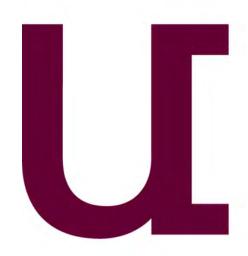
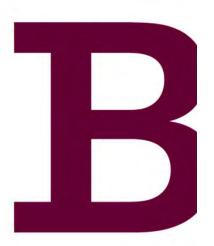
UNIVERSITY^{OF} BIRMINGHAM





Air Filled emulsions & Hydrophobins





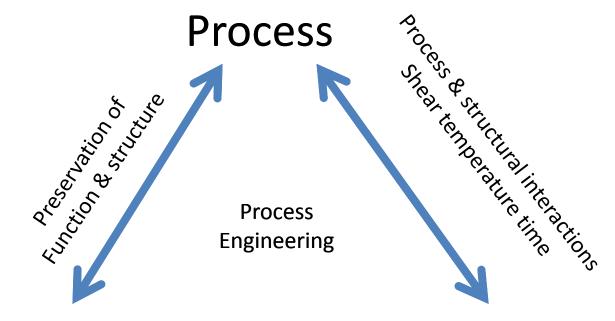




How do we get to here?







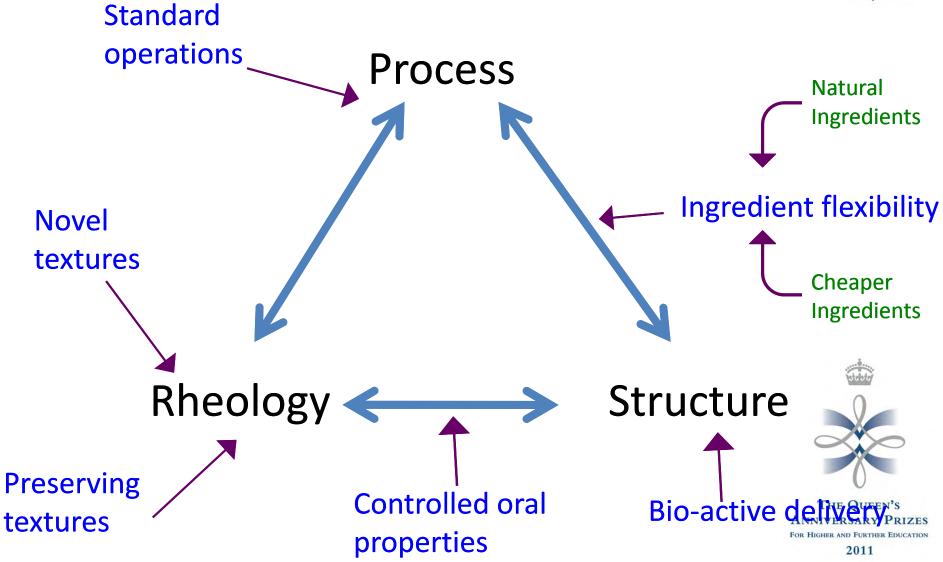
Rheology

Material Science & Structural Properties

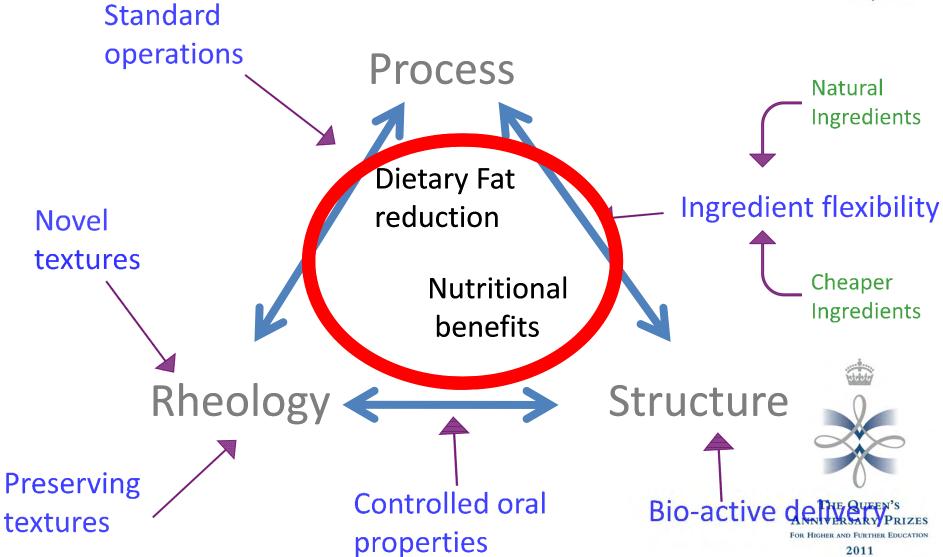
Structure





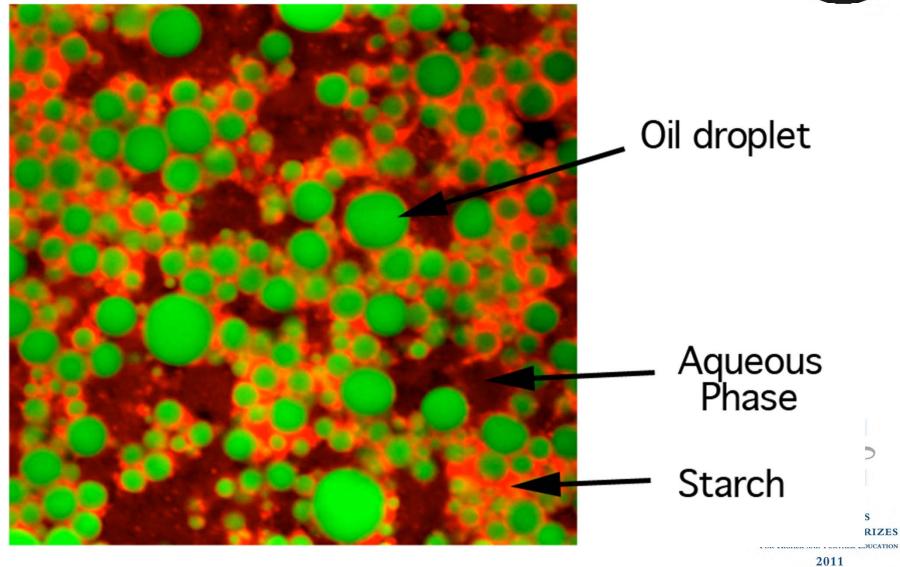






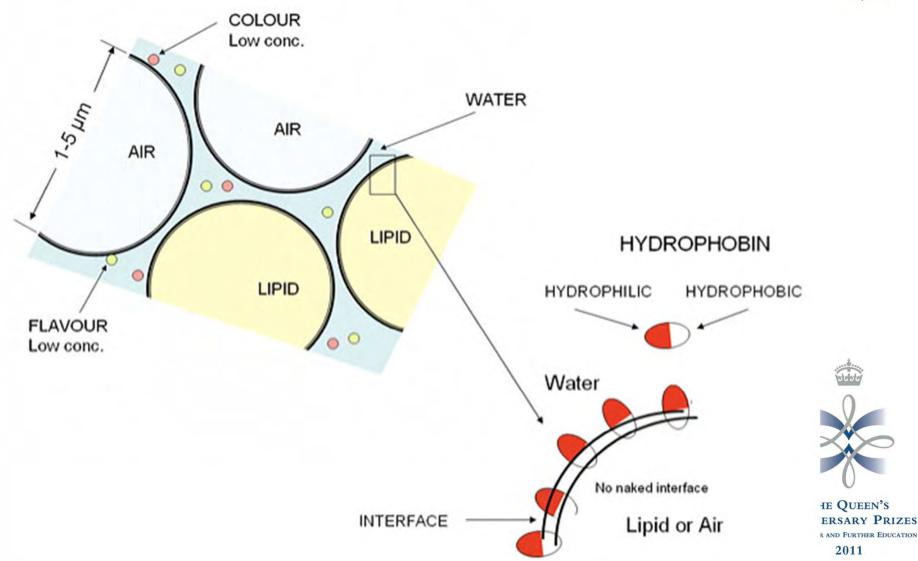
One current approach: reduced dietary fat intake





