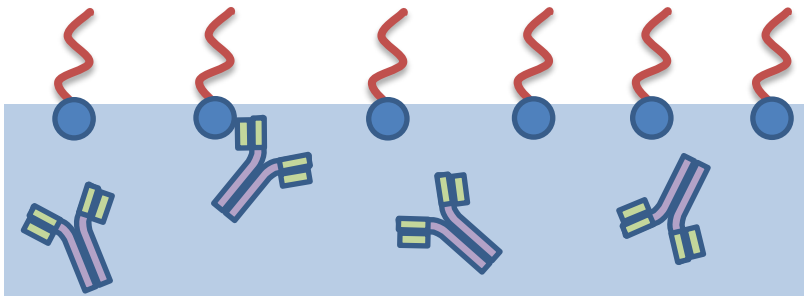
An Investigation of the Stabilizing Effects of Surfactants on Biotherapeutics

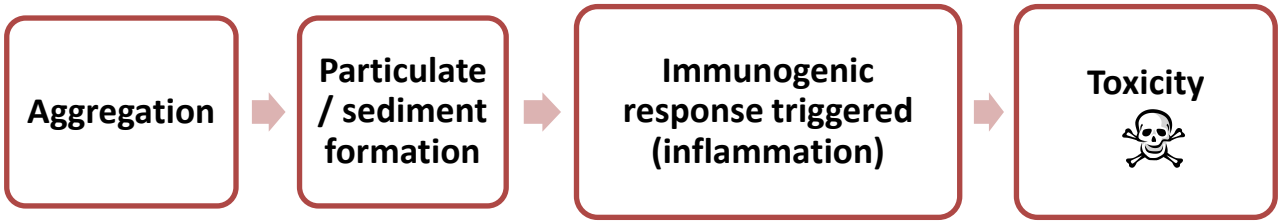


Marianna Fleischman
SURF Program 2013
University of Delaware

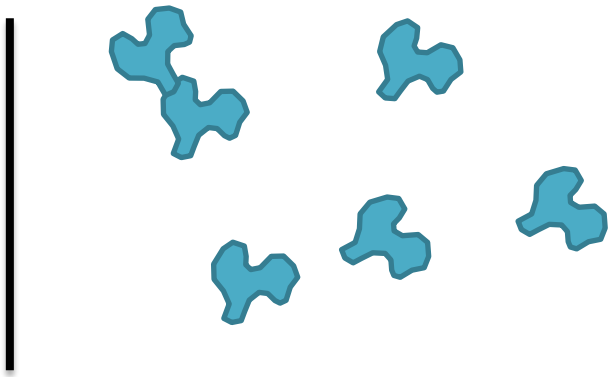
Advisors: Drs. Ron Jones, Tatiana Perevozchikova, and Katie Weigandt

Our Research

The objective of this research is to find out more about why/how non-ionic surfactants reduce aggregation.



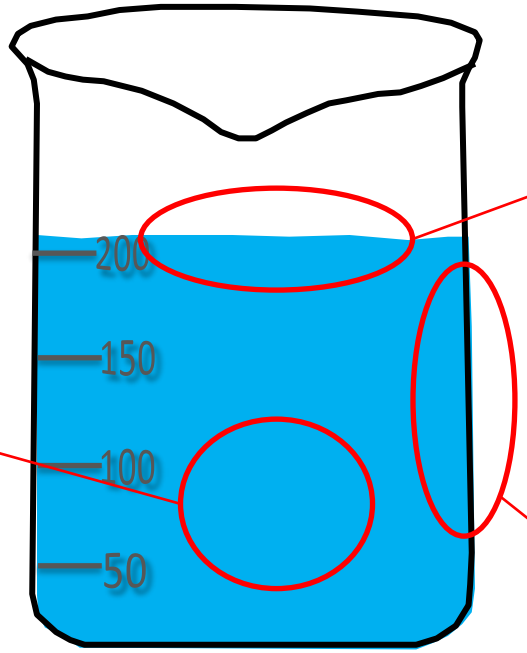
- Protein adsorption to interfaces



- Interactions between proteins in bulk solution

Our Research

Potential locations for aggregation:



Bulk material
Small-Angle Neutron Scattering (SANS)

