

UNIVERSITY OF  
BIRMINGHAM



# U Air Filled emulsions & Hydrophobins

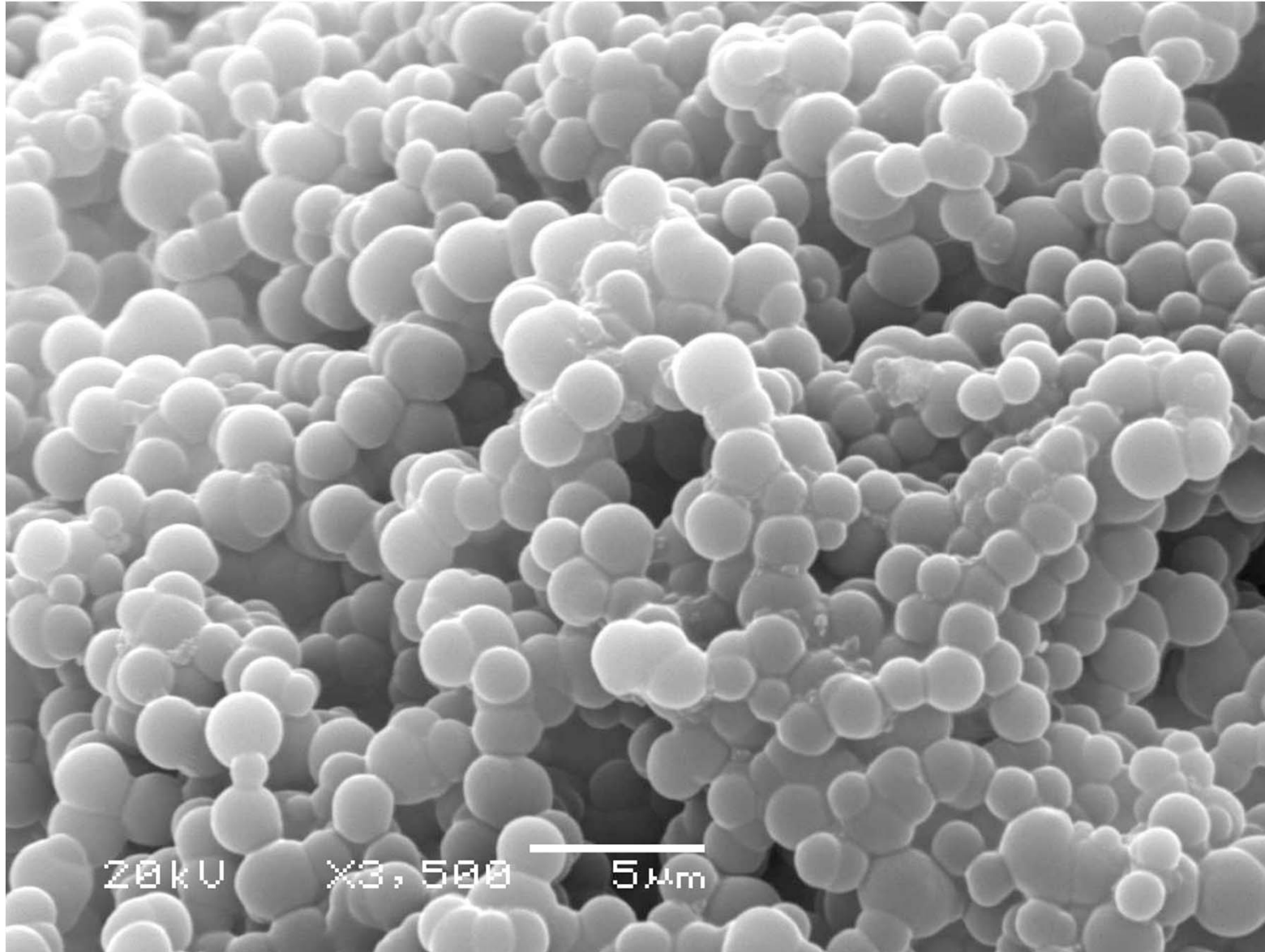


Phil Cox



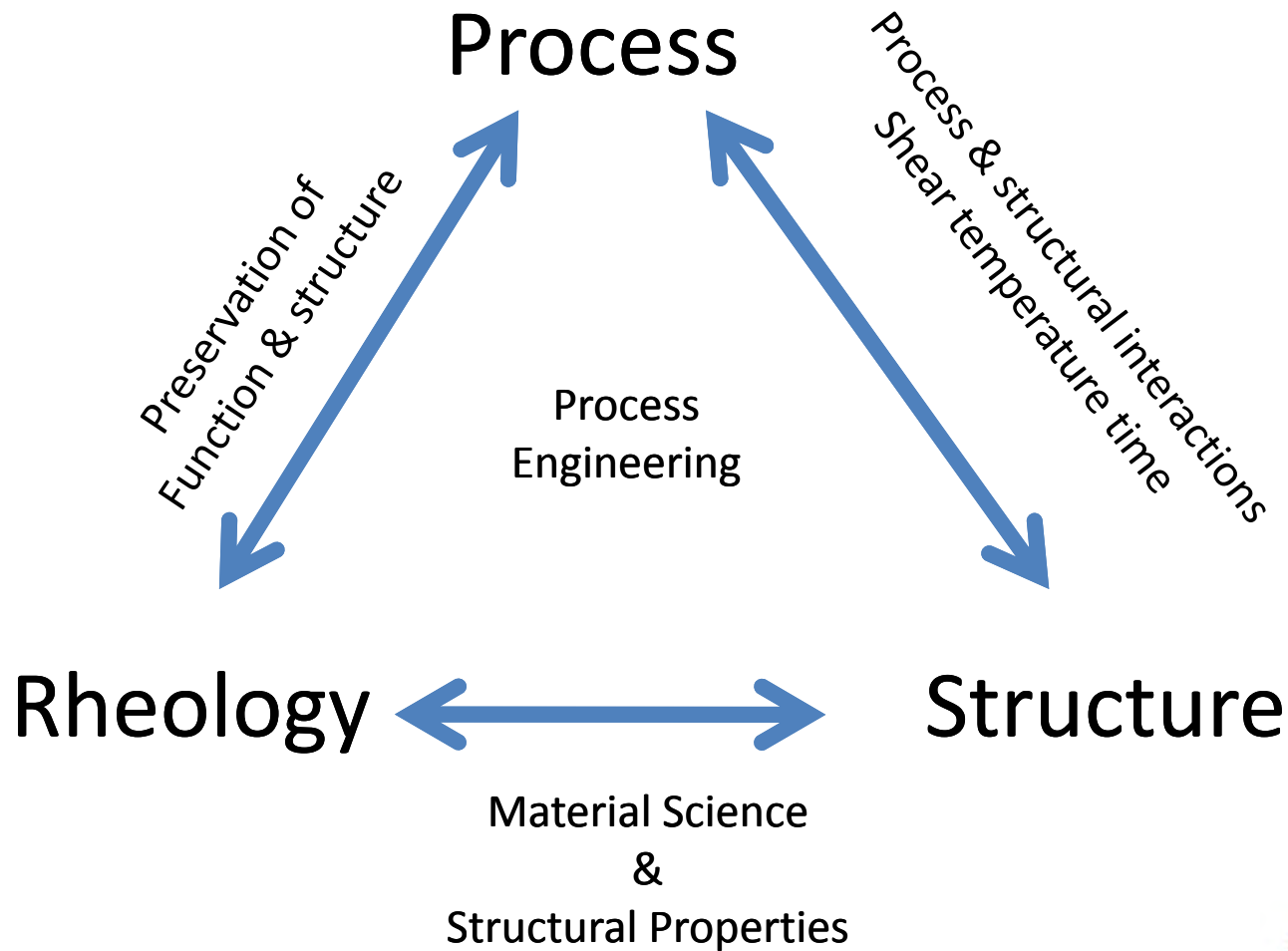
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# How do we get to here?



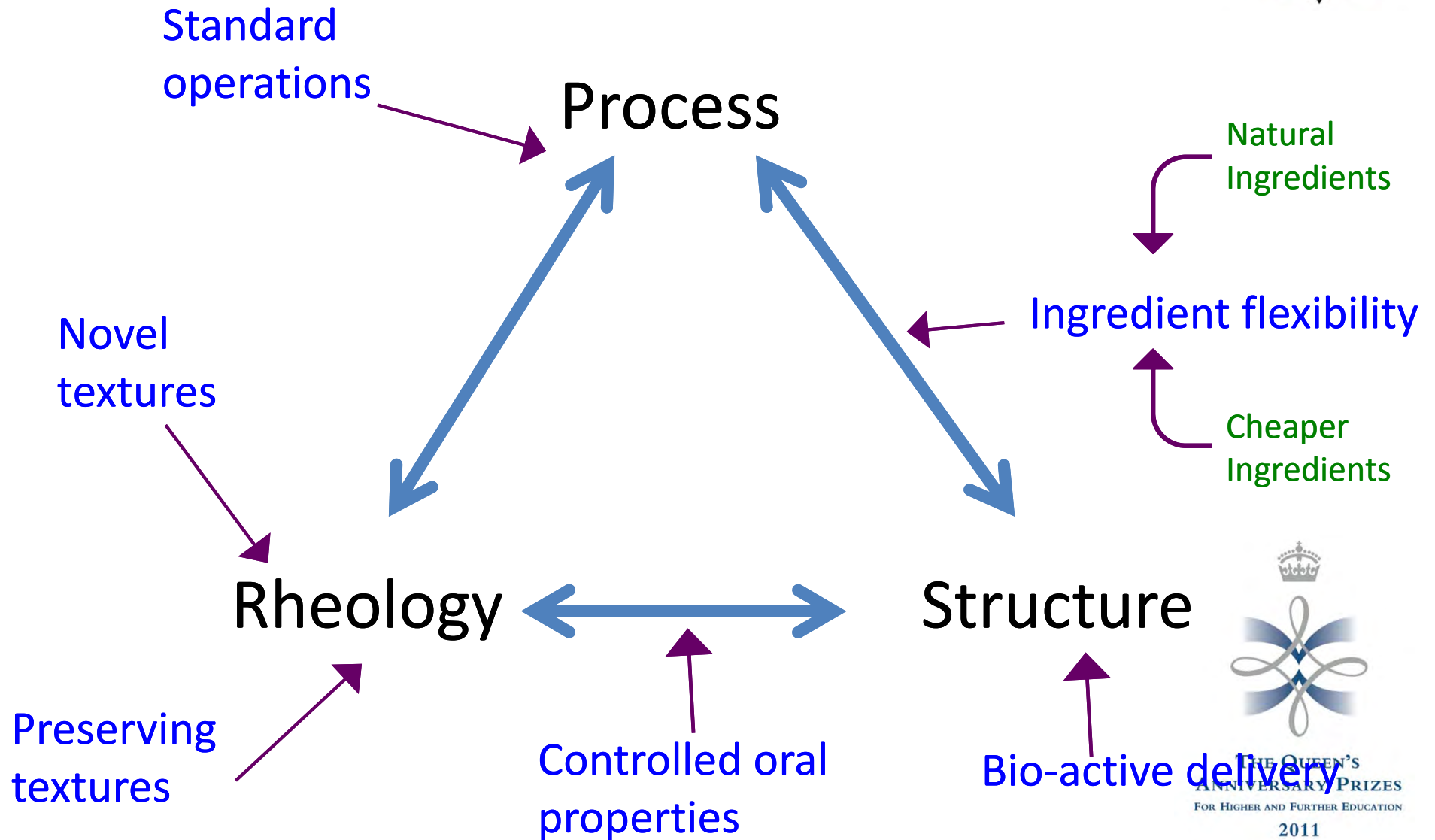
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# Micro-structural approach

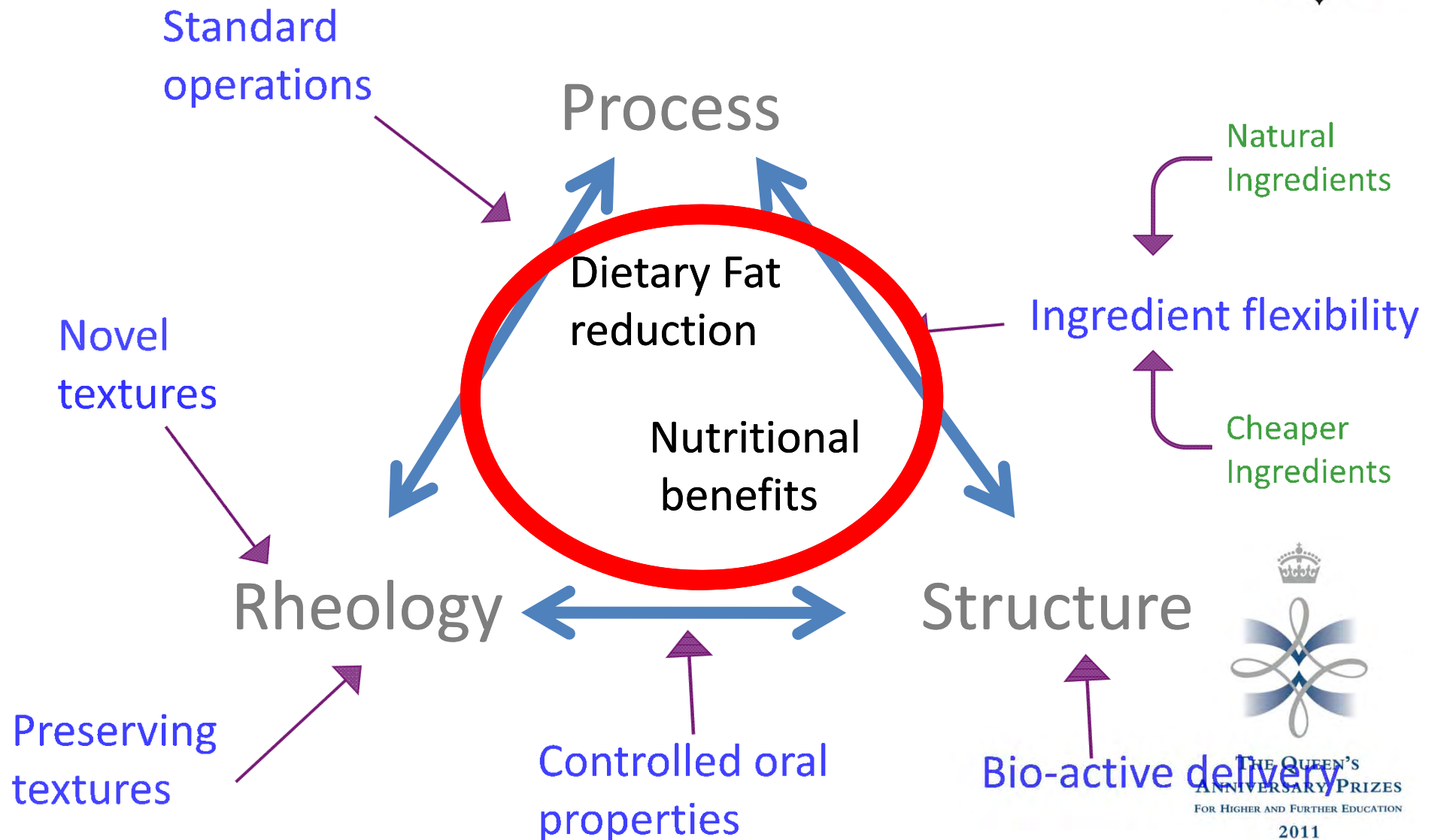


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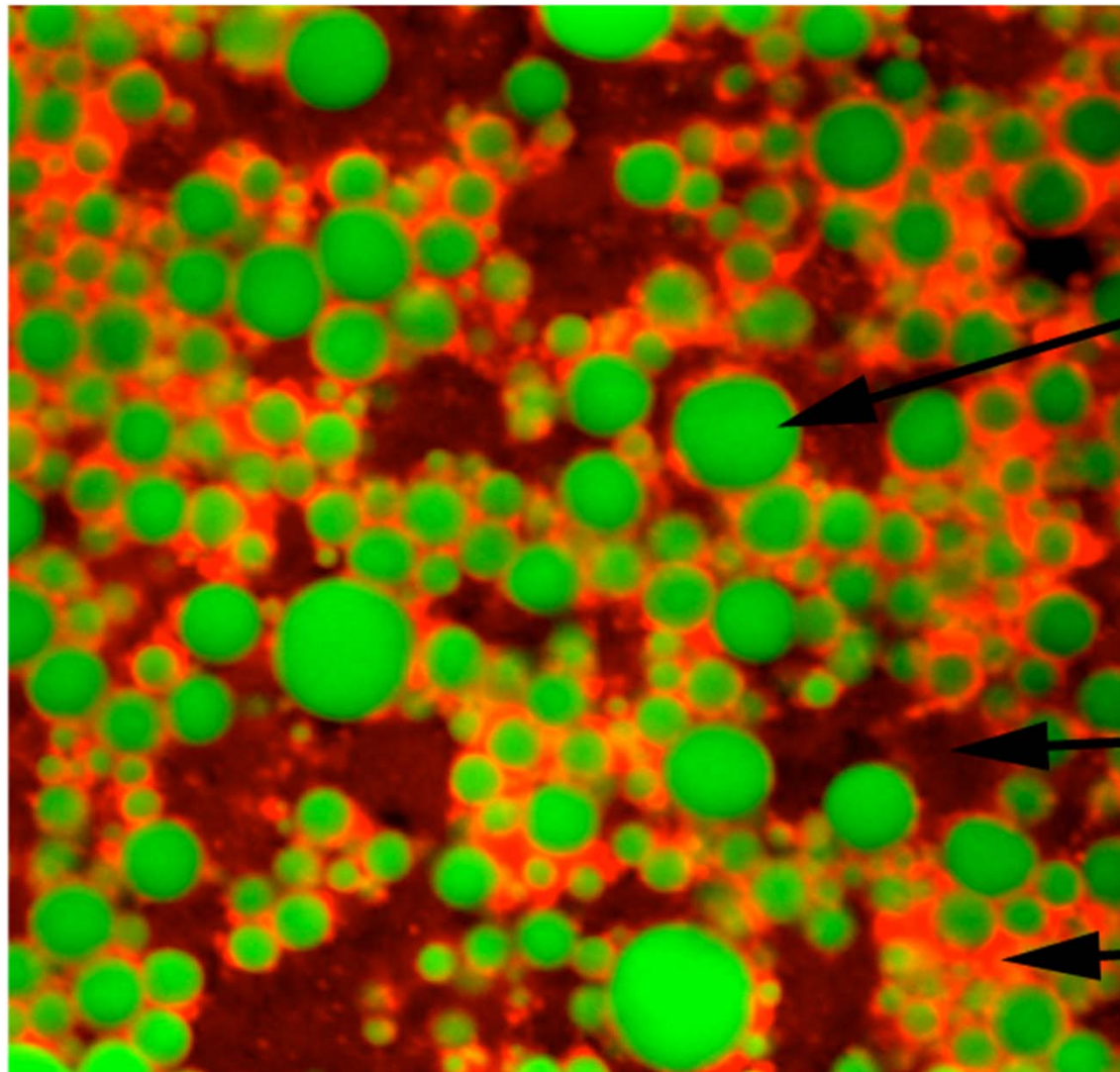
# Micro-structural approach