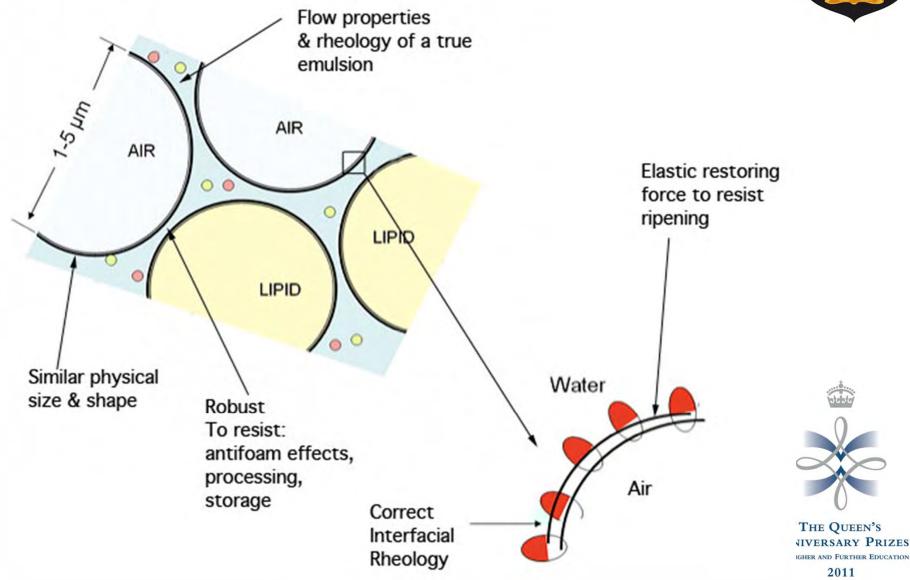
Air filled emulsions





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

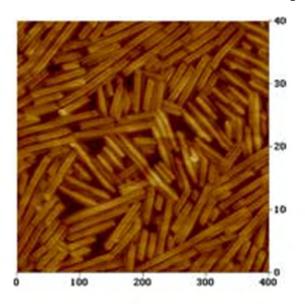
Acc.V Spot Magn Det N/D 2 μm 2.00 kV 3.0 8561x SE 5.7

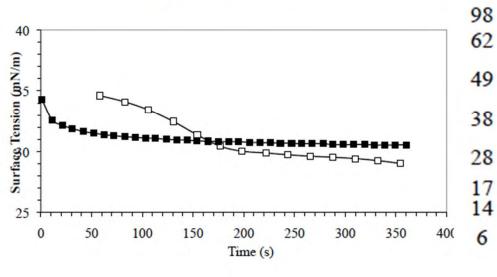
N.B. These are emulsions not foams!

Hydrophobins

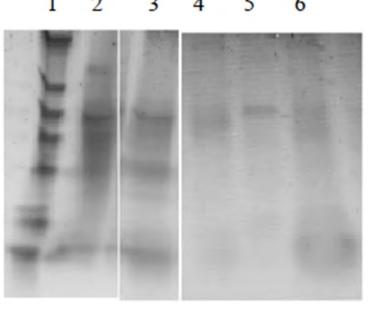
kDa







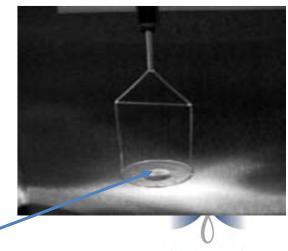




Hydrophobins



- 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 Range?



