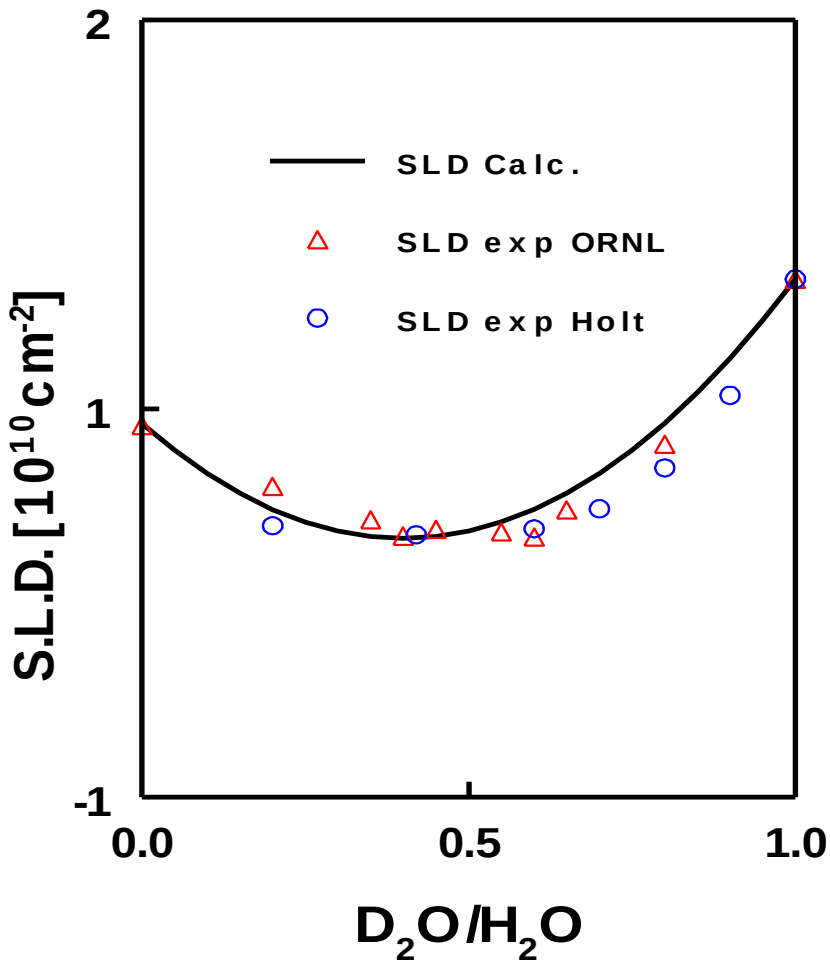
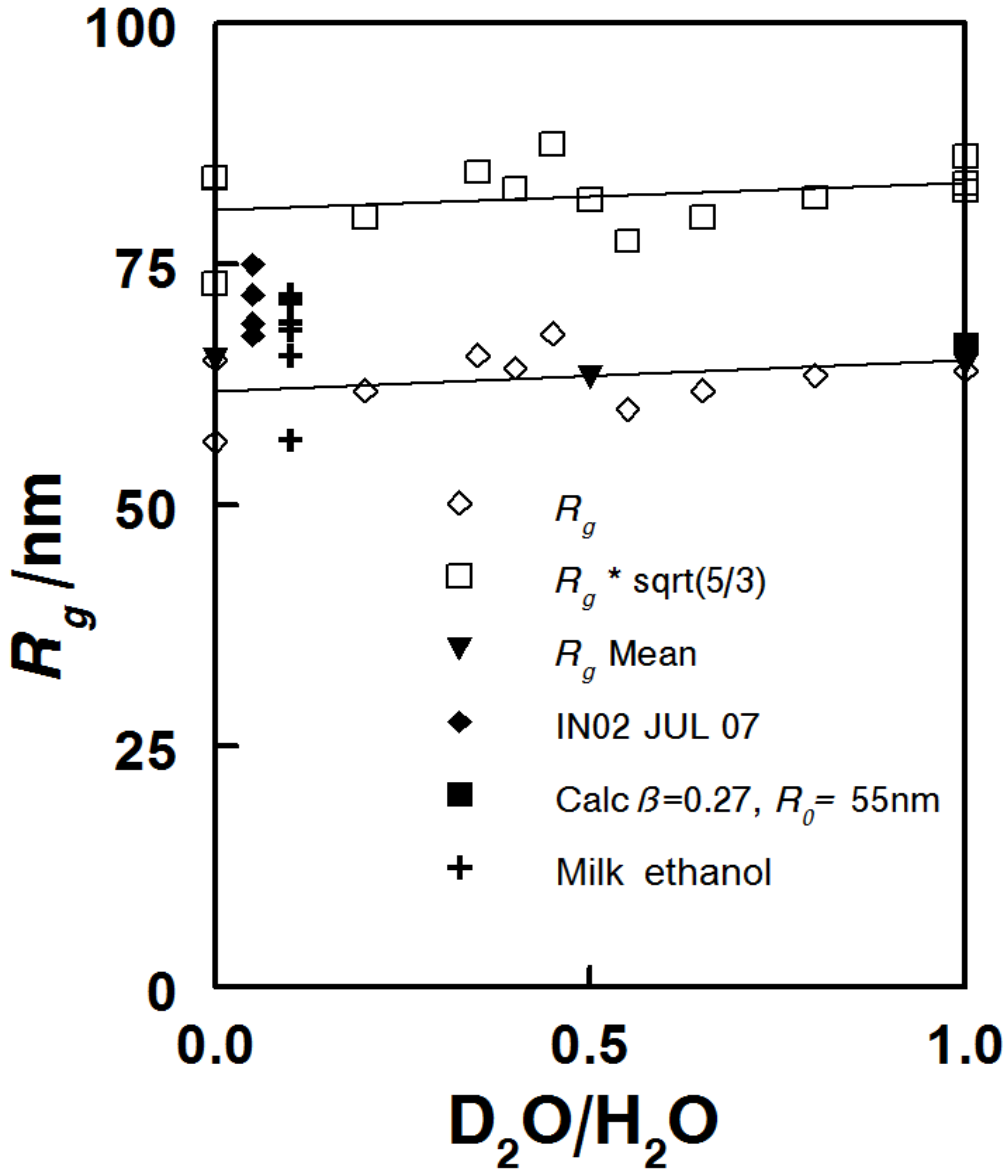
Scattering length density