# Micro-structural approach



# One current approach: reduced dietary fat intake

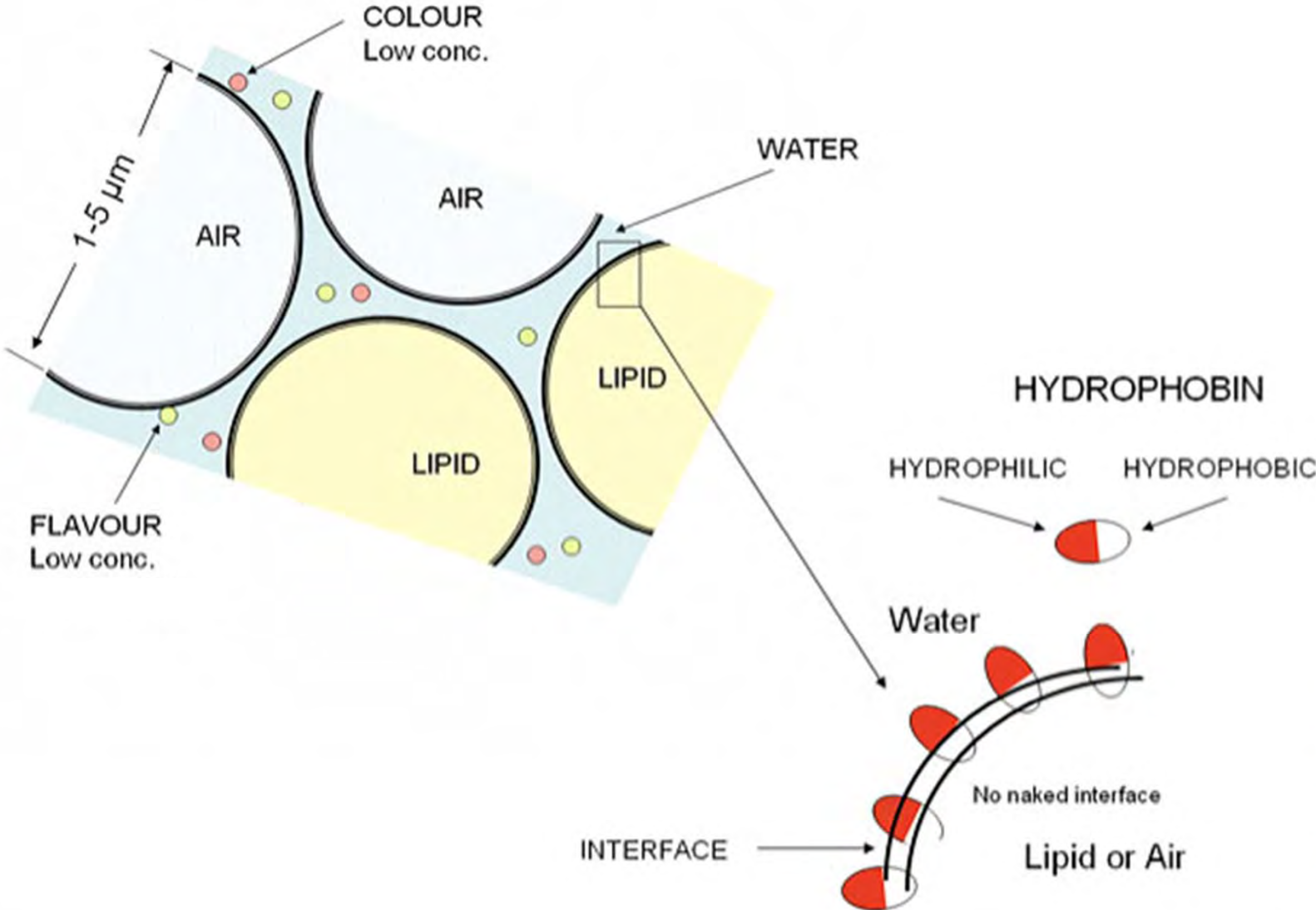


Oil droplet

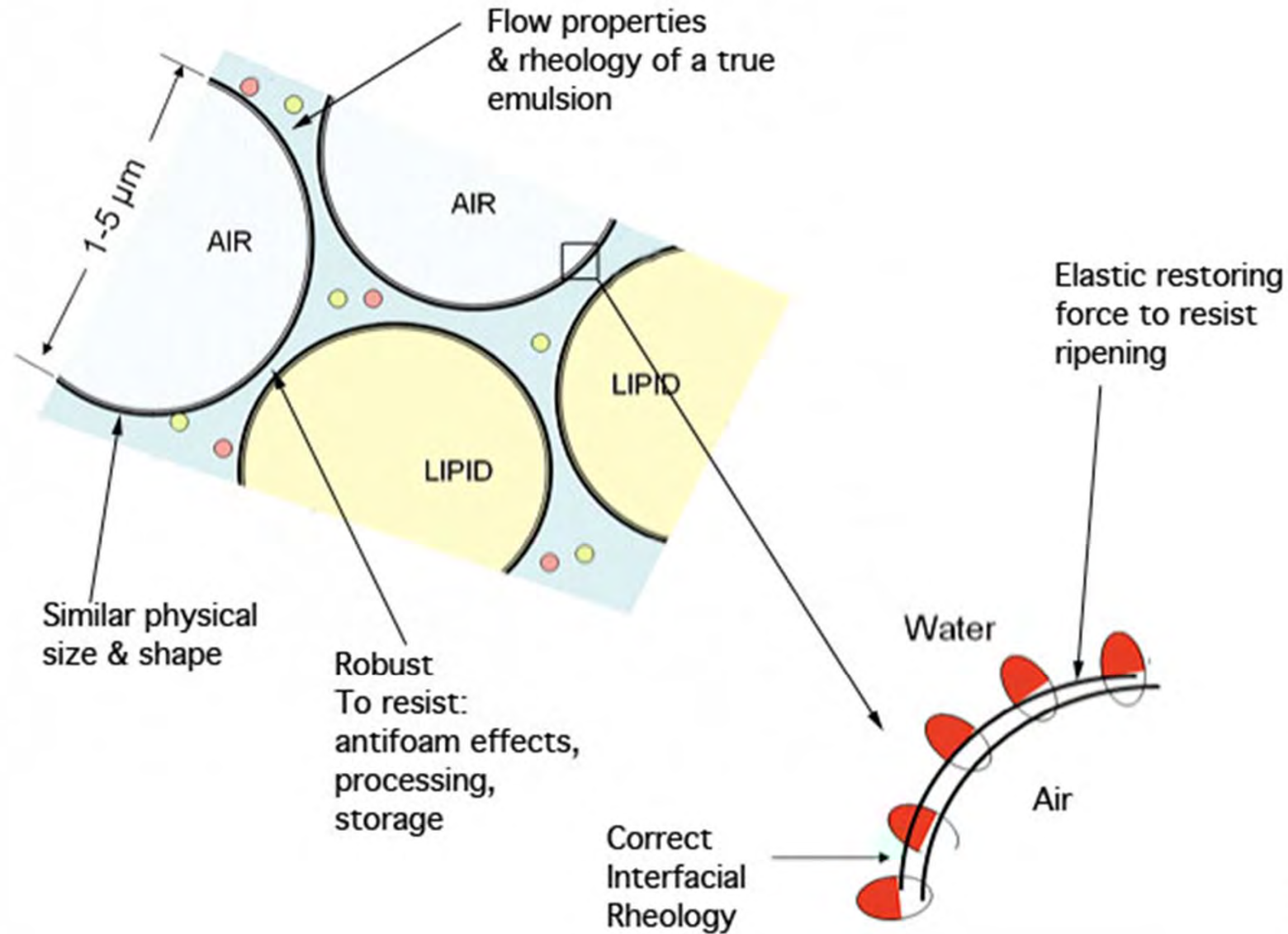
Aqueous  
Phase

Starch

# Air filled emulsions



# Micro-structural Design

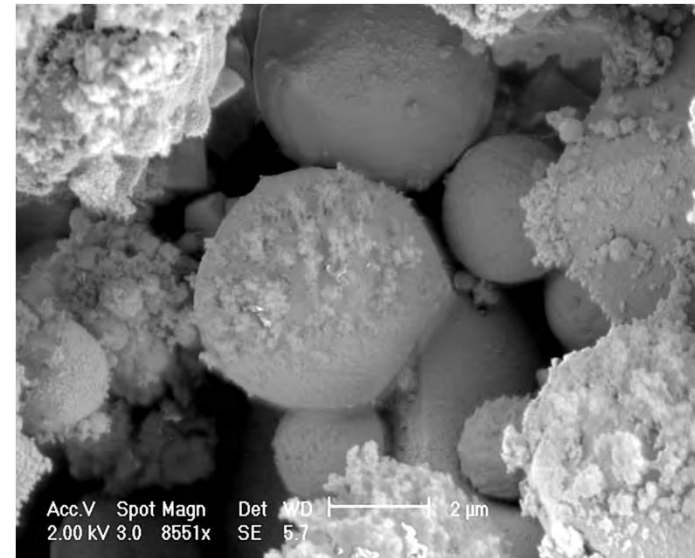




# Criteria for Air Filled Emulsions



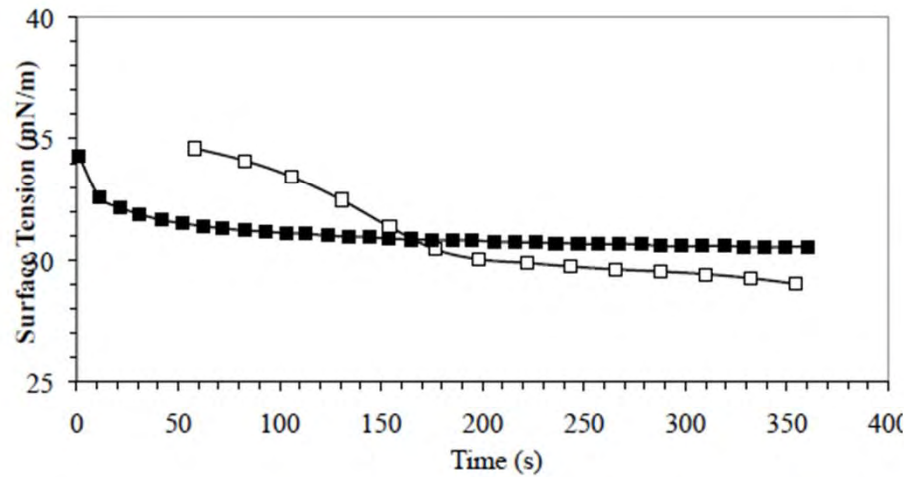
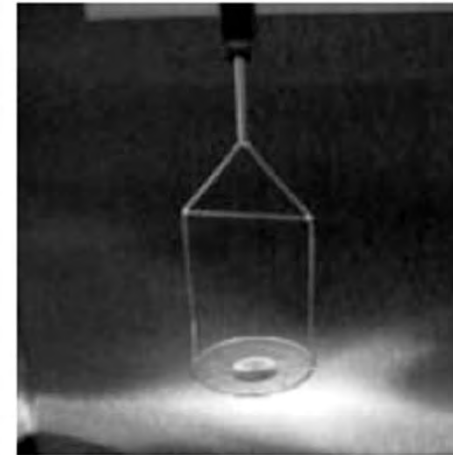
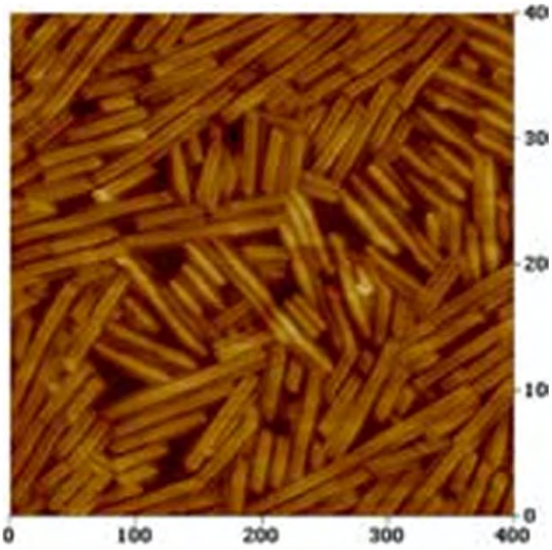
- Reduction in fat content of emulsified foods
- Maintenance of oral and organoleptic properties
- Use of natural materials
- Ingredient flexibility
- Manufacturing & shelf stability



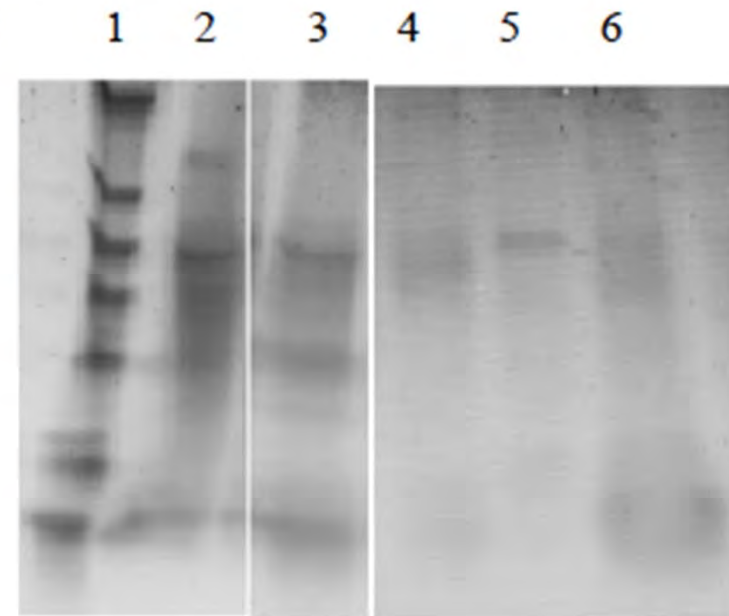
N.B. These are emulsions not foams!