Rate of Assembly

Class II

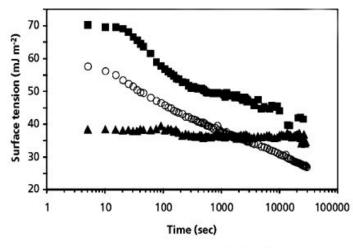


Solubility



What does this mean?





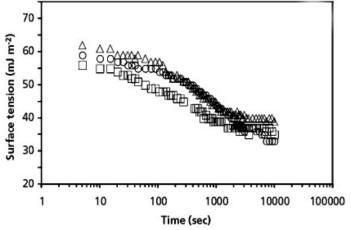


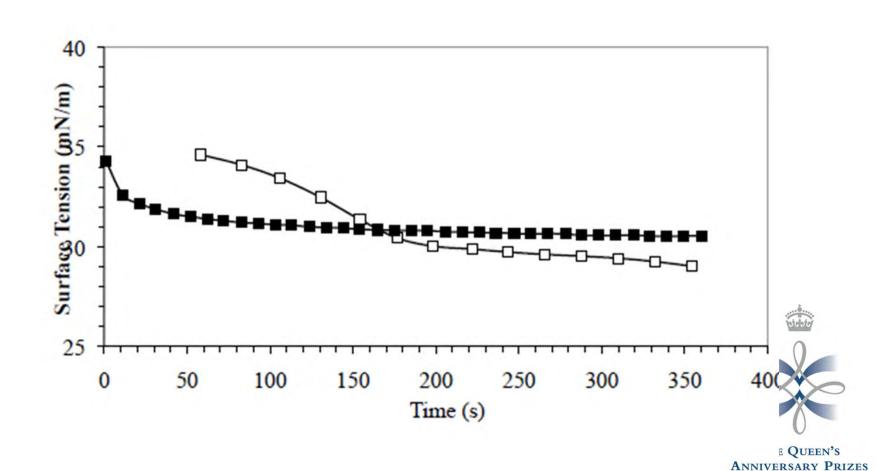
Figure 1. Mixed hydrophobins assembly kinetics at an oil water interface: ■ - Class II hydrophobin HFBI; ▲ - Class II hydrophobin HFBII; ○ - Class I hydrophobin SC3; △ - THE QUEEN'S Mixture of HFBII & SC3; □ - Mixture of HFBII & SC3. (reproduced from Askolin *et al.*, 2006)

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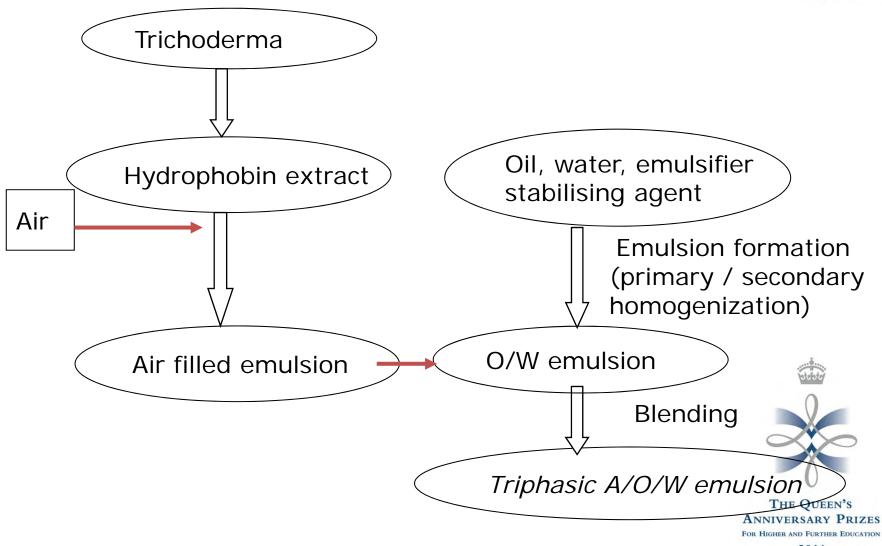
For Higher and Further Education 2011