Actually this means we got the composition right

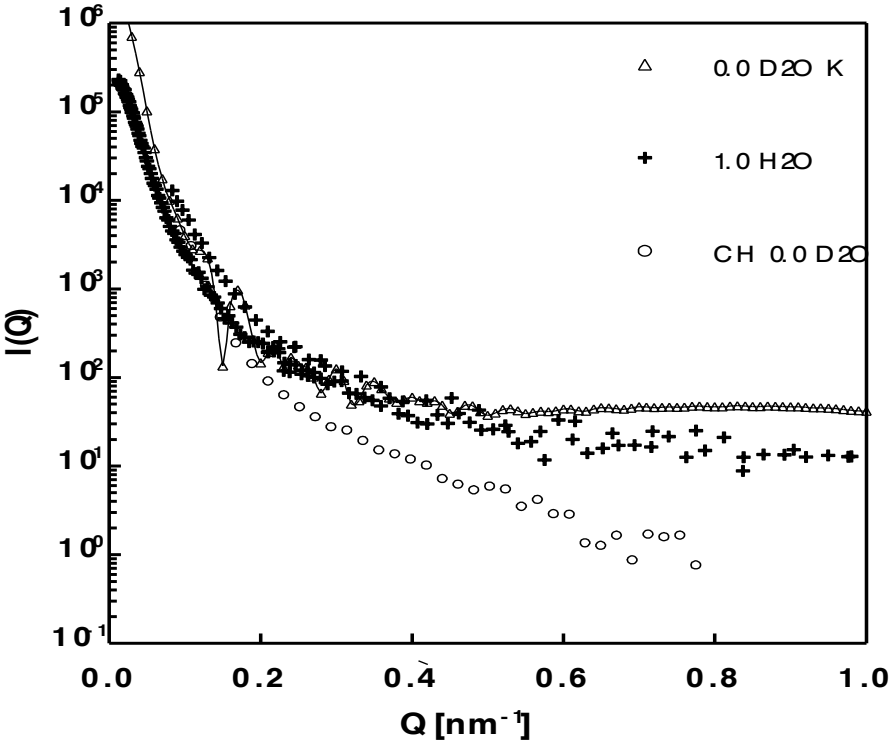


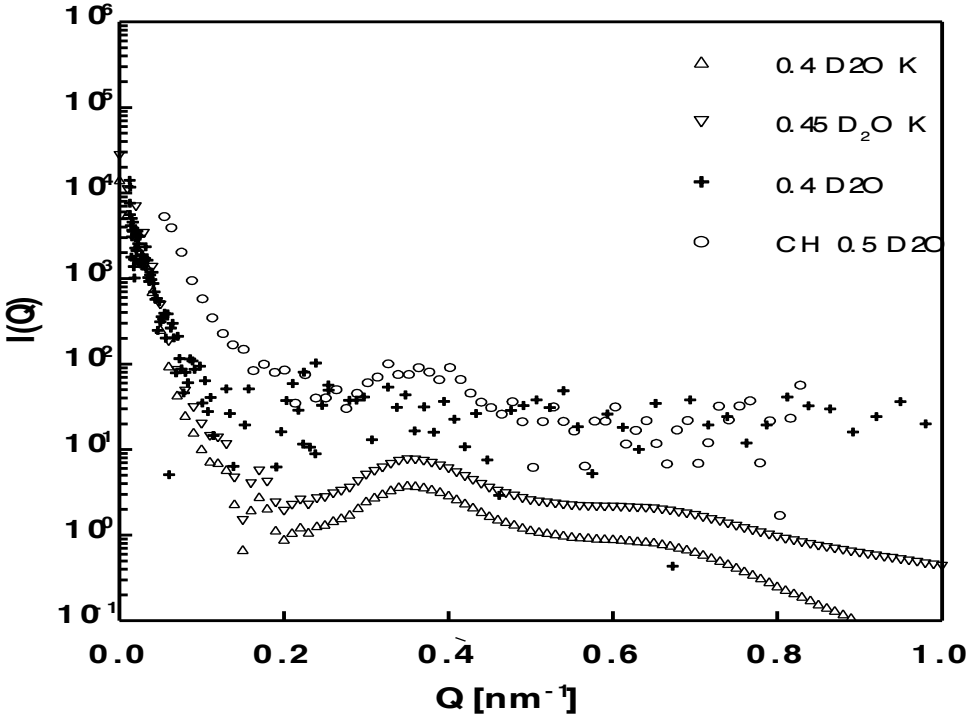
Optical moment of Inertia

Means: CM are homogeneous