# Hydrophobins



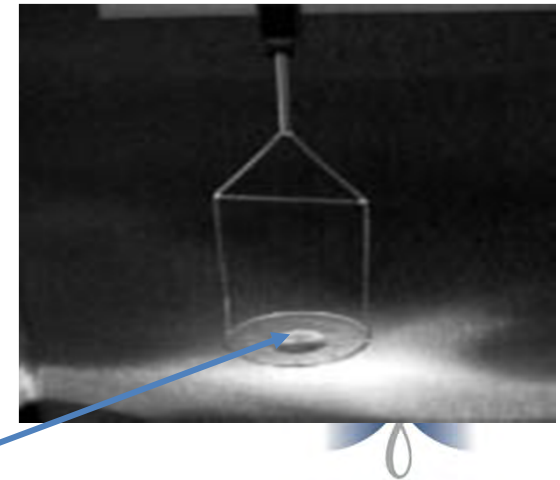
kDa  
98  
62  
49  
38  
28  
17  
14  
6



# Hydrophobins

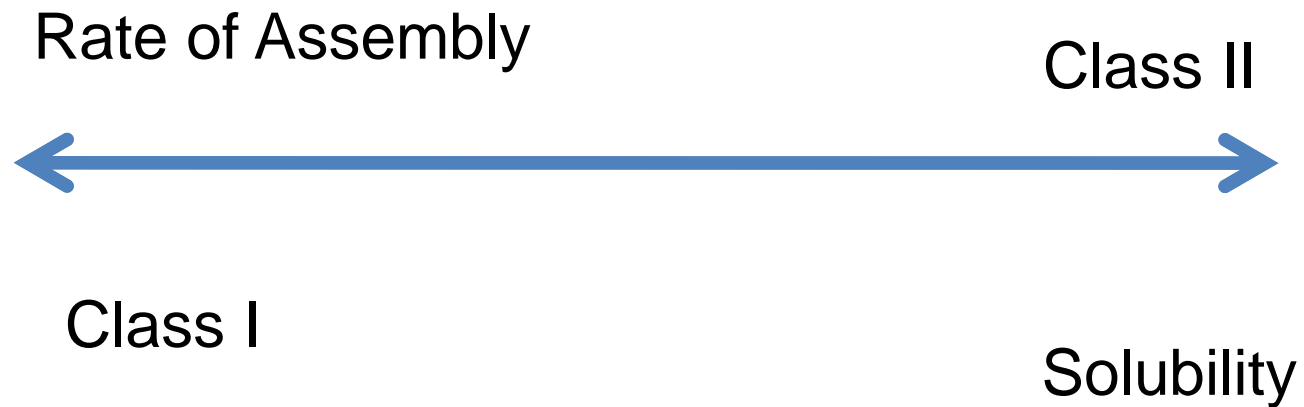


- Ubiquitous to filamentous fungi.
- Small cysteine-rich protein (4 disulfide bonds).
- Chemical and thermal stable.
- Self-assemble at hydrophobic/hydrophilic interfaces (e.g. air/water & oil/water).

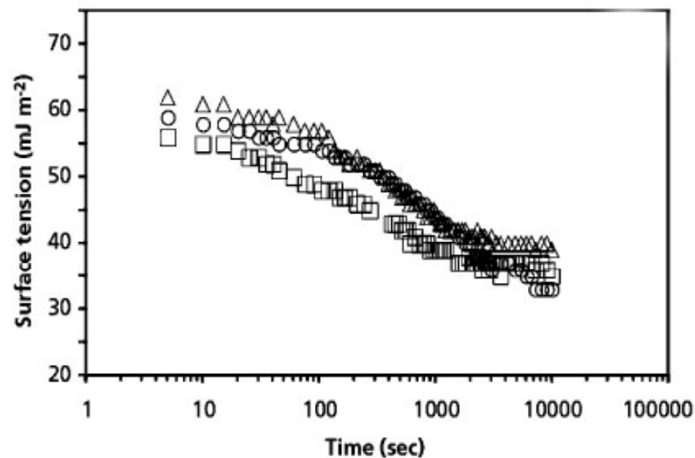
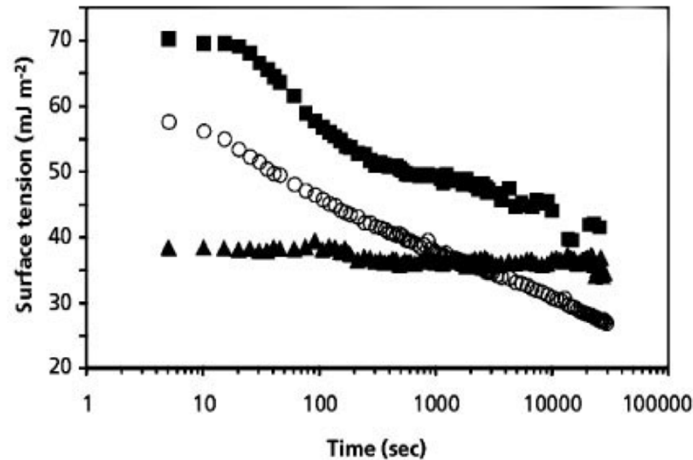


**Hydrophobin film suspending water**

# Hydrophobin Range ?



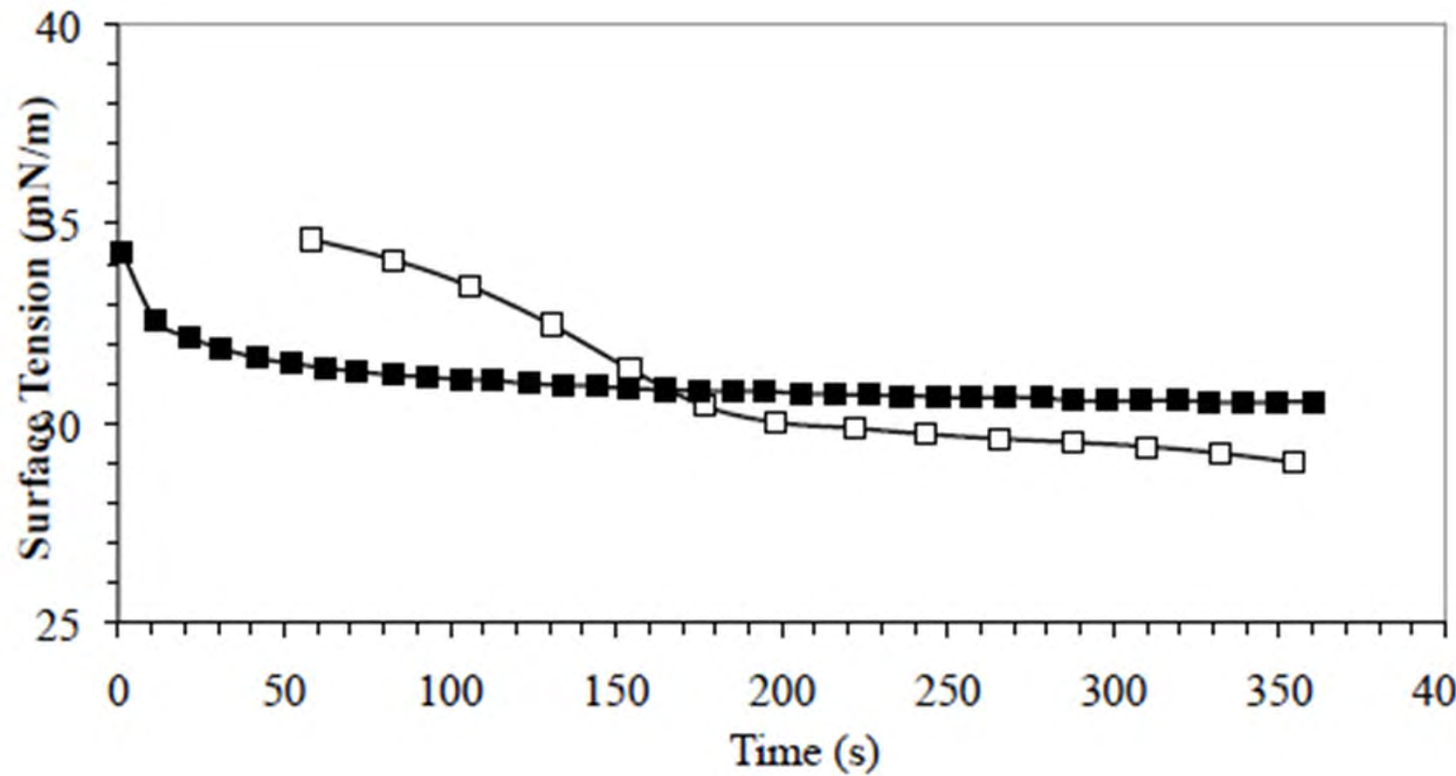
# What does this mean ?



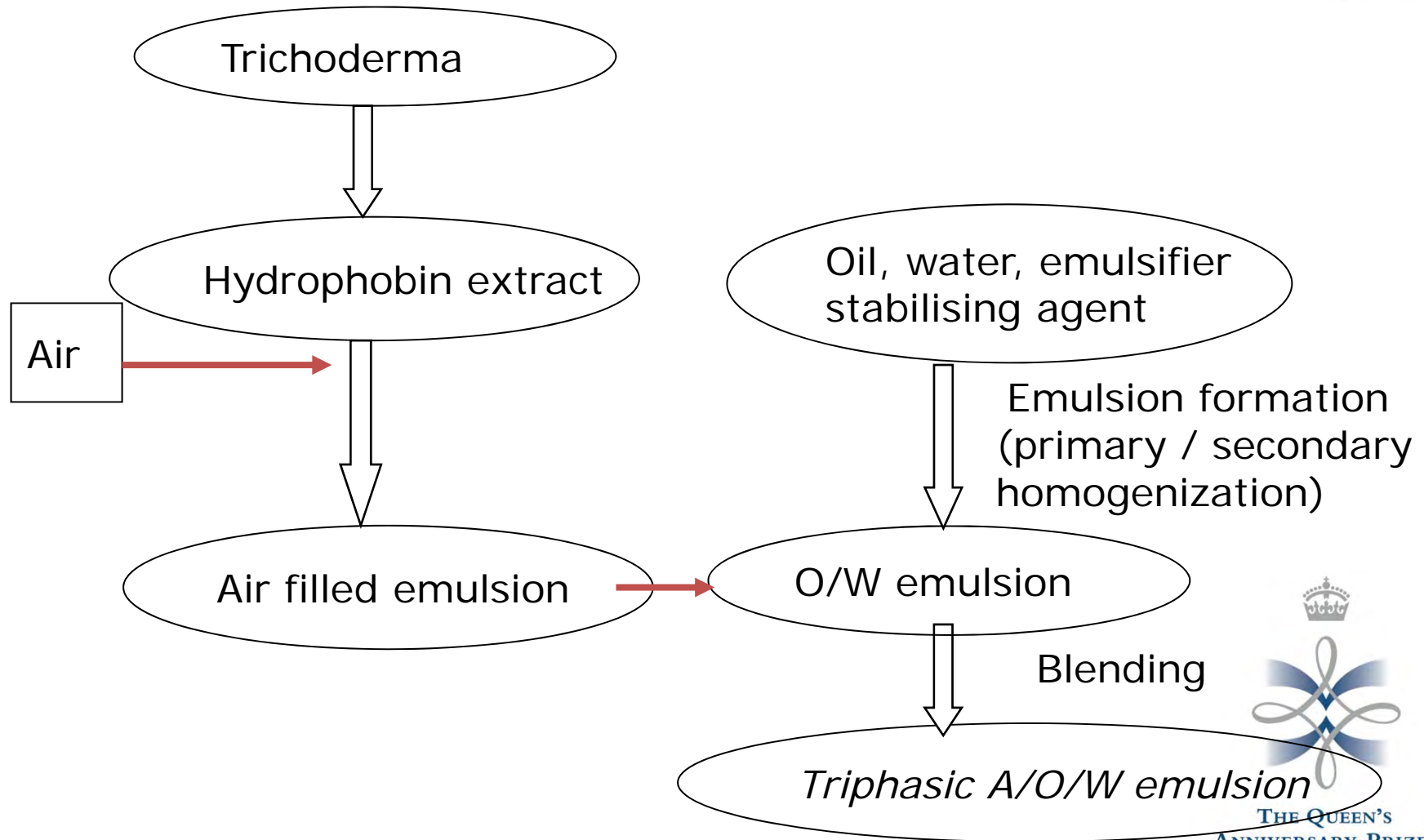
**Figure 1.** Mixed hydrophobins assembly kinetics at an oil water interface: ■ - Class II hydrophobin HFBI; ▲ - Class II hydrophobin HFBI; ○ - Class I hydrophobin SC3; △ - Mixture of HFBI & SC3; □ - Mixture of HFBI & SC3. (reproduced from Askolin *et al.*, 2006)