Interfacial assembly & Mixed Proteins



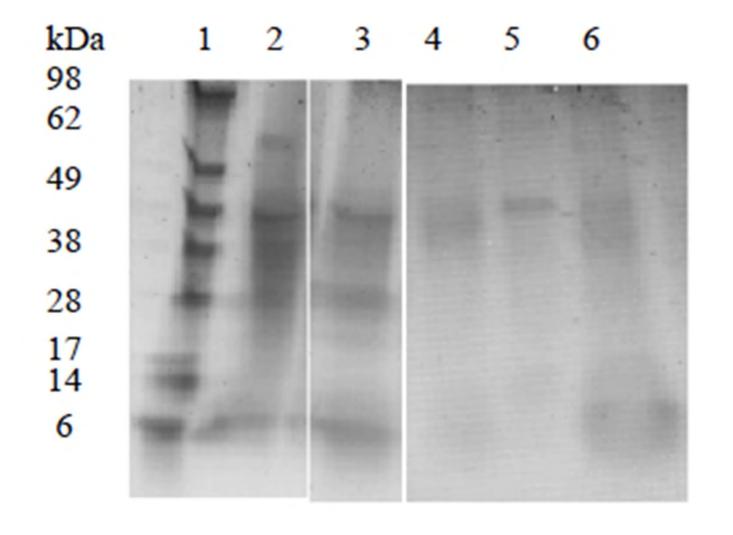
The Birmingham Approach





Hydrophobin Rich Extract

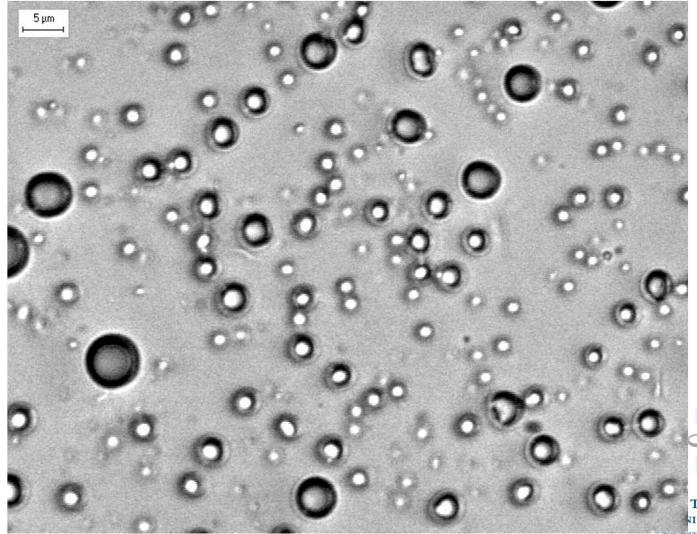






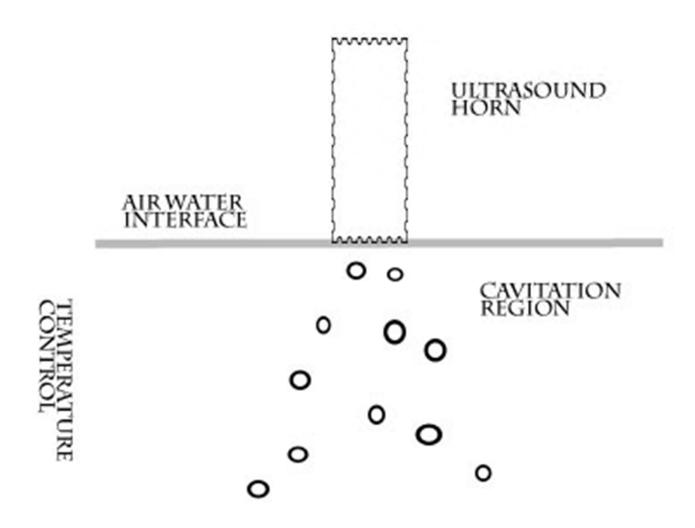
Microstructural reality





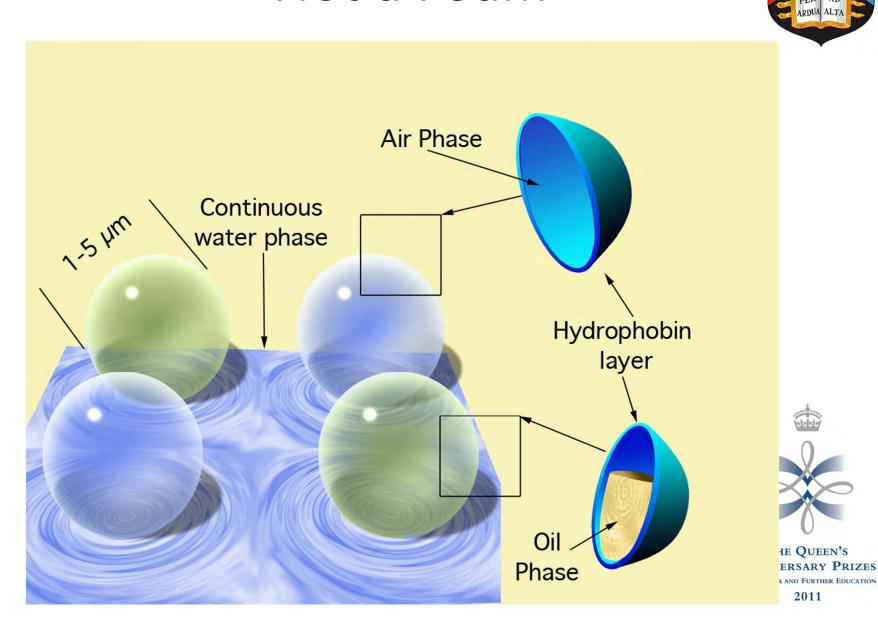
VIVERSARY PRIZES

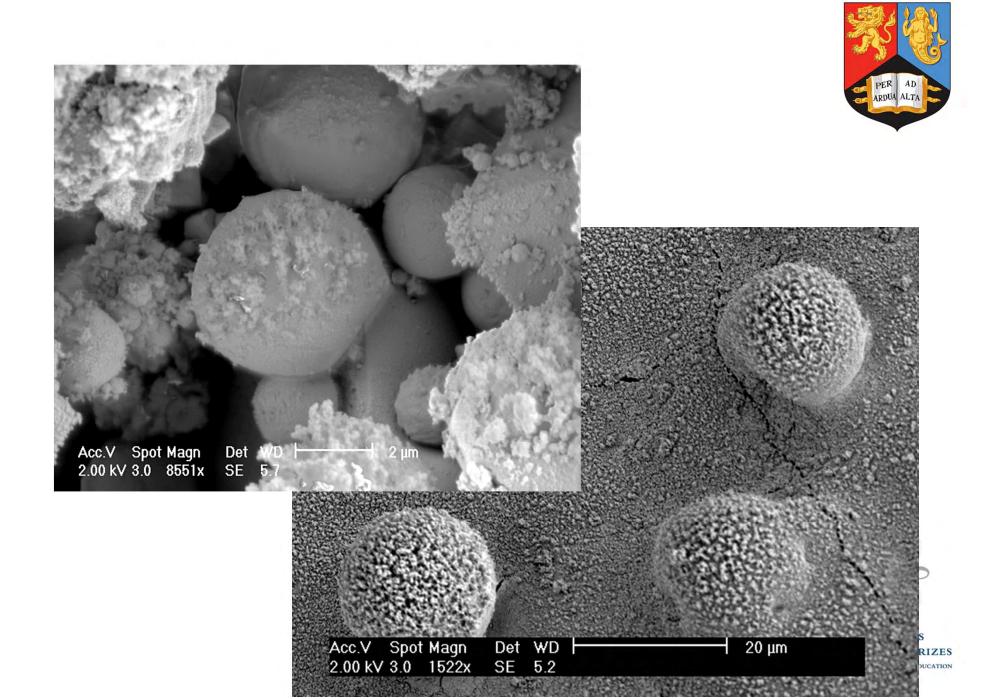
Method: Ultrasonic templating



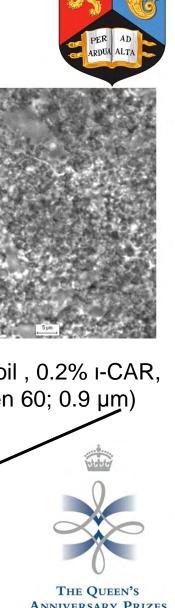


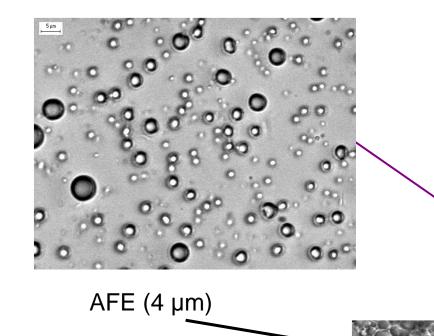
Not a Foam



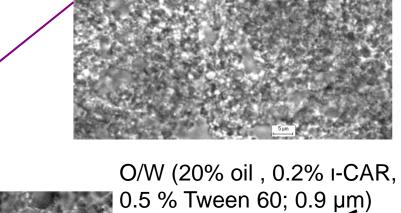


Triphasic structures





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