H2O and 40% D2O





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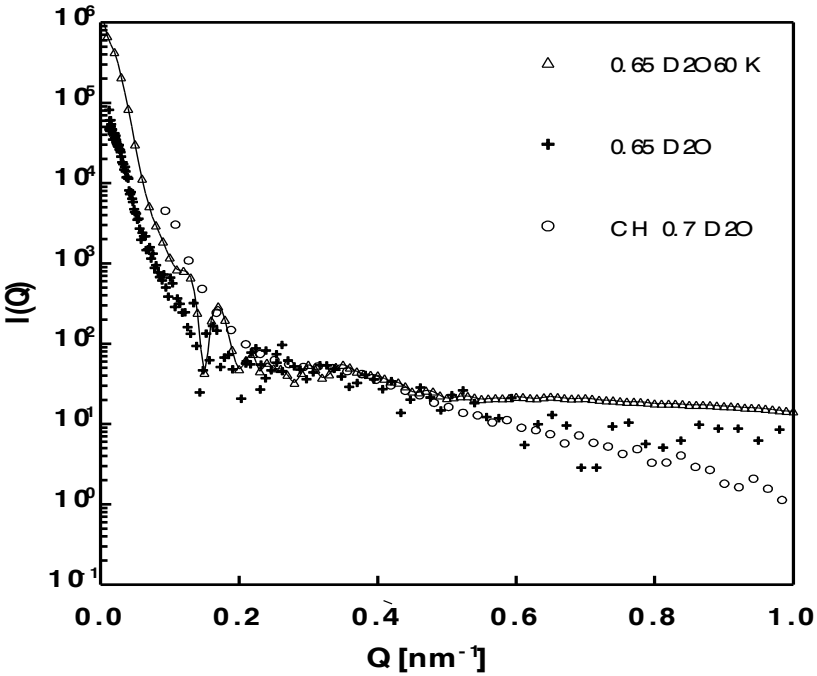
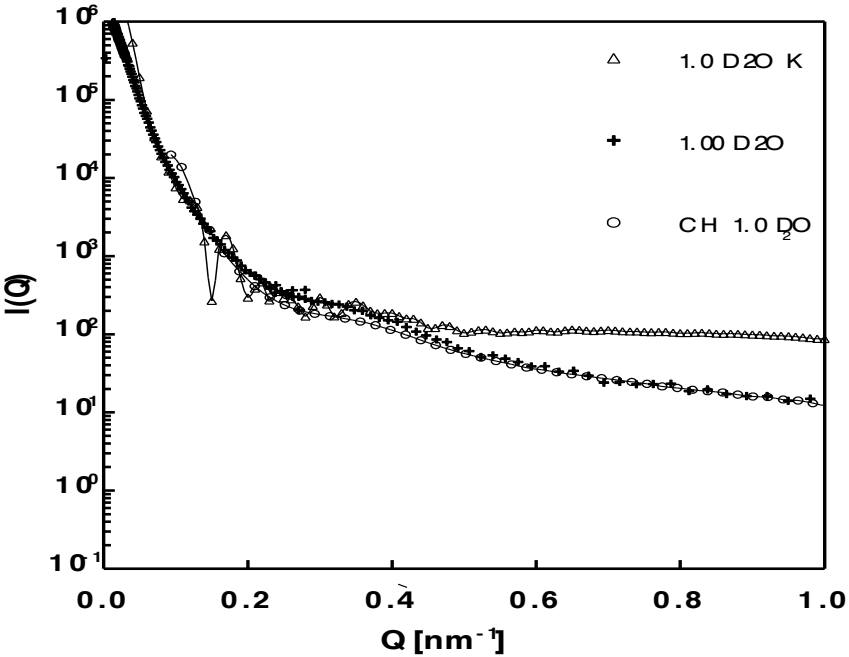
60% D2O 100% D2O