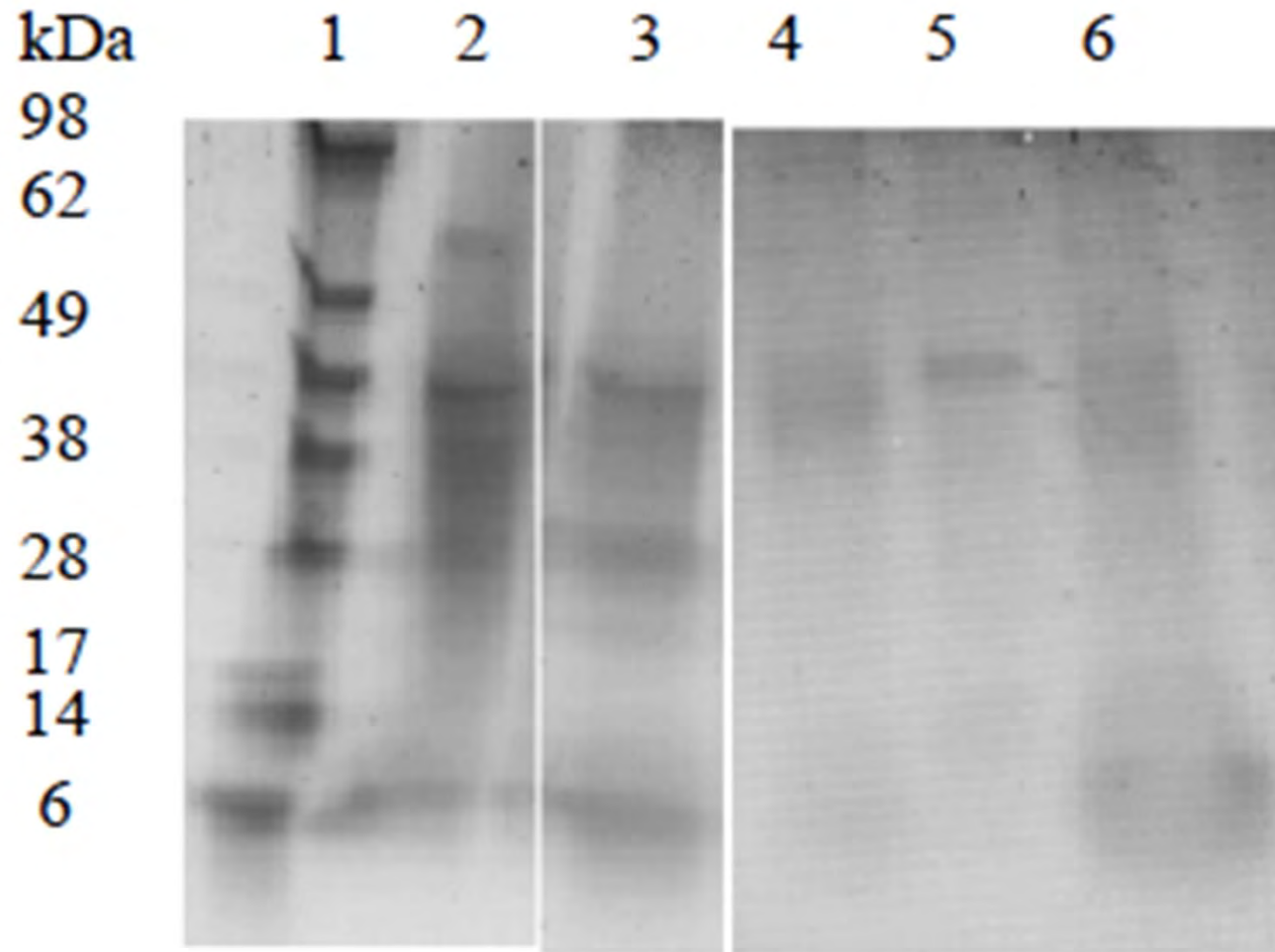
# Interfacial assembly & Mixed Proteins



# The Birmingham Approach

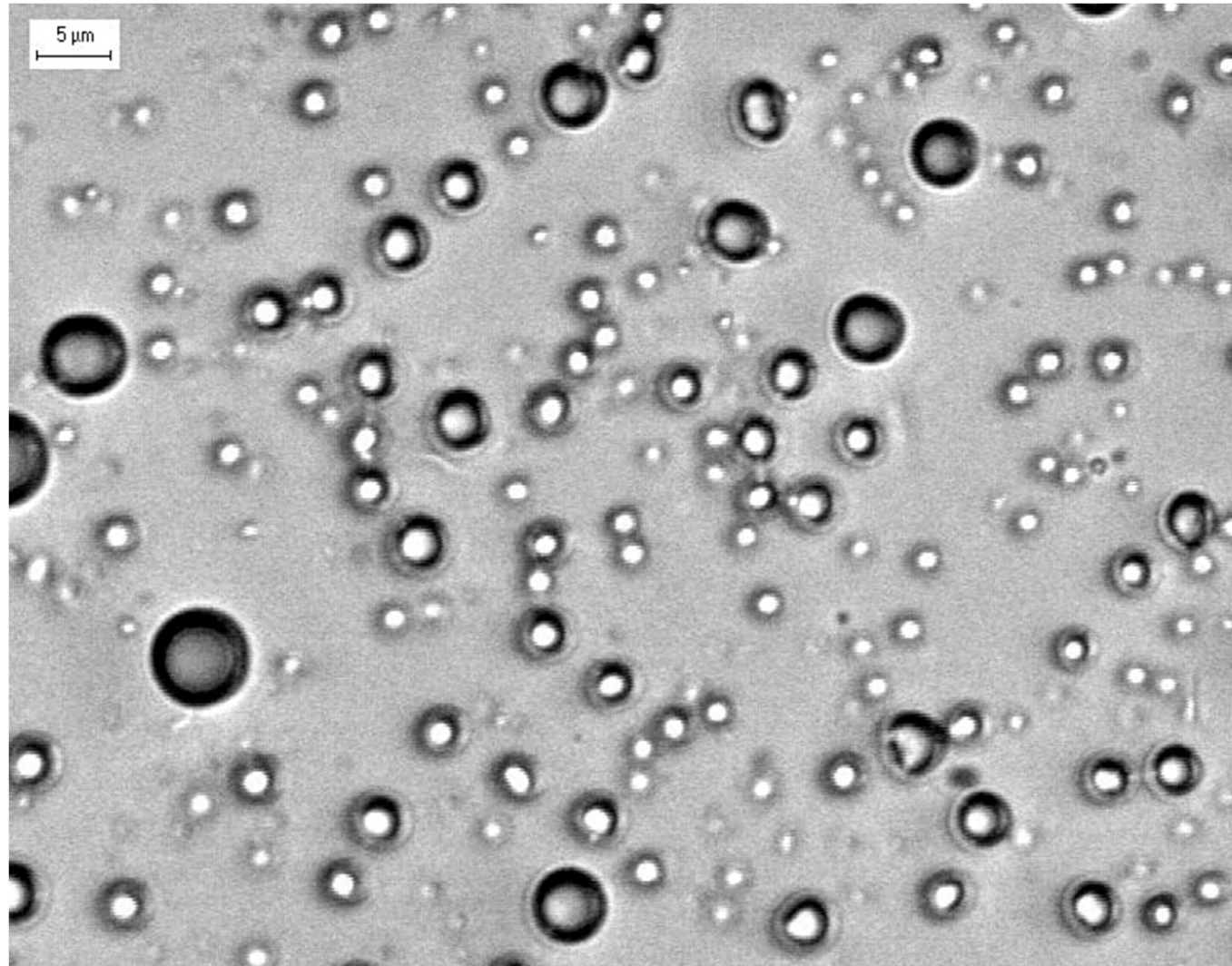


# Hydrophobin Rich Extract



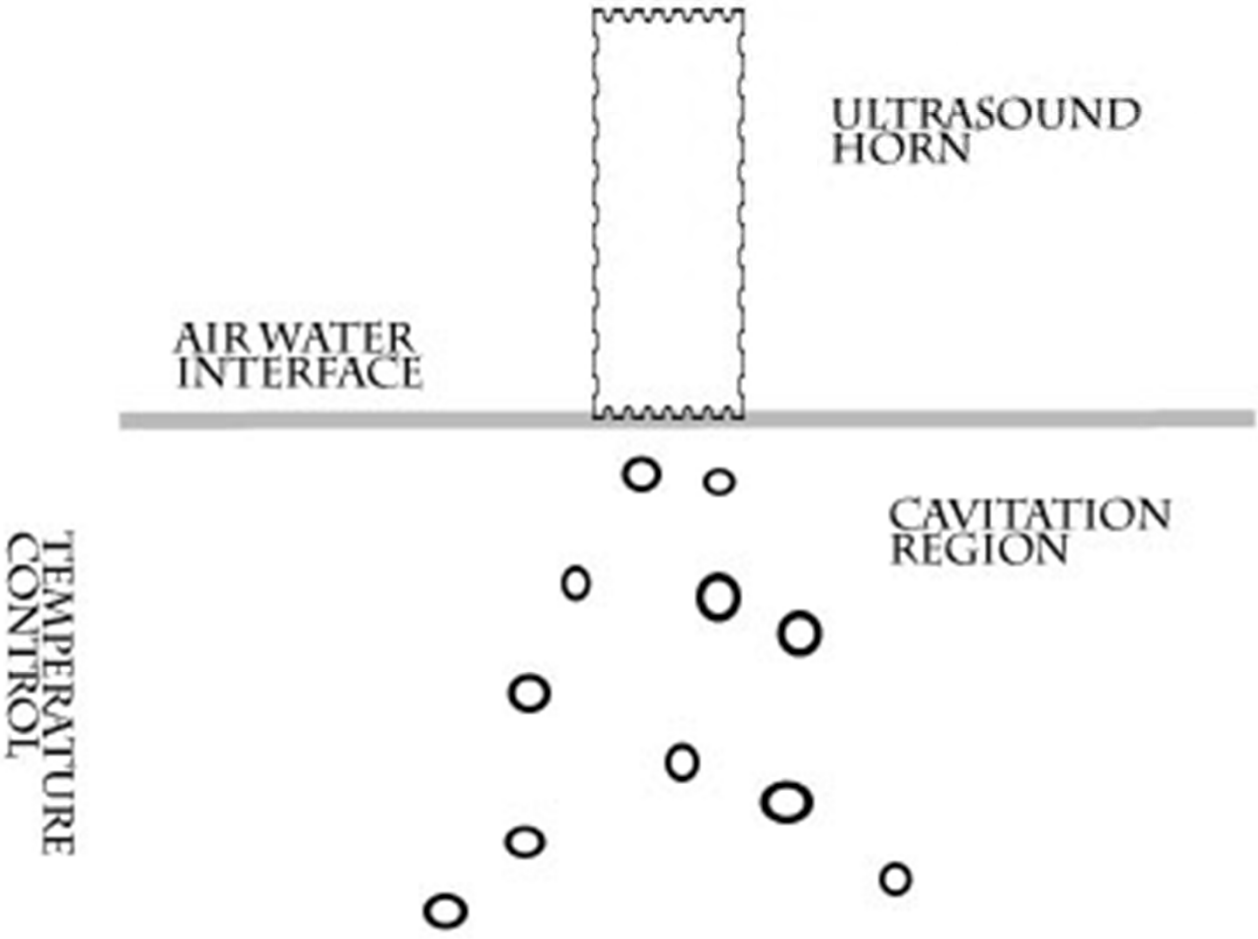


# Microstructural reality



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# Method: Ultrasonic templating

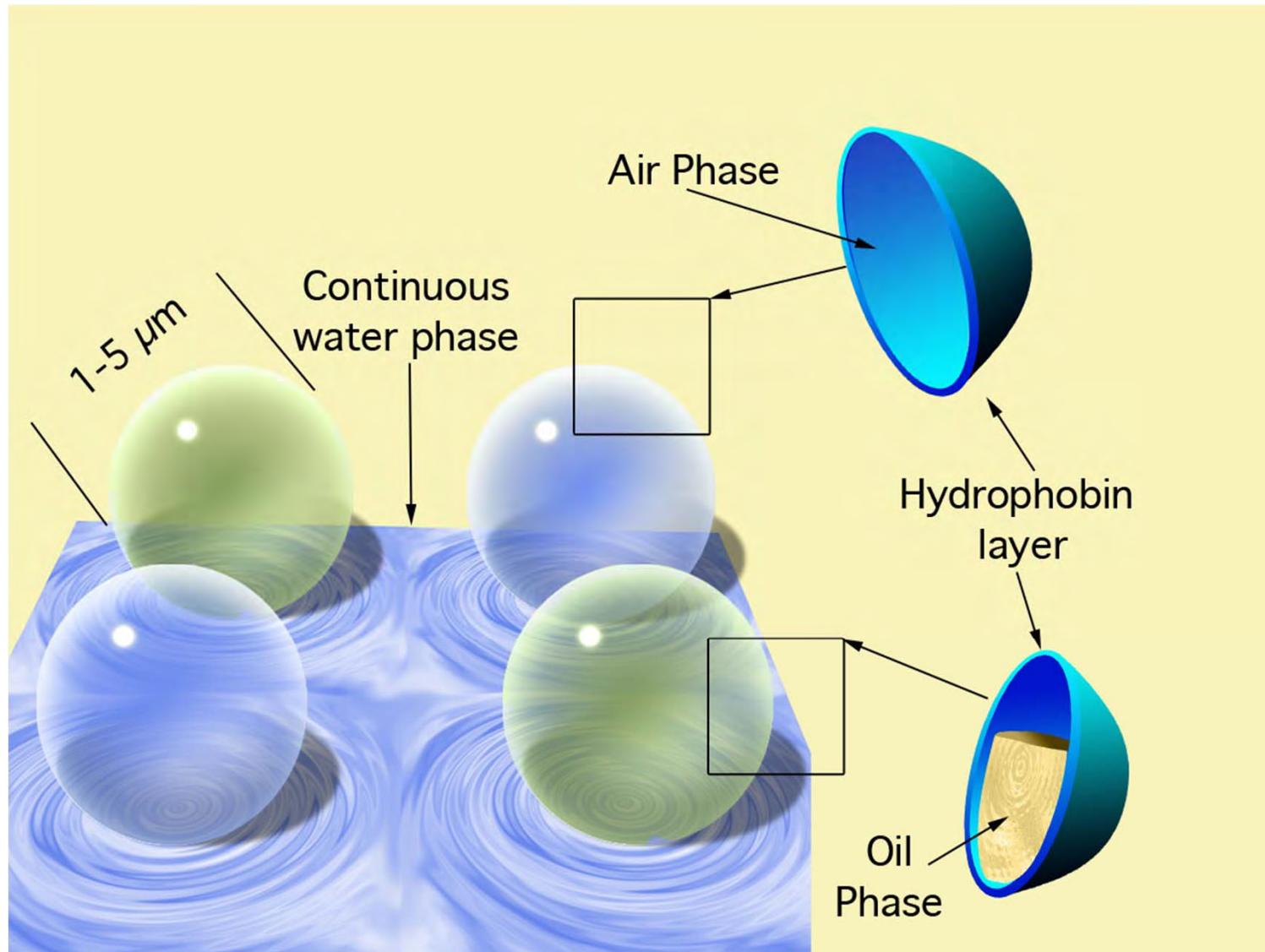


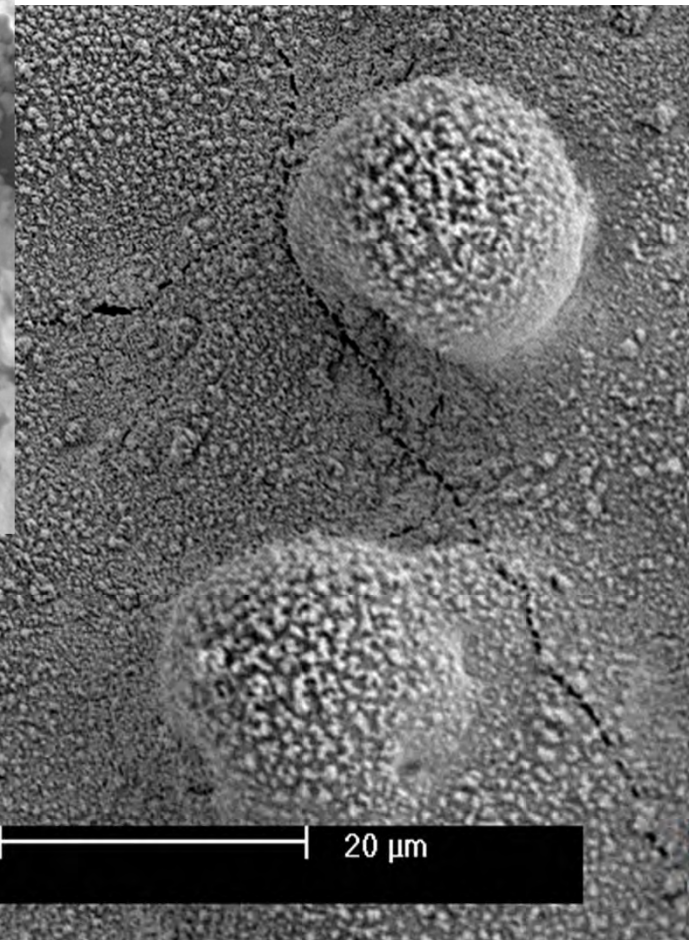
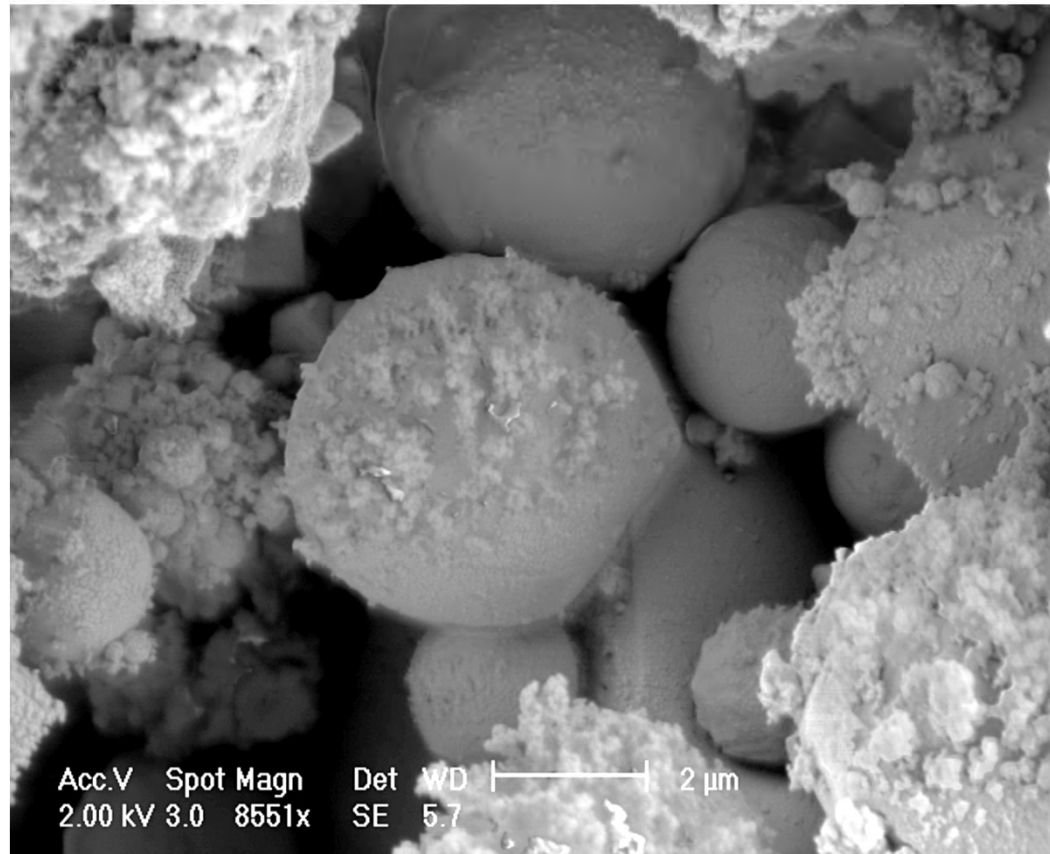
TEMPERATURE  
CONTROL