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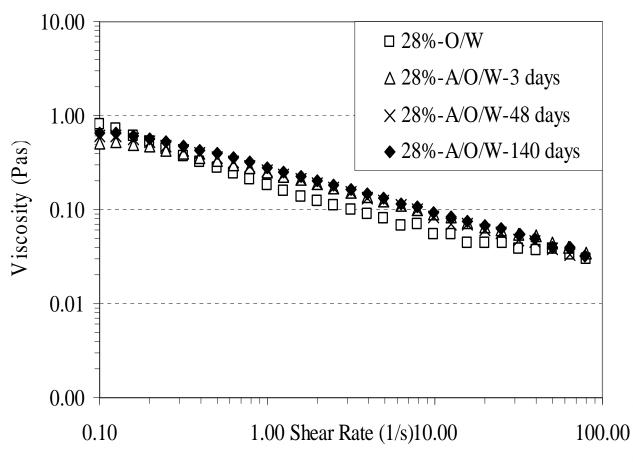
- What is the rheology
- What is the stability
- What is the tribology

Oxidation of lipids



Rheological matching





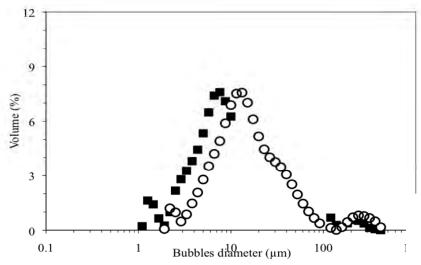


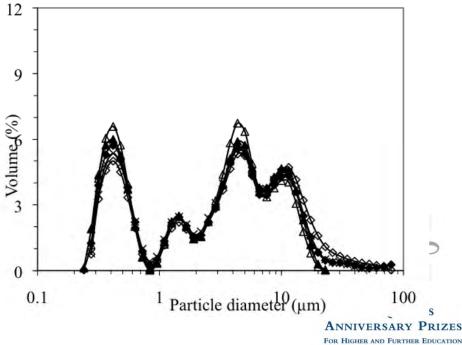
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Triphasic size measurement



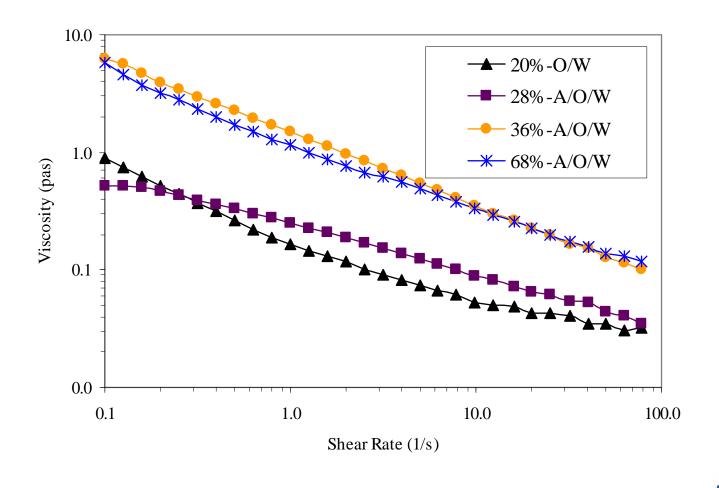
2011





Viscosity with Phase Volume

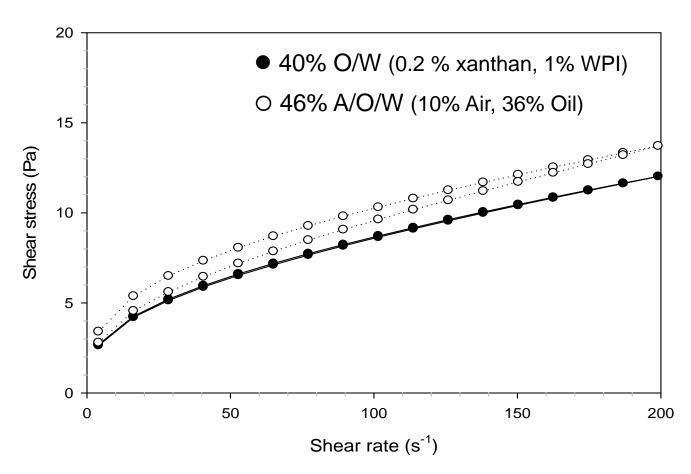






Weak gel formation







Hysteresis loops obtained for 5 day old emulsions at Piers Prize For Higher and Further Education 2011