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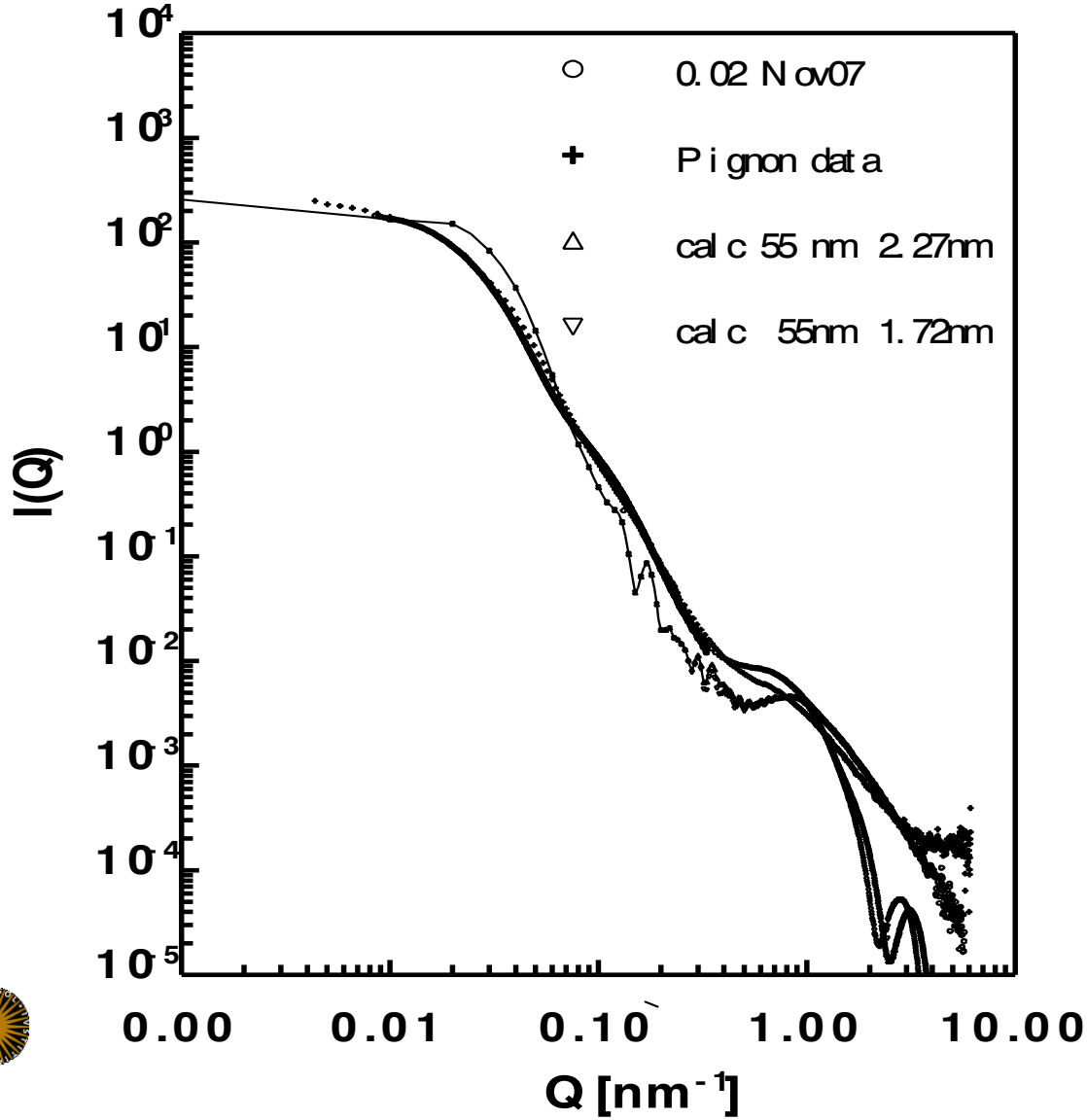
SAXS