TEMPERATURE  
CONTROL

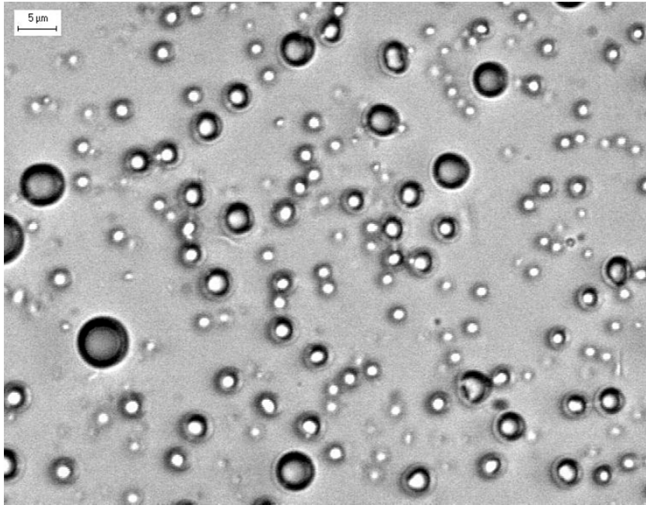


# Not a Foam

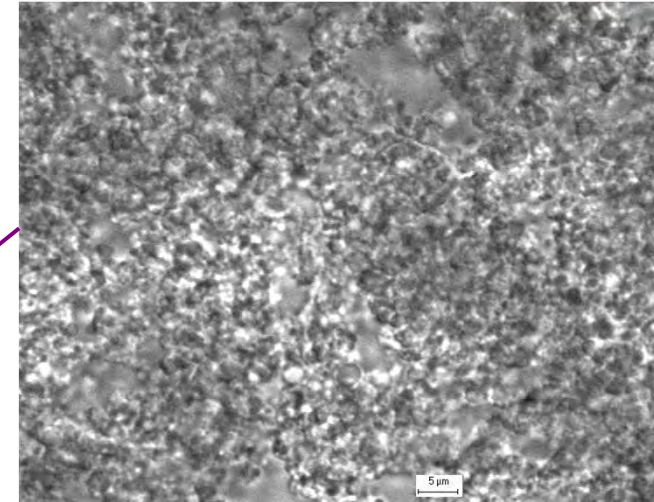




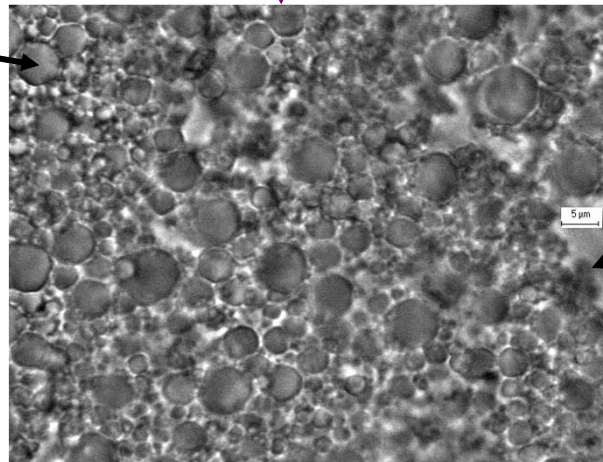
# Triphasic structures



A/E (4 μm)



O/W (20% oil , 0.2% I-CAR,  
0.5 % Tween 60; 0.9 μm)



36% A/O/W (20% air  
and 16% oil)



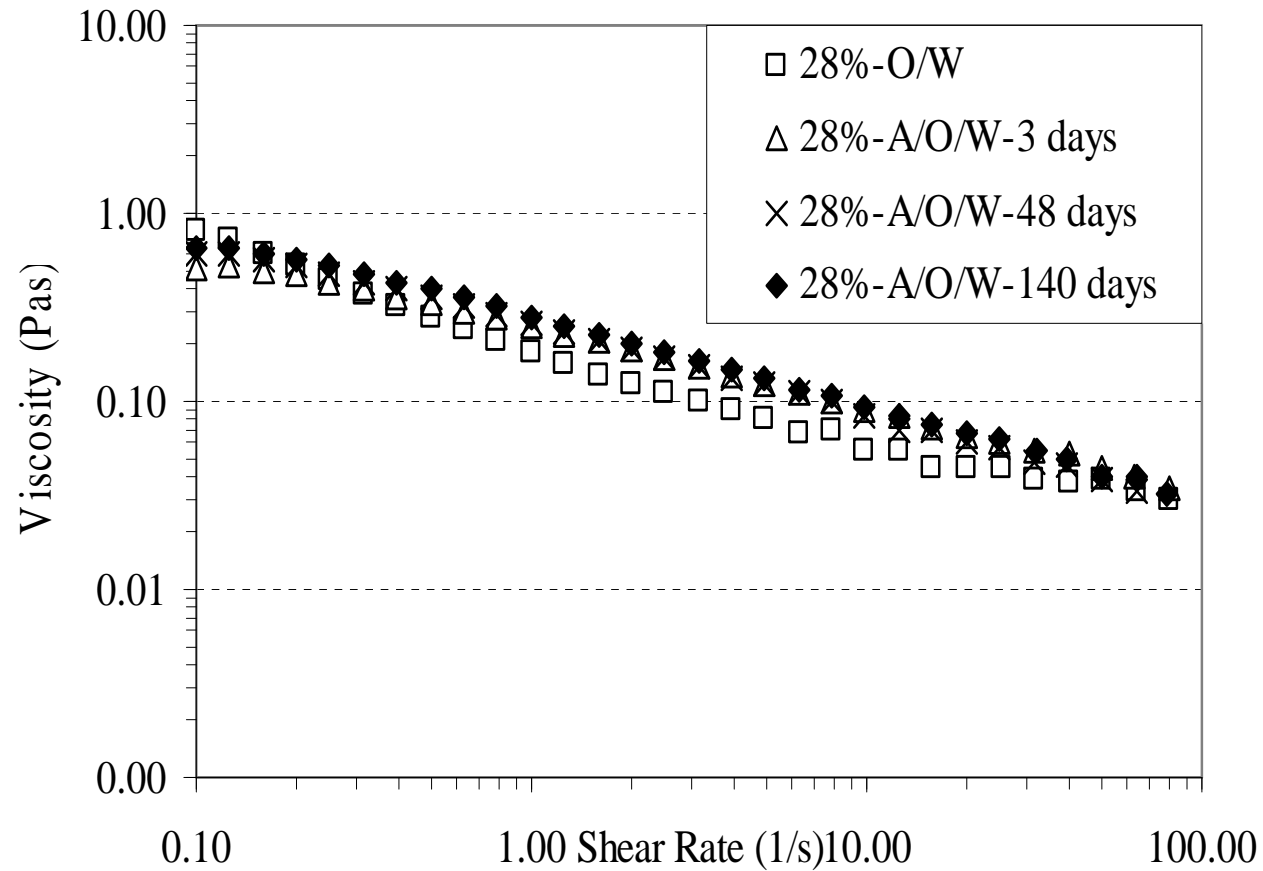


- What is the rheology
- What is the stability
- What is the tribology
  
- Oxidation of lipids

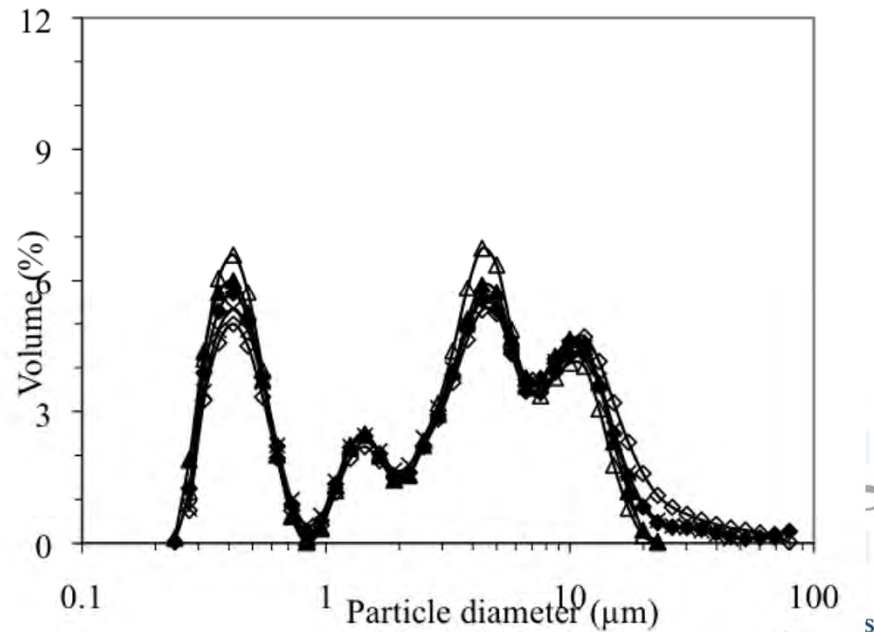
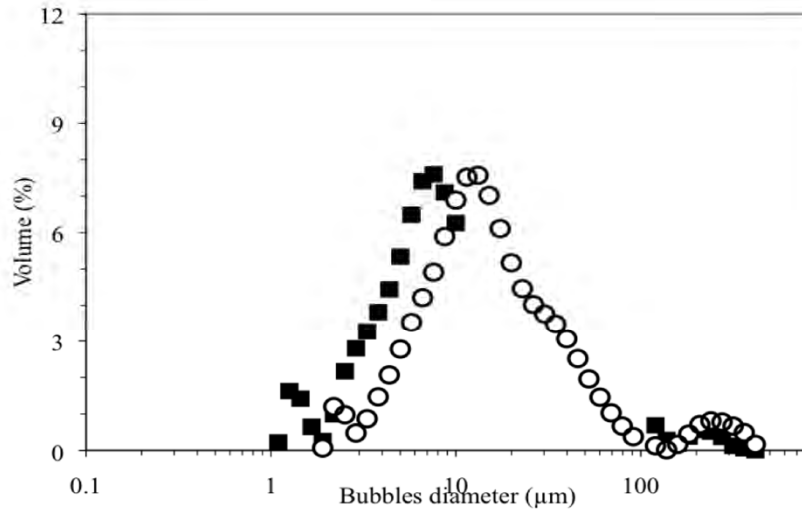