Creaming - Triphasic

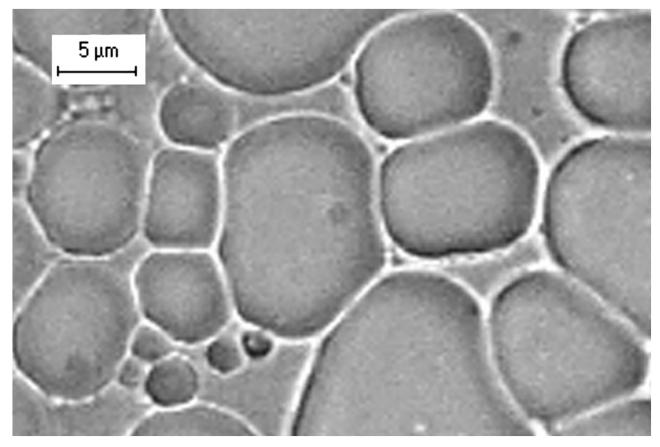






Elastic interfaces

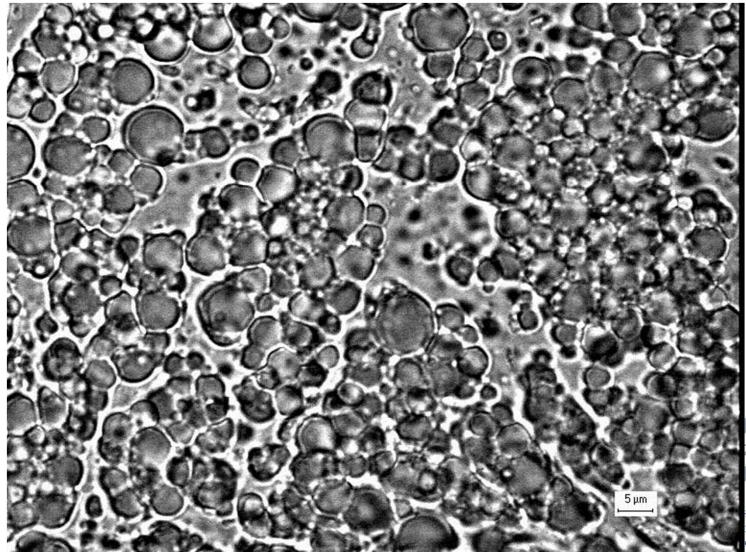






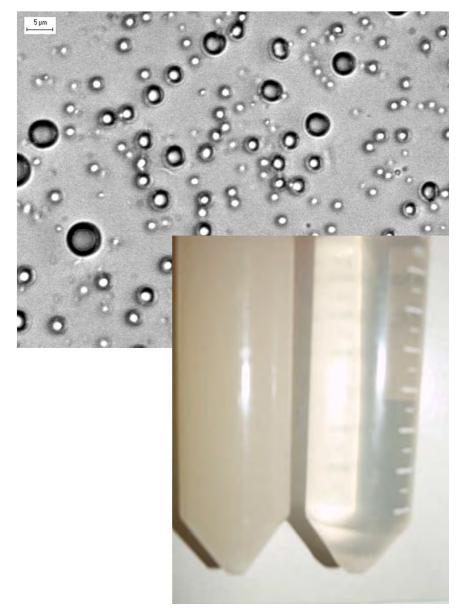
Ostwald Ripening 140 days

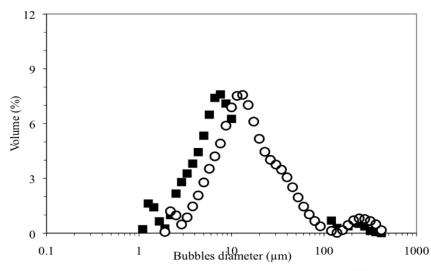




AFE Stability

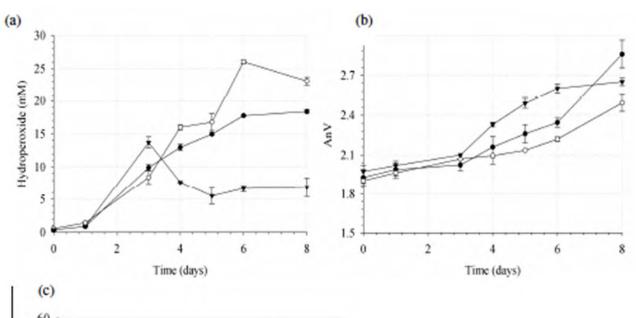




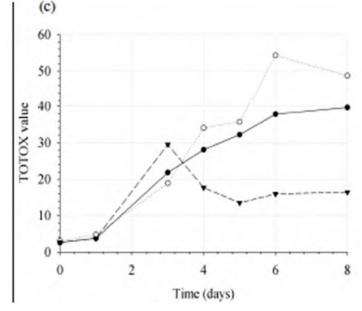




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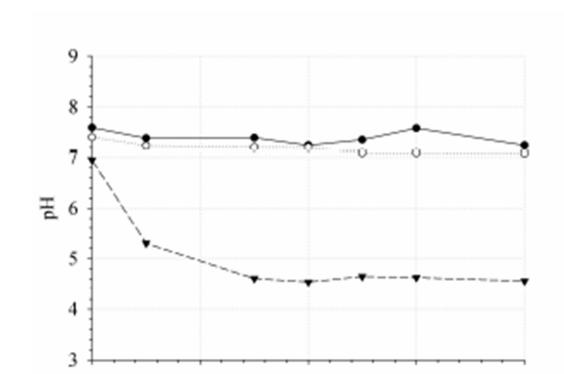


28% A/O/W (10% air, 18% oil) 28% O/W

20% O/W (used for the 28%)

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Hydrophobin at all interfaces