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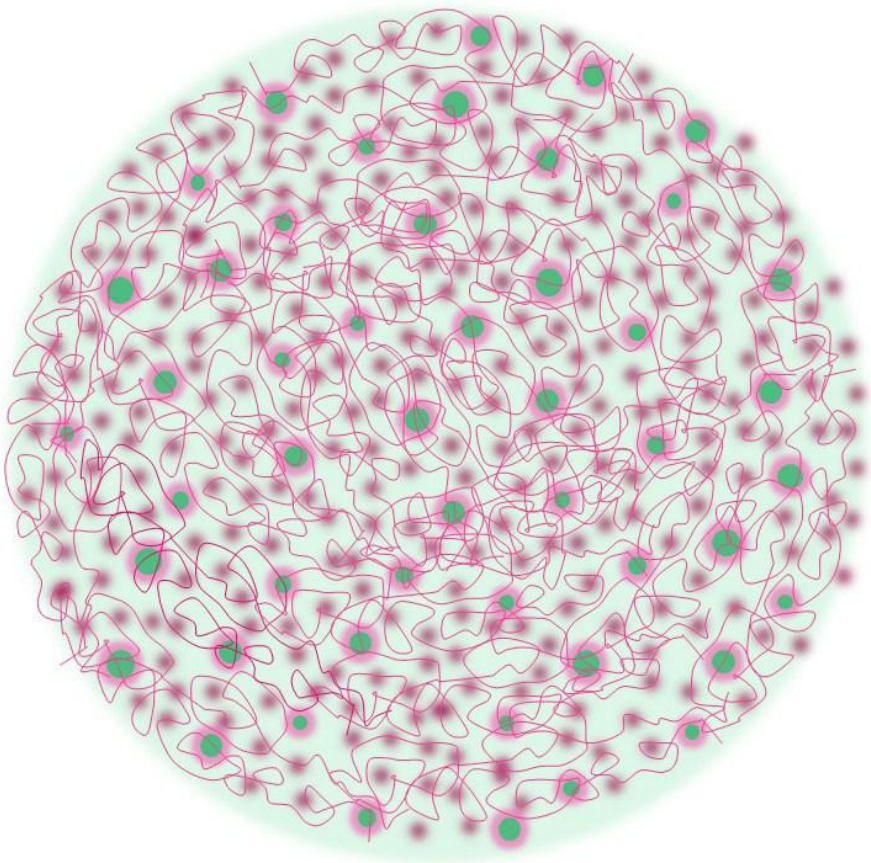
Advances in Colloid and Interface Science 2012

Parameter	symbol	Cow 68 (Pooled milk) [units]	Experimental
Casein micelle center rad.(ln-norm)	R_{10}	55 (60) [nm]	DLS
Polydispersity	β	0.27 (0.35) [-]	DLS
Volume averaged radius	R_3	61.1 (71.3) [nm]	Viscosity, DLS
Radius of gyration	R_{gyr}	90 (135) [nm]	SANS, SLS
DLS radius (λ=541,Θ=90)	R_{hydr}	80 (100) [nm]	DLS
Voluminosity	-	4.4 [ml g^{-1}]	Viscosity
Mass density (hydrated)	-	1.078 [g ml^{-1}]	Analytical UC
Casein micelles per liter	N_{cm}	1.14 10^{17} [L^{-1}]	Calculated
Nanocluster	r_{nc}	1.72 to 2.27 [nm]	SANS/SAXS
Nanoclusters/ Casein micelle	N_{nc}	285 (372)	Calculated
Correlation length	ξ	9.3 [nm]	SANS
Packing fraction NC/prot. Clust.	-	0.4 / 0.2	estimated
Protein clusters	R_{prot}	2 nm	SAXS
Correlation length	ξ_{prot}	3 nm	SAXS