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# Rheological matching

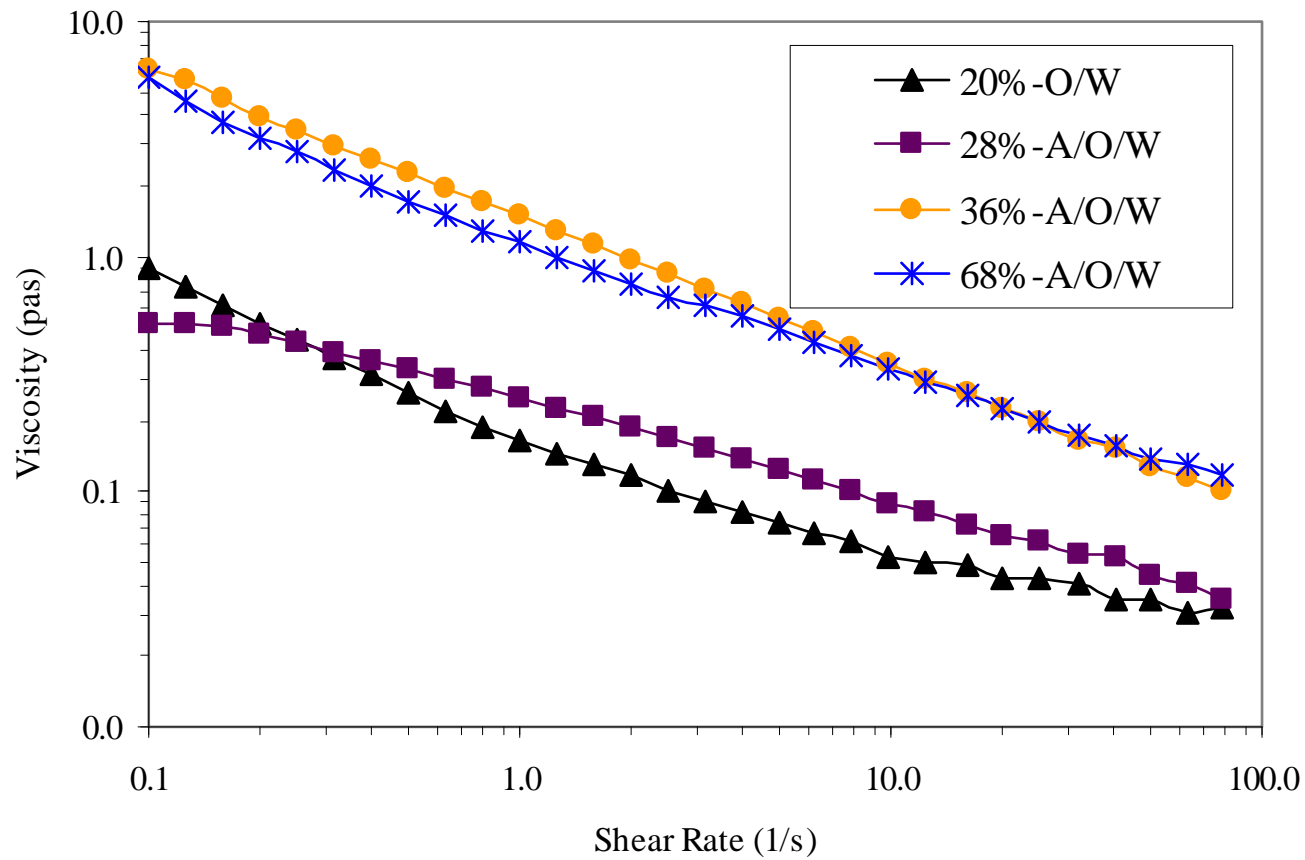


# Triphasic size measurement

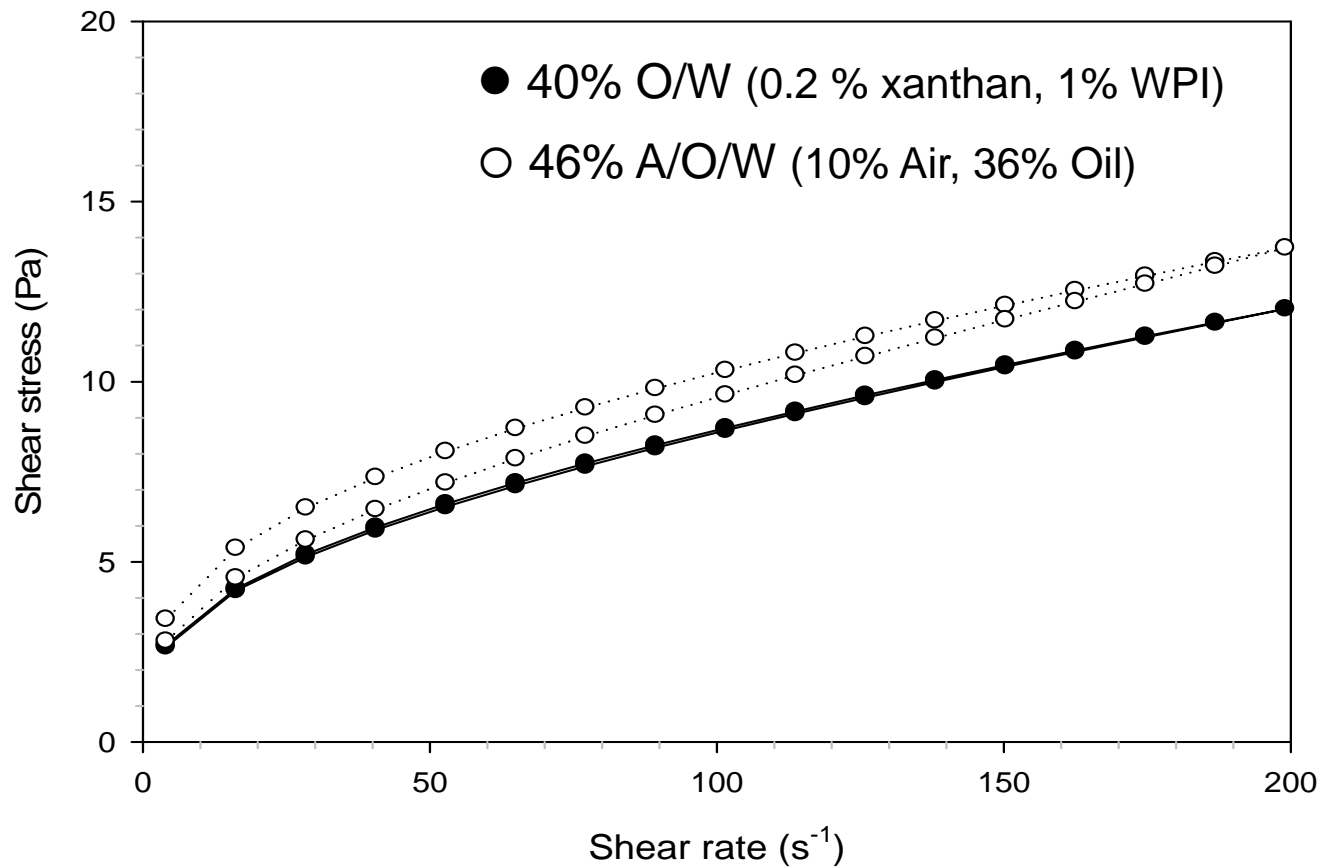




# Viscosity with Phase Volume



# Weak gel formation



Hysteresis loops obtained for 5 day old emulsions at 20° C

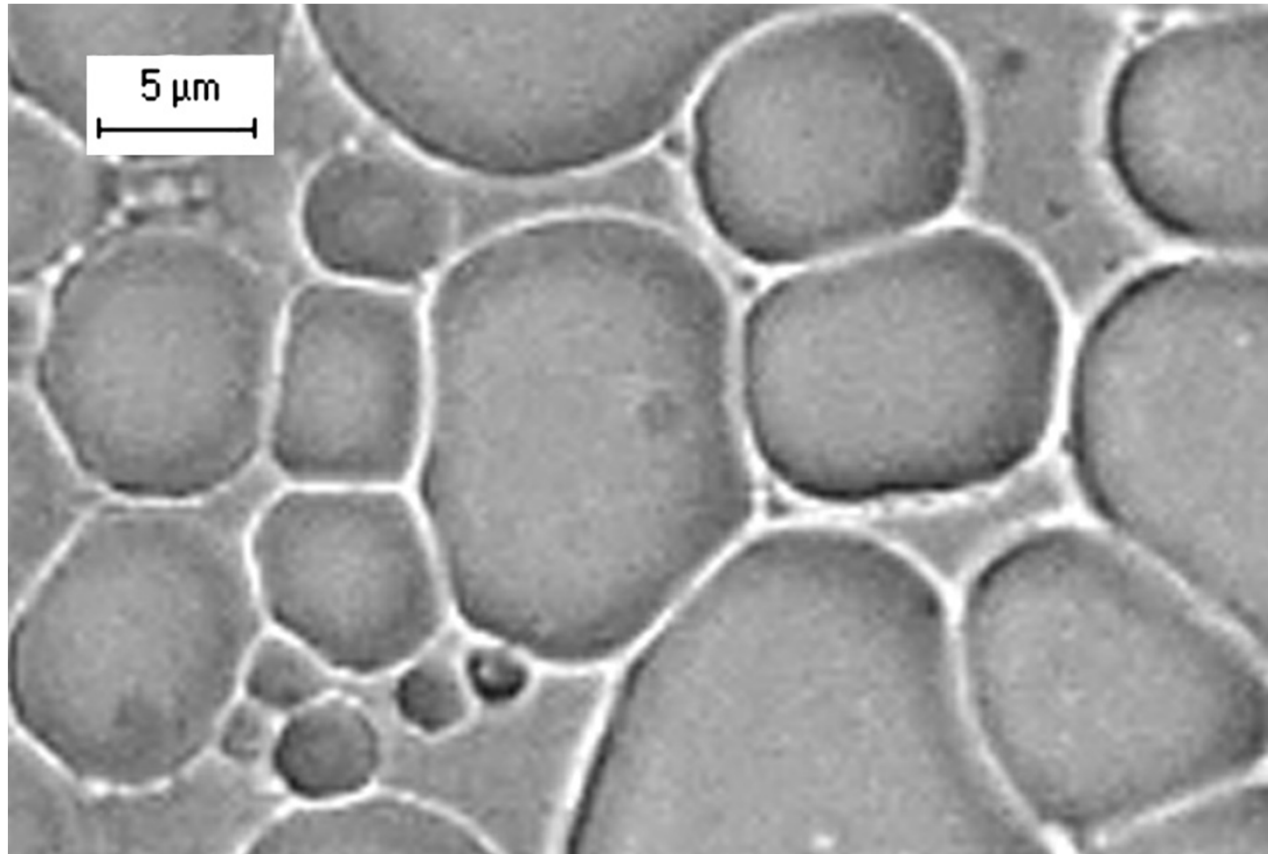


# Creaming - Triphasic



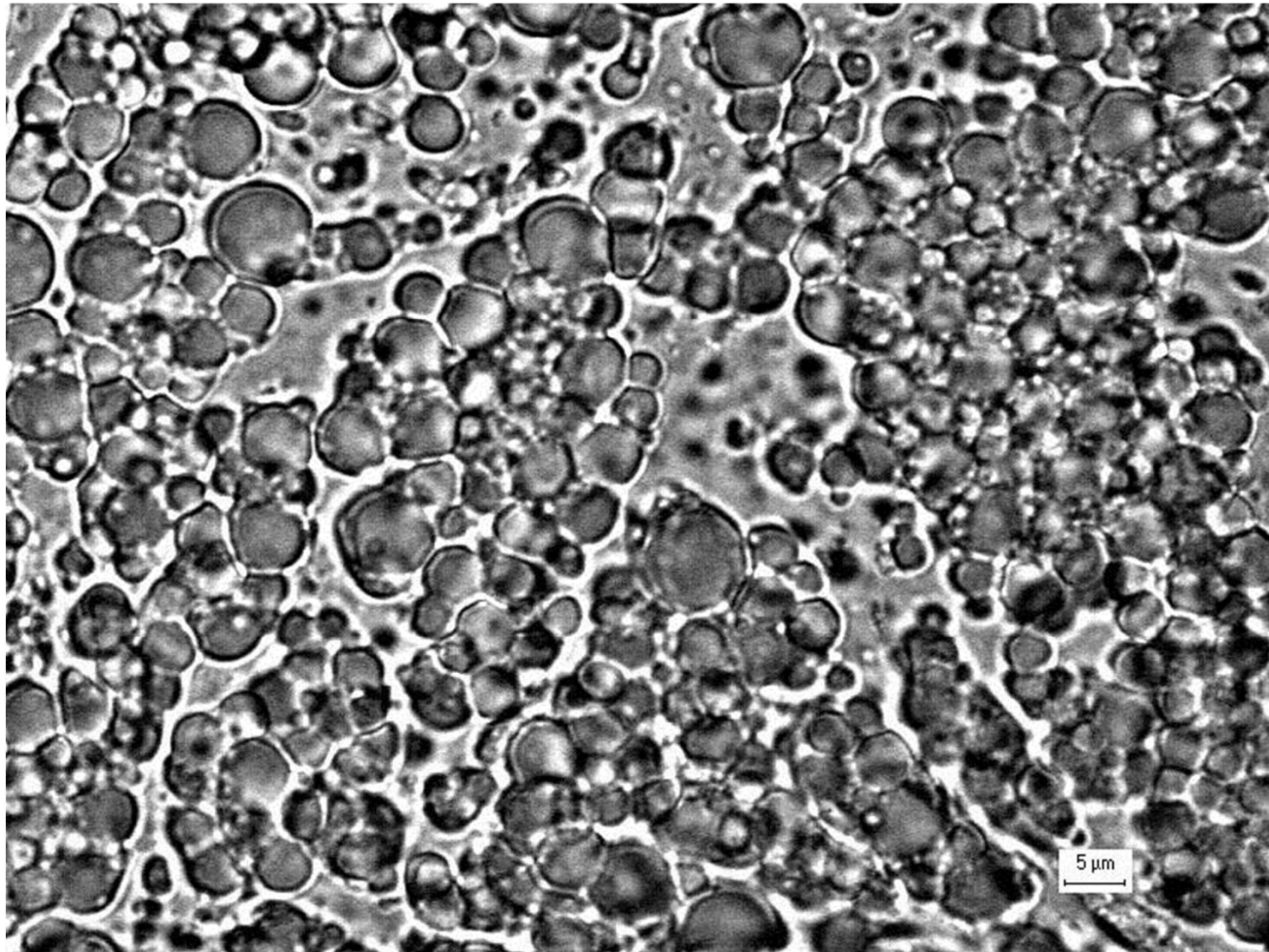
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# Elastic interfaces

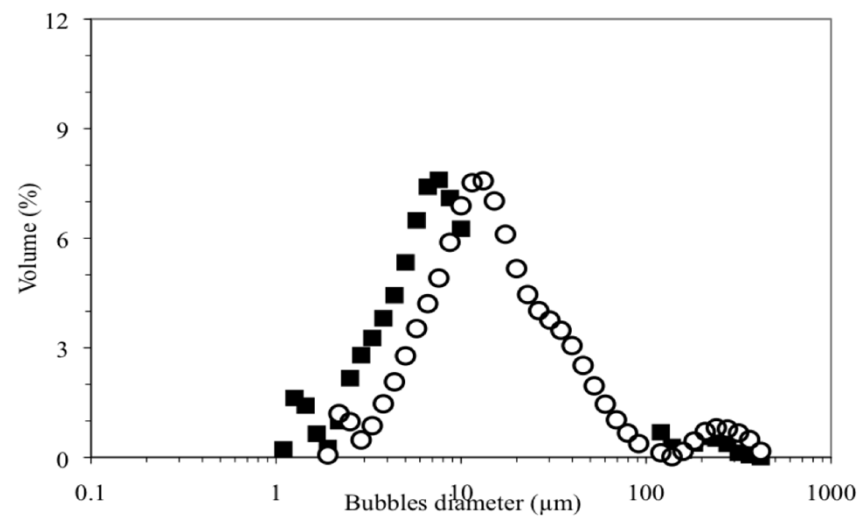
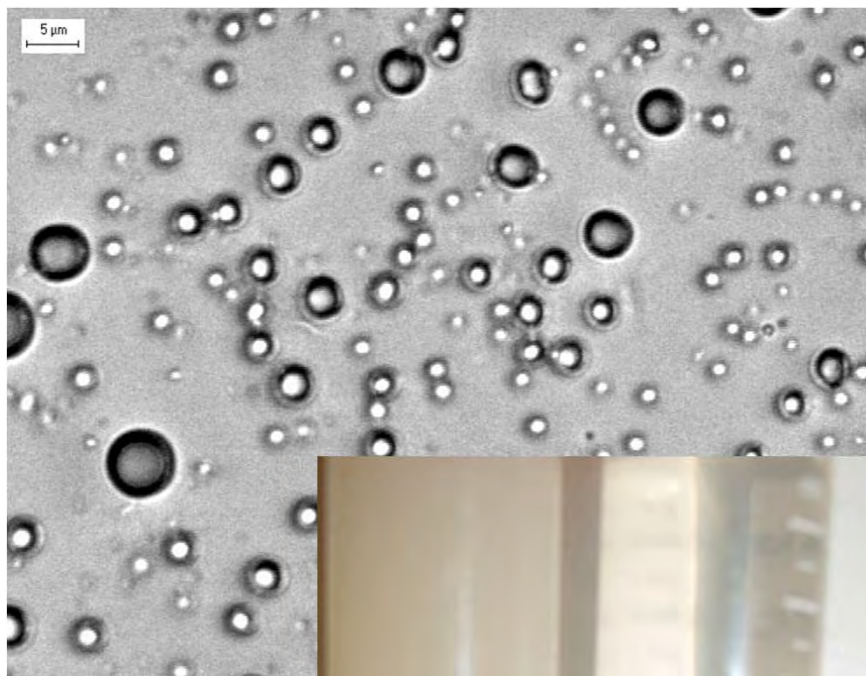


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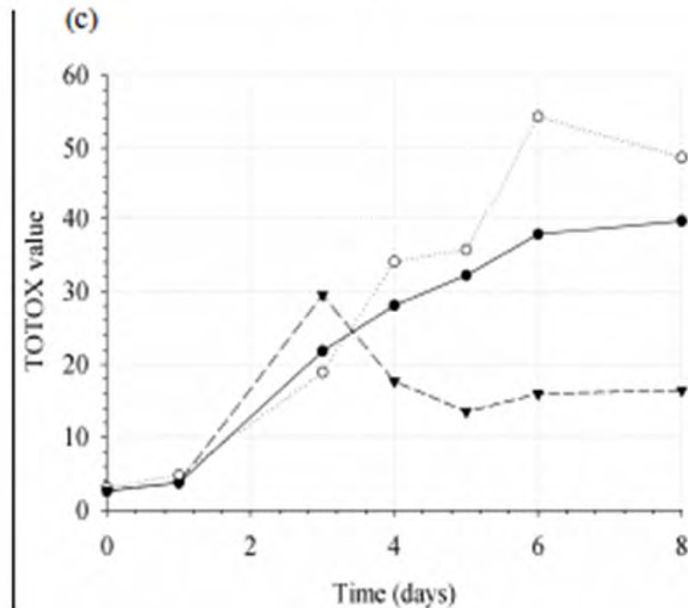
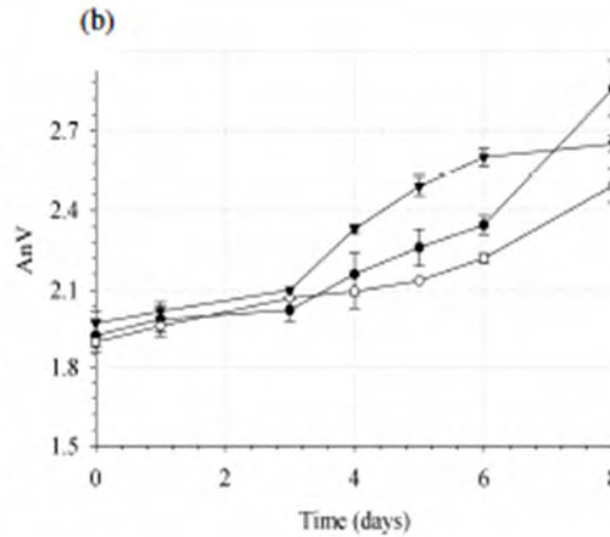
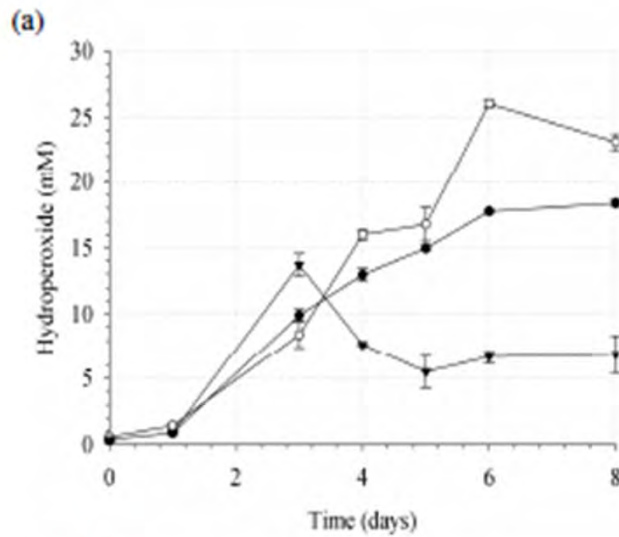
# Ostwald Ripening 140 days



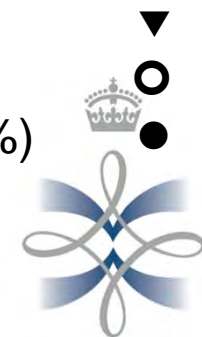
# AFE Stability



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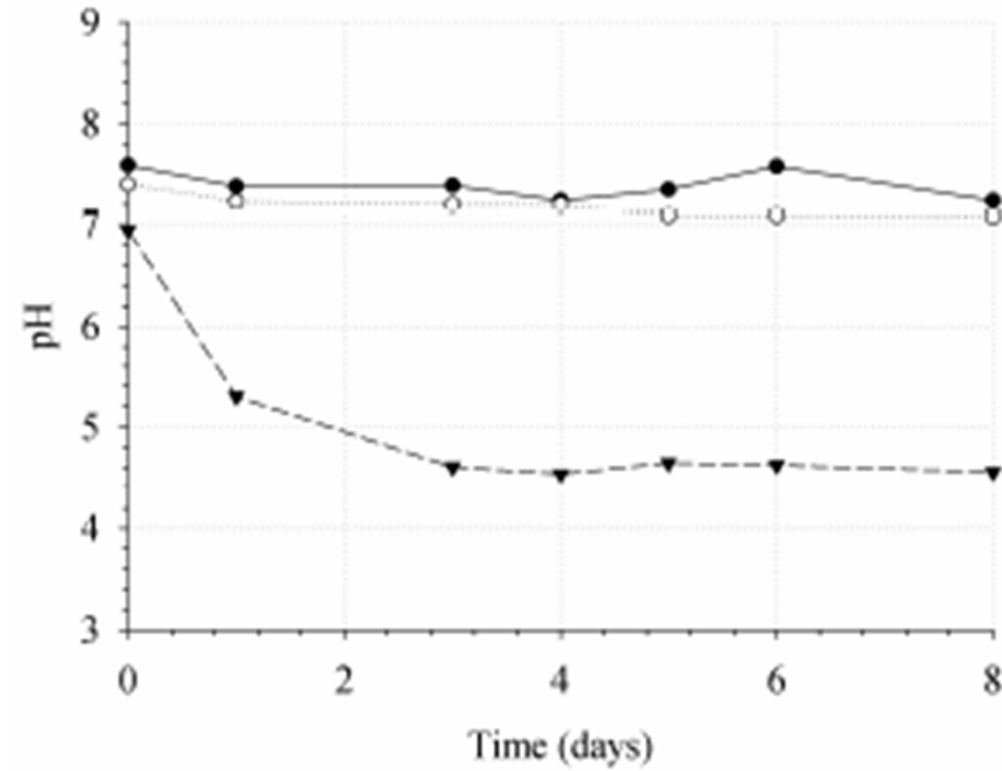


28% A/O/W (10% air, 18% oil)  
 28% O/W  
 20% O/W (used for the 28%)



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Hydrophobin at all interfaces