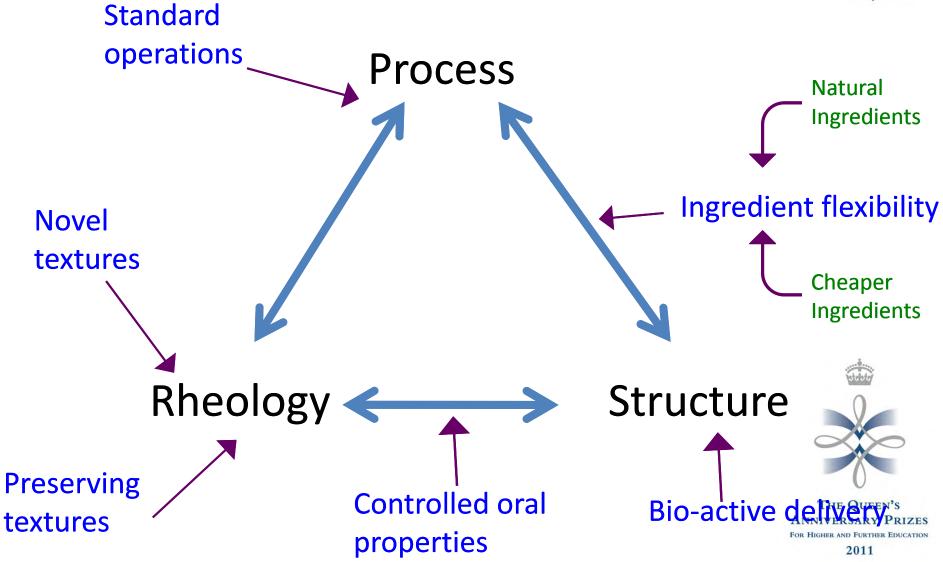
Time (days)



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







The new approach

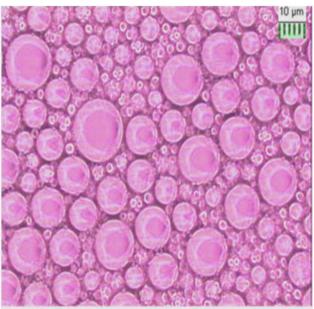
PER AD ALTA

- 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

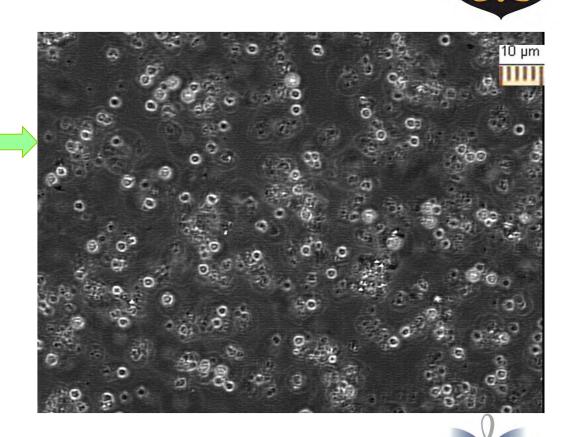


Particle Size Distribution 10 Volume (%) 2 0.01 100 1000 3000 0.1 Particle Size (µm)

Sonochemically-prepared Ovalbumin microbubbles

Sonication condictions

5% w/v aqueous BSA 50°C initial temperature 25 cc/min O₂ 20 kHz, 3 min

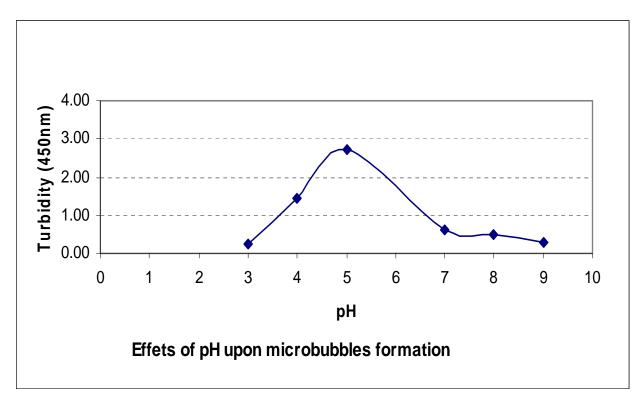


Light microscopy (X 40) of the BSA-microbubbles

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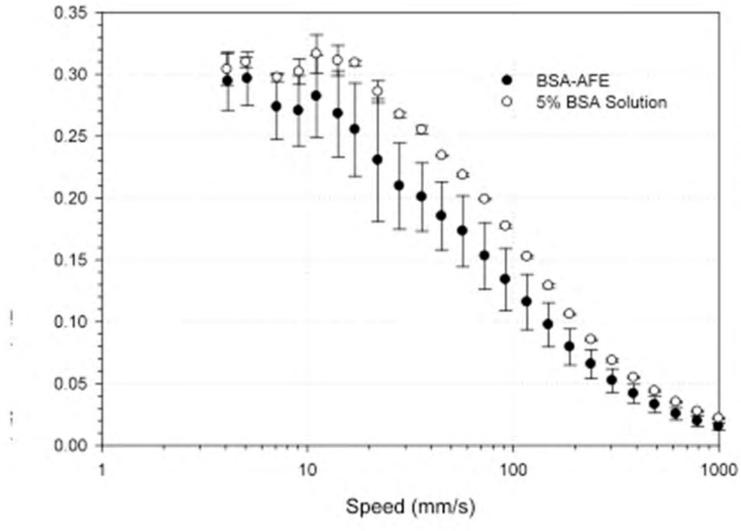




- ⋄ pH 5 seems to be the optimal pH
- √s[buffer] ⇒ ionic strength: no bubbles with BSA-sodium phosphate
 Buffer pH7, 0.2M but bubbles were observed at the same pH, 0.01M



BSA Tribology



Effect of unit operations

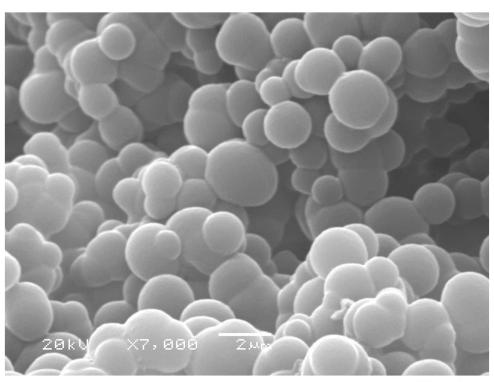




BSA-AFE



Autoclave (121 ° C /15min)