Artist impression of casein micelles based on predictive calculation

Advances in Colloid and Interface Science 2012



Homogeneous distribution of matter

285 NC 2nm Radius

NC 18.6 nm apart

Protein matrix

small clusters 2 nm

No submicelles, no channels no …..

¾ H20 ¼ PROTEIN 7% NC

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Mother nature is not sloppy

- CM constant in time
- CM quite mono-disperse in size
- Polydispersity scales with size
- Variation between cows
- Not between race?!
- Relative protein composition is constant
- Genetics??
- Cows were strongly related!!

Now what´s the use of it?

- Materials science approach
- Zero fat cheese The golden cheese
- Zero fat Ice cream
- High protein drinks
- Functionalized skim milk powders

- Patent applications
- Confidential work for clients

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- @ deKruif

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Martha

Protein-Protein interactions

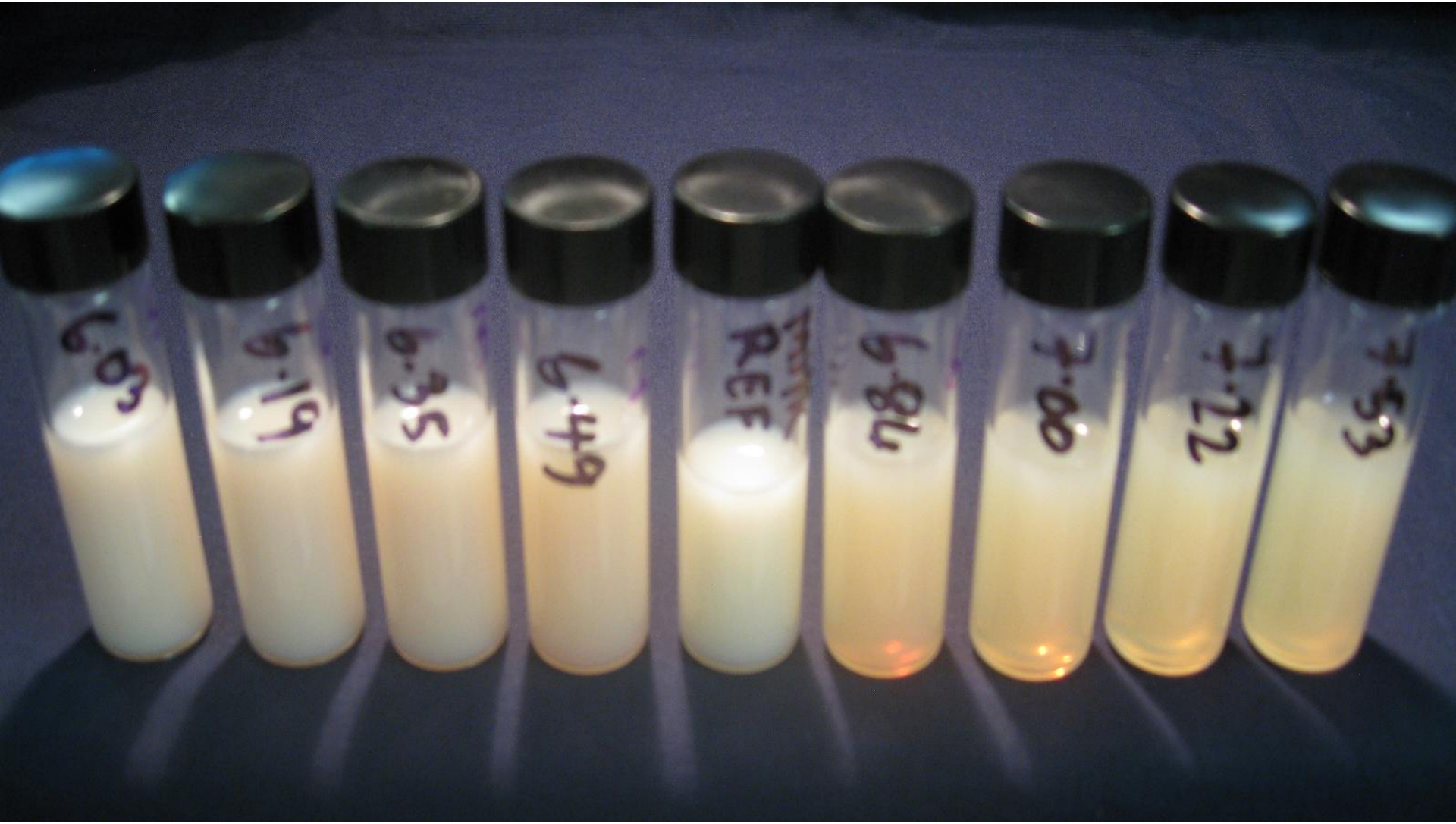
@Fonterra with Skelte Anema

Charge (distribution) matters

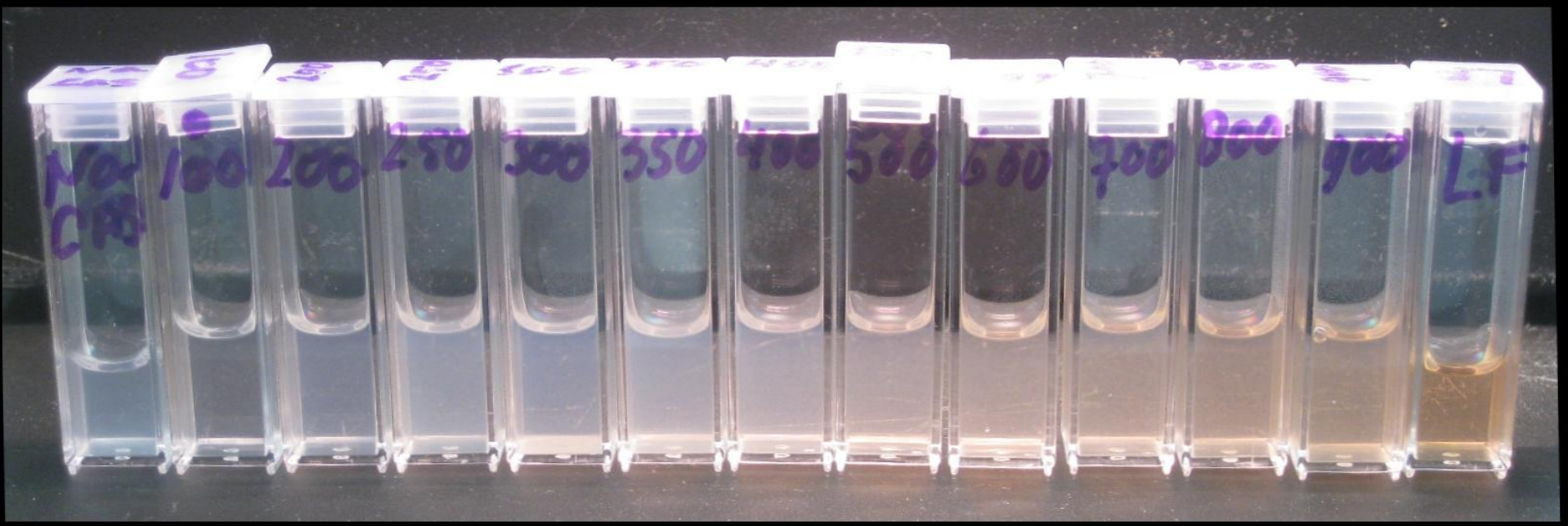
A simple illustration

Mixture of Lactoferrin and casein micelles

Skim milk + Lactoferrin (pH)



Na – caseinate + Lactoferrin Complexation



245 Skelte G. Anema and C. G. (Kees) de Kruif
Interaction of Lactoferrin and Lysozyme with Casein Micelles
| Biomacromolecules 2011, 12, 3970−3976

246 Skelte G. Anema2 and C. G. (Kees) de Kruif Co-acervates of lactoferrin and caseins
Soft Matter, 2012, 8, 4471

Alice in Wonderland
The Cheshire cat

'But I don't want to go among mad people,'
said Alice.

'Oh, you can't help that,'
said the cat.

'We're all mad here.'
Lewis Carroll

Reprints??

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