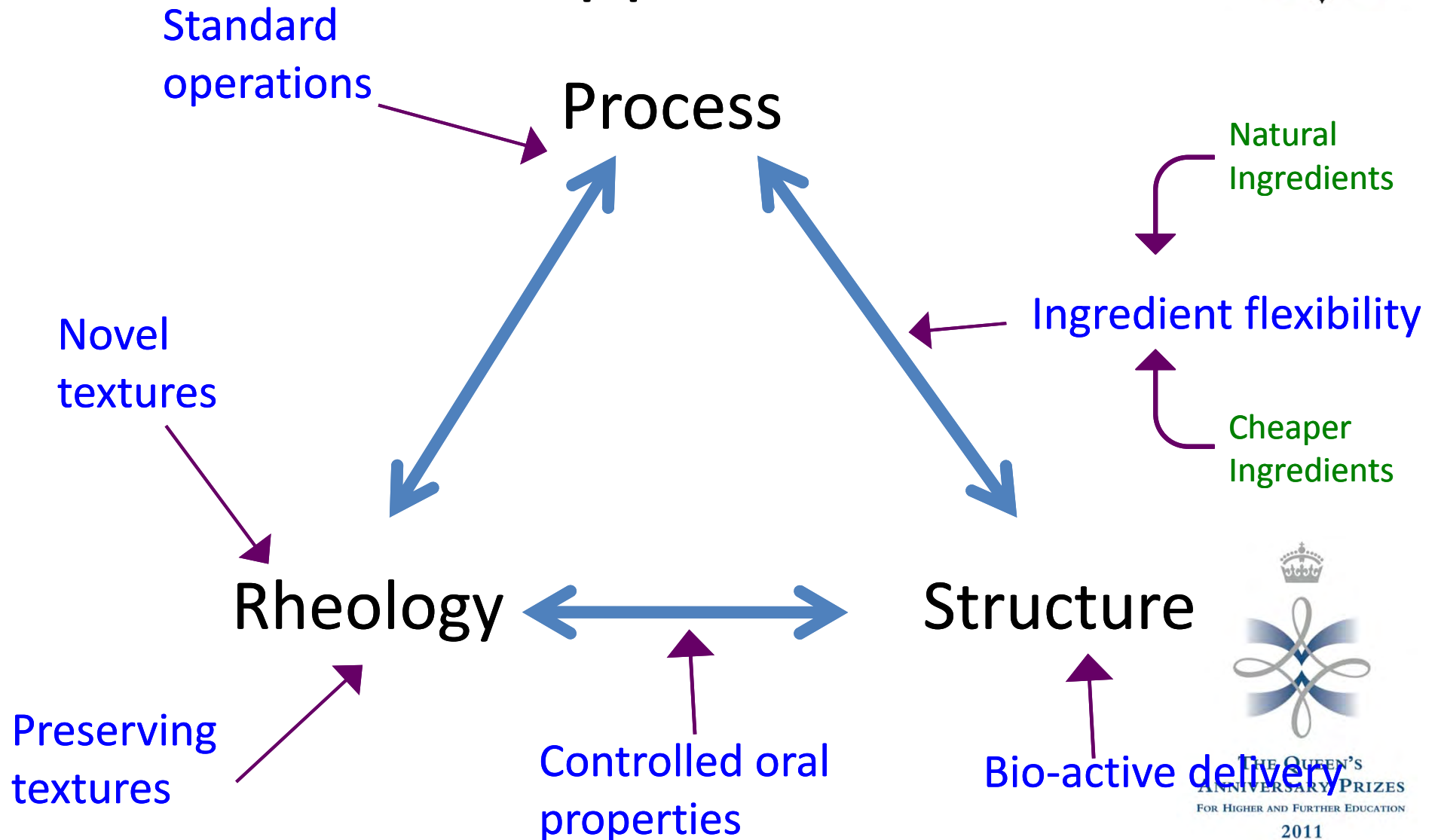


28% A/O/W (10% air, 18% oil)  
28% O/W  
20% O/W (used for the 28%)





# Micro-structural approach



# The new approach

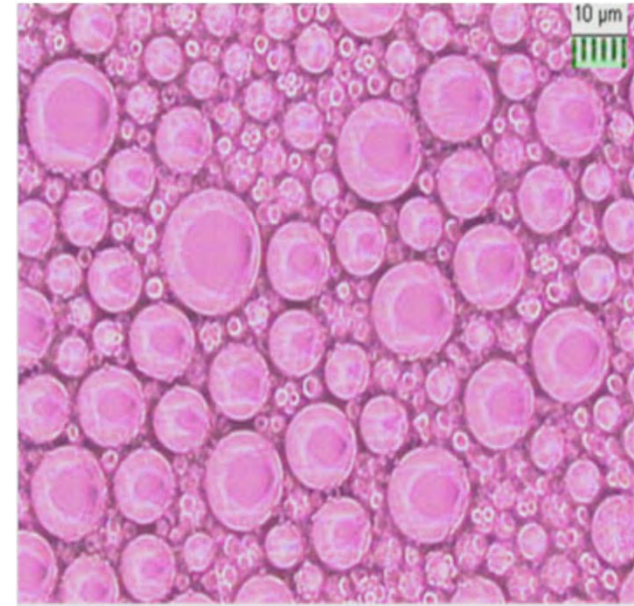
- Need for self assembling proteins
- Need for the same or similar interfacial properties
- Compliant with the cavitation templating process
- Cross bonding is essential (cysteine group mediated)
- Cheap would be really really really nice !
- Functionalise cheap or even waste stream proteins



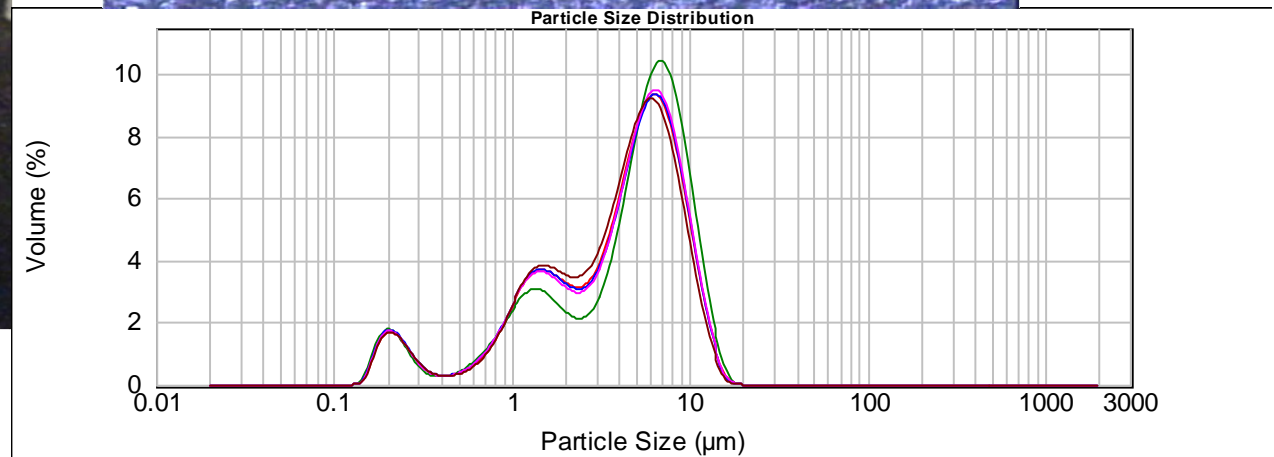
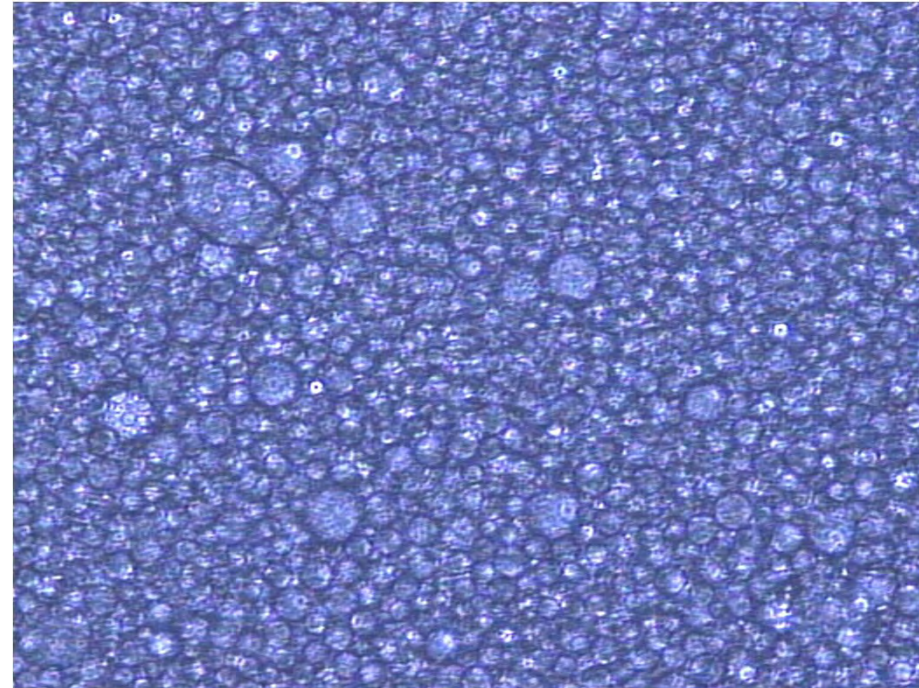
# Possible Targets



- Zein
  - Difficulties with solubility
- Soy
  - Cross bonding is slow
- BSA, Ovalbumen, Whey Proteins
  - Meet the criteria above



# BSA

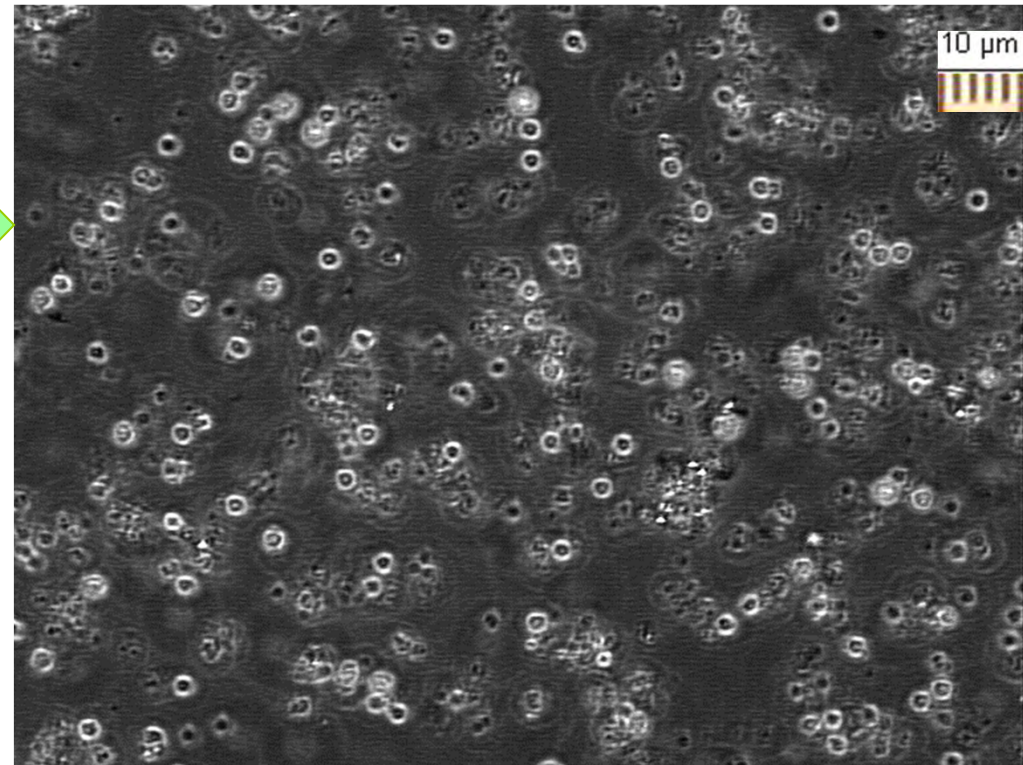


# Sonochemically-prepared Ovalbumin microbubbles



## Sonication conditions

5% w/v aqueous BSA  
50°C initial temperature  
25 cc/min O<sub>2</sub>  
20 kHz,  
3 min



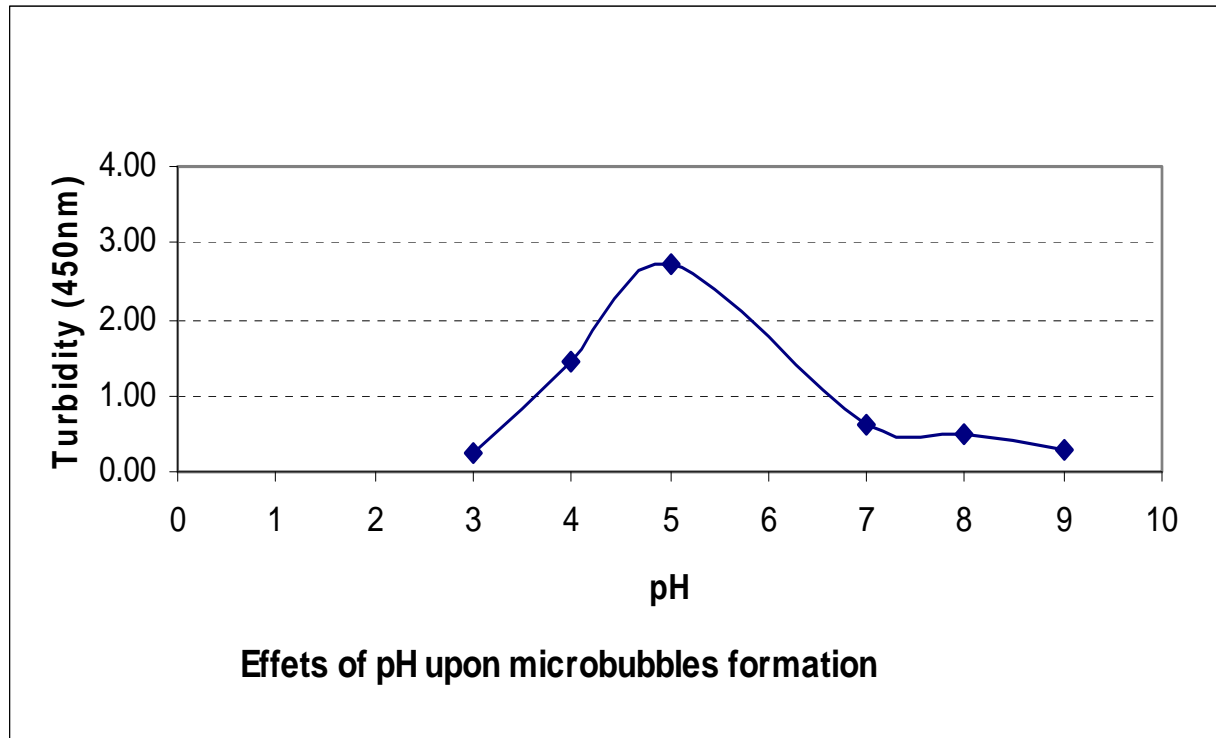
Light microscopy (X 40) of the BSA-  
microbubbles



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# Ionic environment

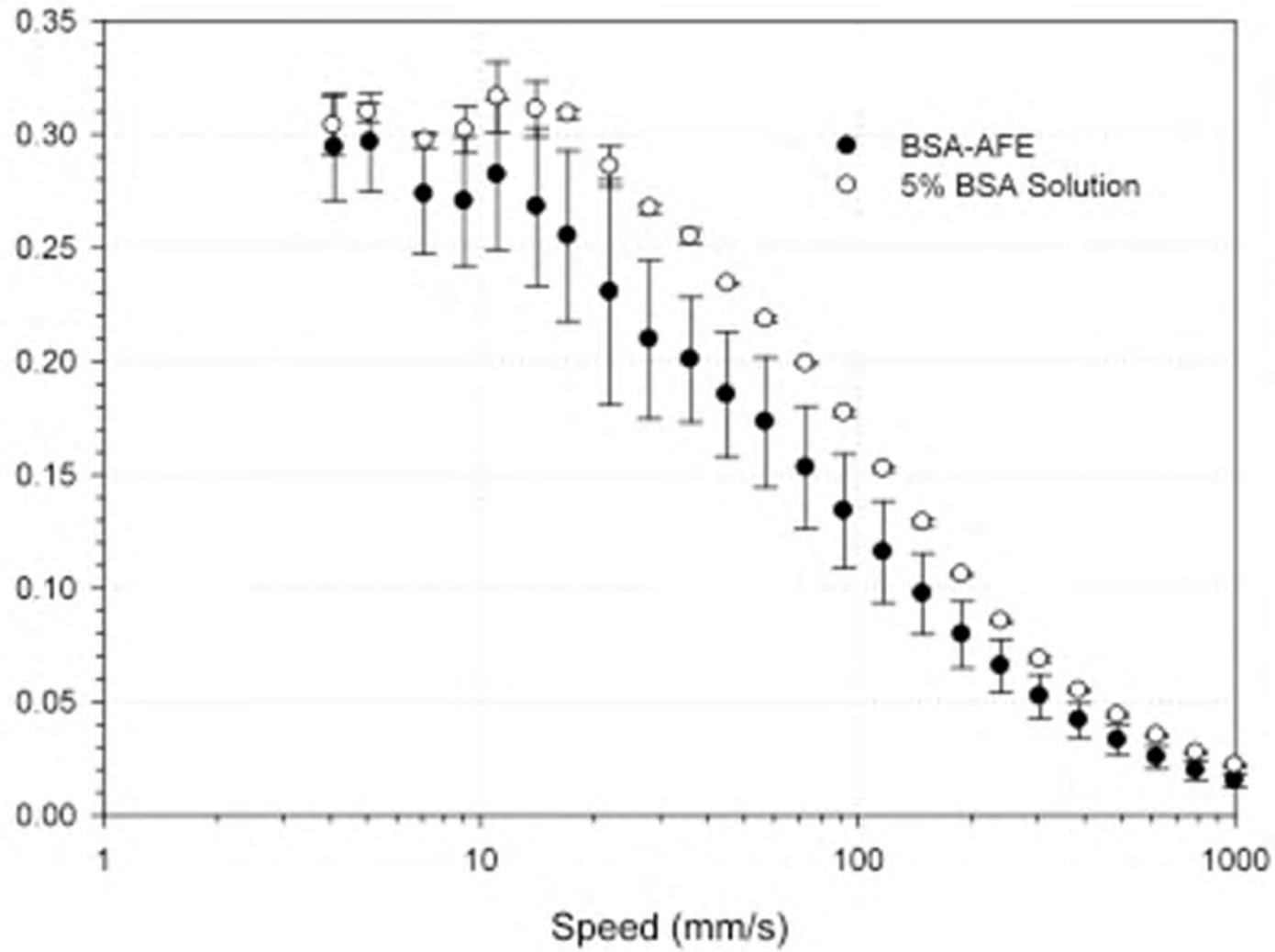


☞ PH: no bubbles were observed at pH 3 and 9;