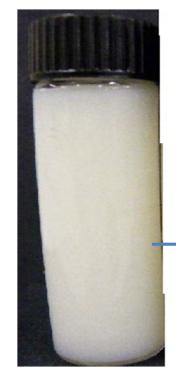


Ovalbumen

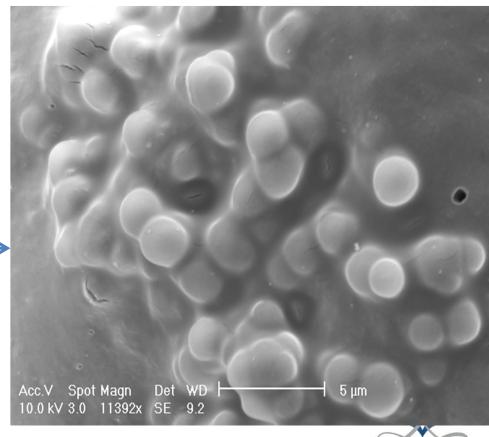








Autoclave (121 ° C /15min



SEM image of EWP- AFE after sterilisation

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New hydrophobins



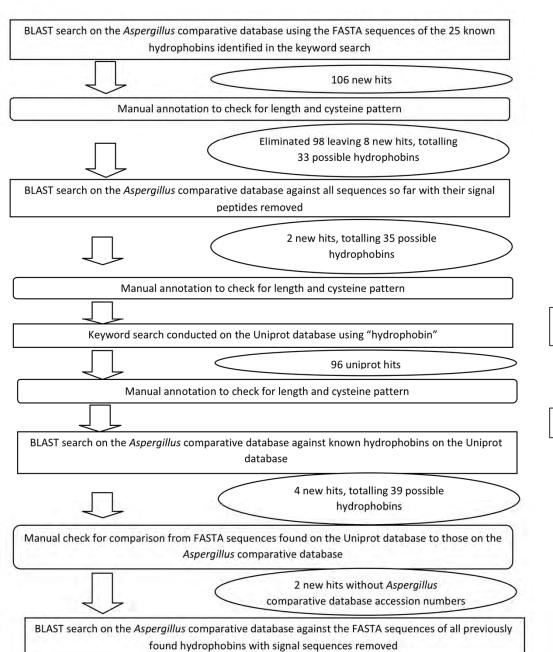
Class I:

$$X_? - C - X_{5-7} - C - C - X_{19-39} - C - X_{8-23} - C - X_{5} - C - C - X_{6-18} - C - - X_{2-13}$$

Class II:

$$X_{?} - C - X_{9-10} - C - C - X_{11} - C - X_{16} - C - X_{8-9} - C - C - X_{10} - C - X_{6-7}$$







 ${\tt BLAST\ search\ on\ the\ } {\it Aspergillus\ } {\it comparative\ database\ against\ the\ } {\it FASTA\ sequences\ of\ those\ identified\ from\ CADRE}$



3 new hits, totalling 44 possible hydrophobins from the *Aspergillus* comparative database

 ${\tt BLAST\ search\ on\ the\ } Asper gillus\ comparative\ database\ against\ the\ FASTA\ sequences\ of\ those\ identified\ from\ the\ previous\ BLAST\ from\ CADRE$

22 new hits, totalling 66 possible hydrophobins from the Aspergillus comparative database and two from Uniprot



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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.



ATEG 04730	1 NP	LAARHGAPICPSGTAYSSMOCC	24
NFIA 038830	1 VP	FSHASNSTSASKSTPSSTVPASLPSPTLSGPNSCPPNKFKQCC	45
ACLA 001890	1 IP	LANTGPSSTPSSSAALATPTLTGPNICPGKRSKQCC	38
sp P79073	1	AVCPTGL-FSNPLCC	14
sp P52754	1 QP	LEDRSNGFSNPQCC	25
cons	1	**	48
ATEG 04730	25 🔼-	VNVADVVSLDCOPPGPL-PSSAAEF	49
NFIA 038830	46 TT	LSQVGDDLLKPLGMVVPLVGAVELSSLVGVSCRMMPDAAPE	88
ACLA 001890		LSQLQTSLLDPVGNVIPLLSGMQLTSLIGLSCRTMSDEHTK	81
sp[P79073]	15 A-	TNVLDLIGVDCKTPTIA-VDTGAIF	39
sp P52754	26 🔼 –	<mark>T_QVLGLIGLDCKVP</mark> SQN-VYDGTDF	50
cons	49	\$30,000,000*C	96
ATEG 04730	50 KE	ICAKIGDKPQCCTLAL-AGQ-AIACQN	76
NFIA 038830		DCANAVMCCDSSA-VGG-DLMQTSCQDFALAKKREREAIERQQ	130
ACLA 001890		DCADALMCCDAAGEVGASNILQTKCEEFDLAKAKEIAAVERAQ	125
sp[P79073]	40 OA	HCASKGSKPLCCVAPV-ADQ-ALLCQK	66
sp P52754		VCAKTGAQPLCCVAPV-AGQ-ALLCQT	77
cons	97	**	144
ATEG 04730	77	PAGMDEDSGSSESESDSGSGGGGDA 1	102
NFIA 038830	131 RR	FSEYQRMMMSQSATPTPTPVGVGLMGSSFSRASATPTRIQGGLL 1	76
ACLA 001890	and the second second		61
sp[P79073]	The second secon	AIGTF	71
sp P52754	78	<mark>AV</mark> G- <mark>A</mark>	81
cons	145		90



2011

Findings



- 74 new HFs identified
- Putative Class 1.5 seen
- Dimers & trimers seen

Looking for non – microbial analogues