☞ pH 5 seems to be the optimal pH

☞ [buffer]  $\Rightarrow$  ionic strength: no bubbles with BSA-sodium phosphate Buffer pH7, 0.2M but bubbles were observed at the same pH, 0.01M

# BSA Tribology



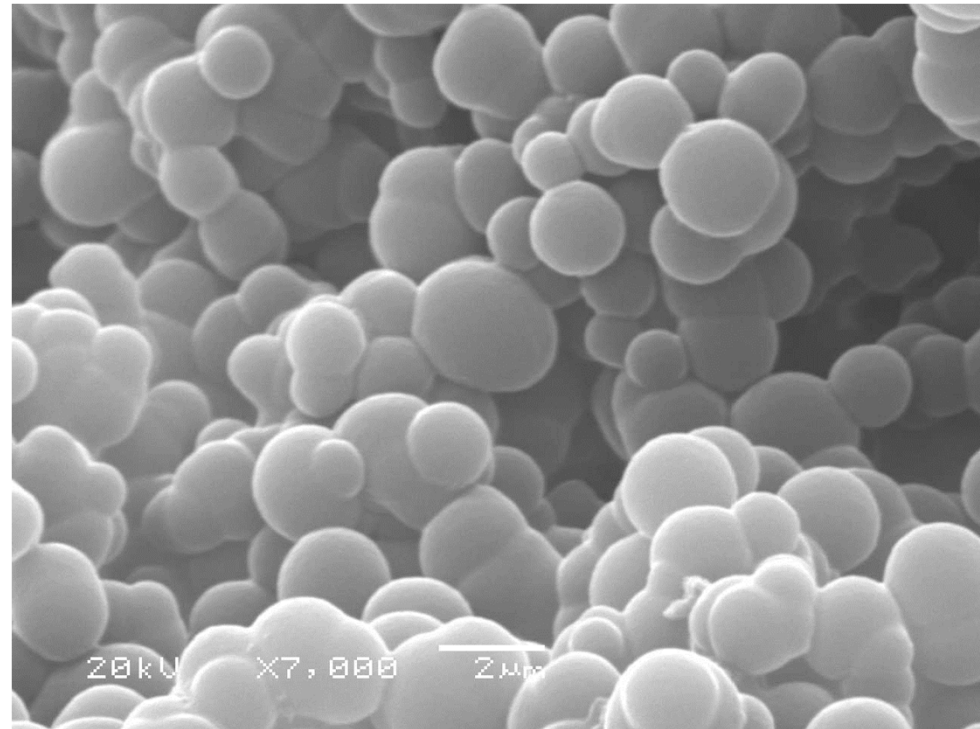
# Effect of unit operations



BSA-AFE



Autoclave  
(121 ° C  
/15min)



SEM image of BSA- AFE after  
sterilisation





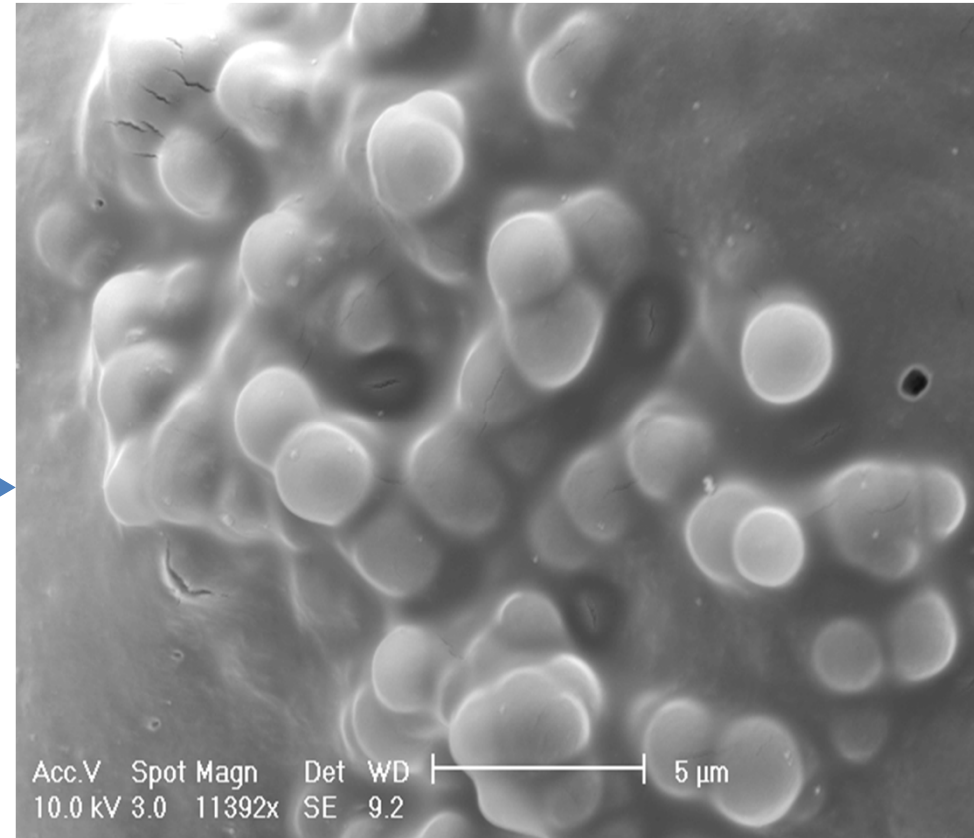
# Ovalbumen



EWP-AFE



Autoclave  
(121 ° C  
/15min)

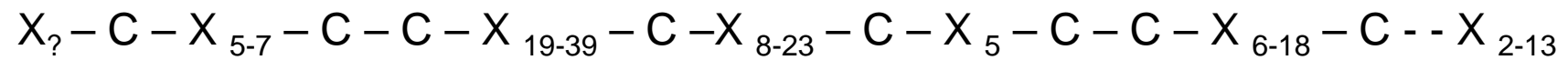


SEM image of EWP- AFE after sterilisation

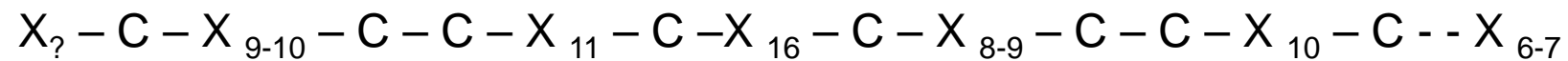
# New hydrophobins



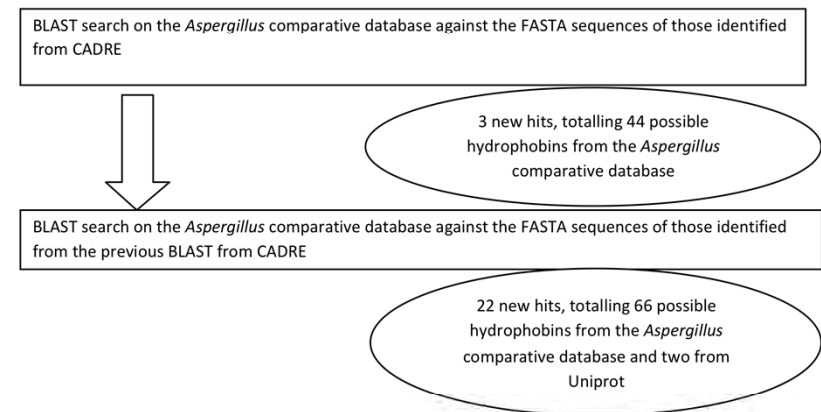
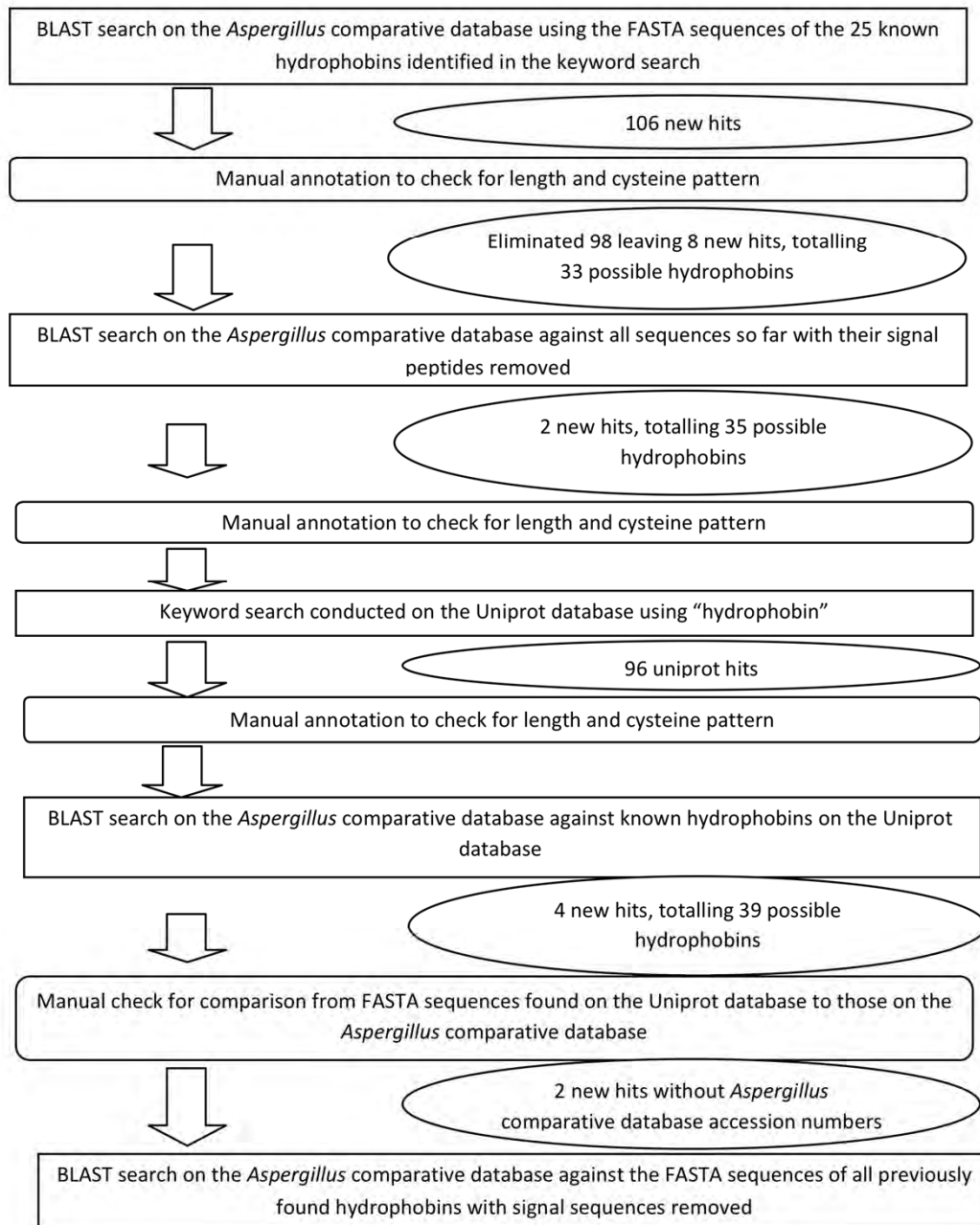
Class I:



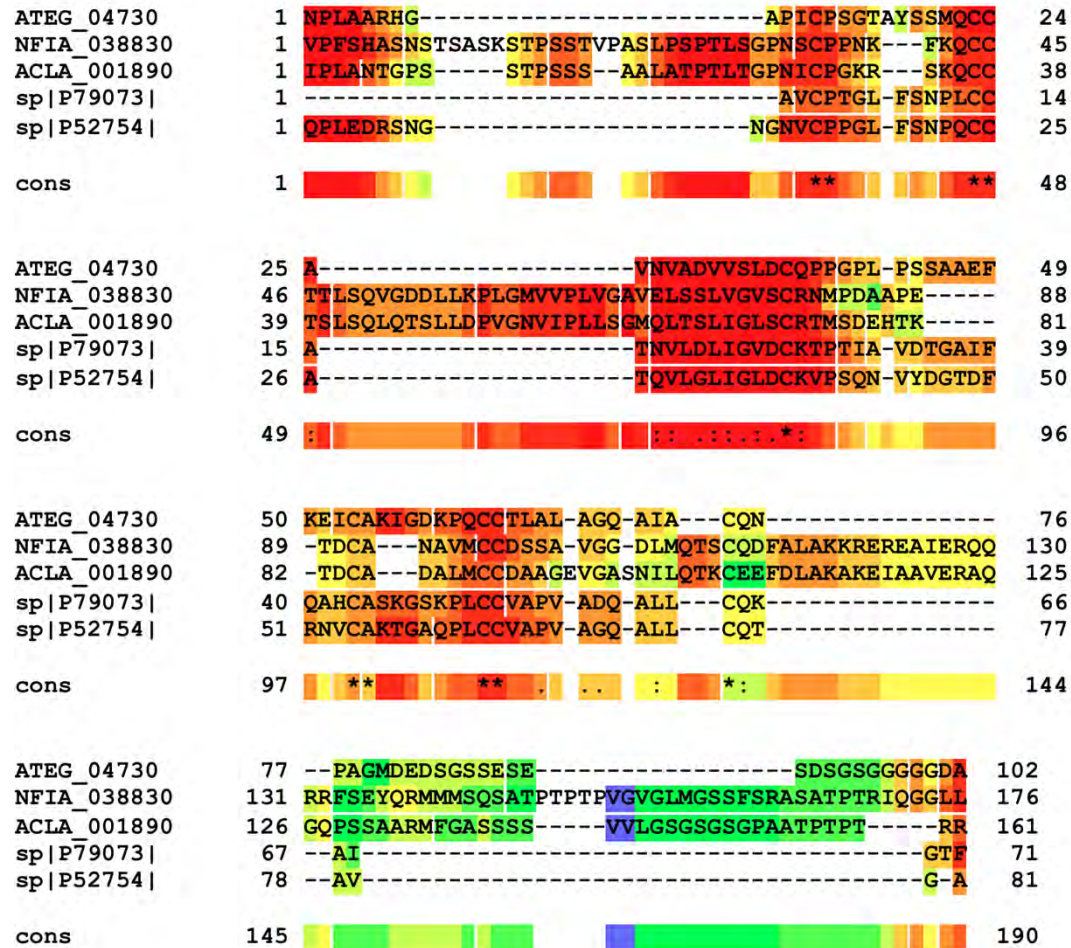
Class II:



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**Figure 2.** TCoffee alignment to suggest ATEG\_04730 is a Class II hydrophobin, comparison is with NFIA\_038830 and ACLA\_001890 both Class I hydrophobins and sp|P79073|HYP2\_TRIRE and >sp|P52754| from *Trichoderma reesei*, both Class II hydrophobins. Brighter (red) colours denote good alignments through to cooler colours with poorer alignments. (\*) shows perfect conservation of a residue, (:) a close match and (.) a less close match.



# Findings



- 74 new HFs identified
- Putative Class 1.5 seen
- Dimers & trimers seen
  
- Looking for non – microbial analogues



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