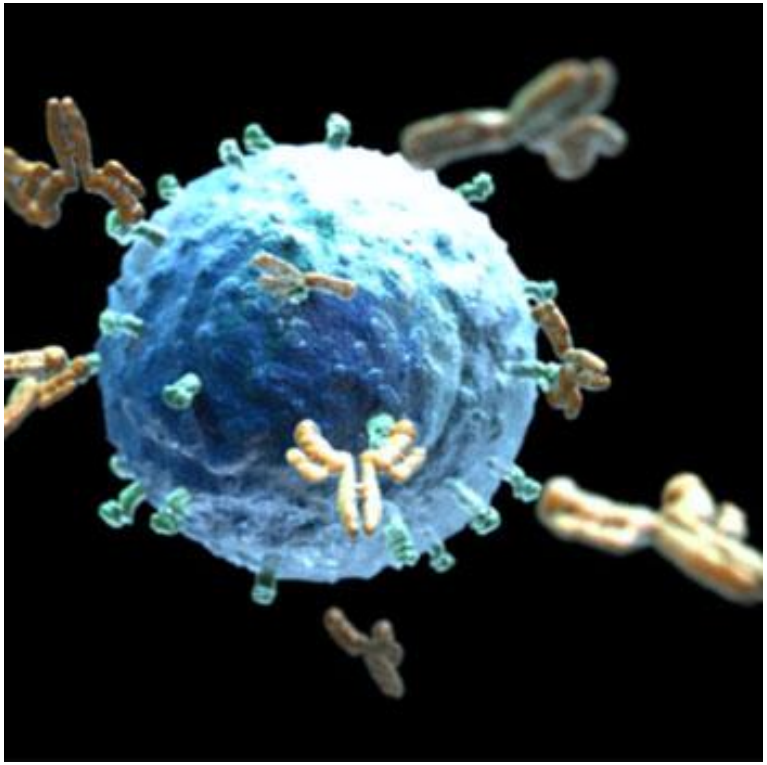
Air-water Interfaces
Langmuir Trough and X-Ray Reflectometry

Micro-Flow Imaging

Solid-water Interfaces
Micro-Flow Imaging

Fluorescence Spectroscopy

Background – Monoclonal Antibodies

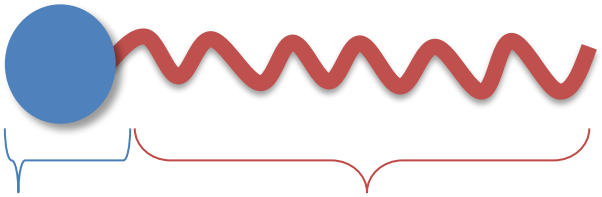


TRES3D Medical and Scientific Animation

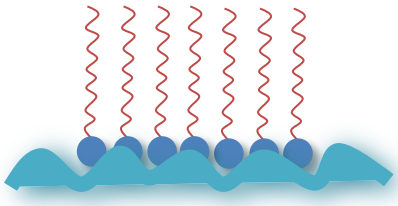
- Target specific antigens
- Can be engineered in a lab
- Effective against autoimmune diseases and cancer
- Specificity reduces side effects

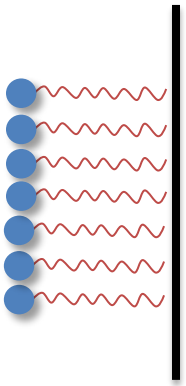
What are surfactants?

Amphiphilic molecules:

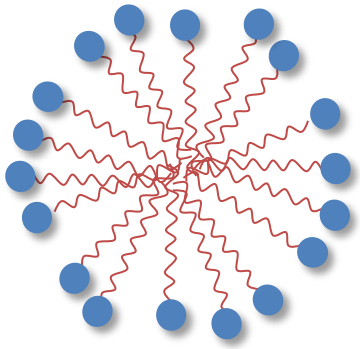


Results in unique behavior:





Forming monolayers at interfaces

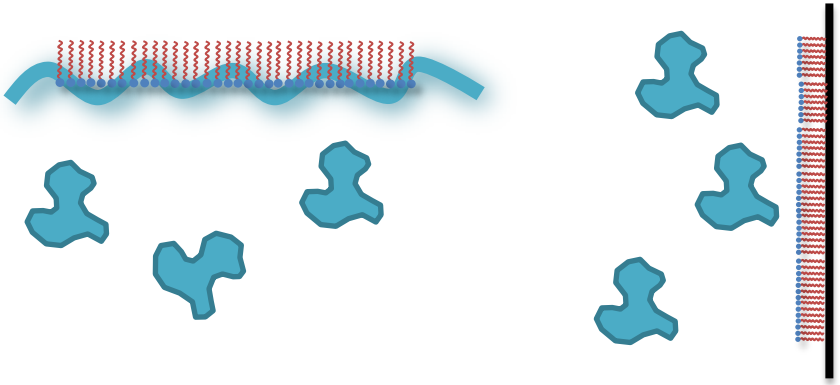


Micellular formation

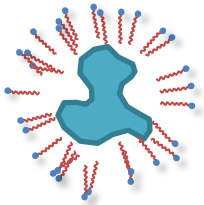
*At or above the critical micelle concentration (CMC)

How might surfactants help?

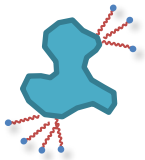
Competitive binding at interfaces



Encapsulation in micelles



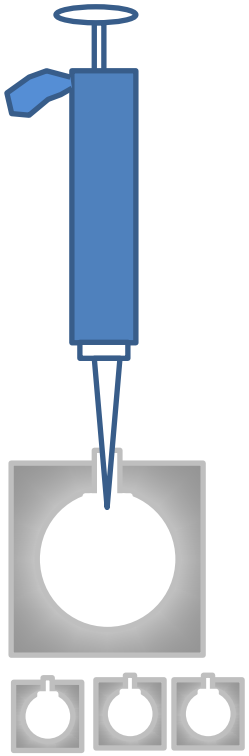
Preferential binding leading to steric hindrance of aggregation



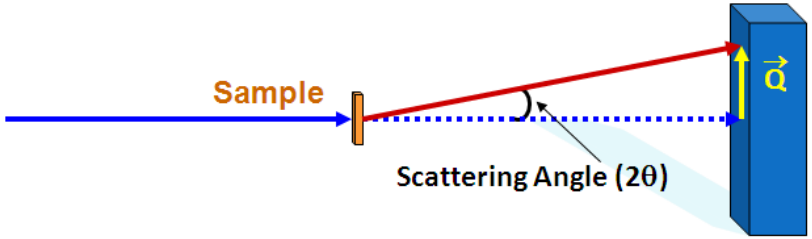
Bulk Solution Investigation: 10m SANS

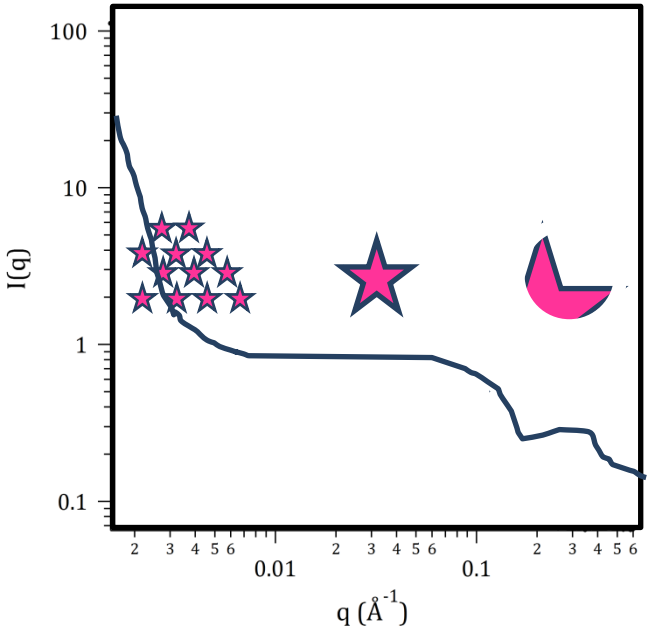
- 4 Surfactants
  - Triton X-100
  - Tween 80
  - Octyl-beta-D-glucopyranoside **(OG)**
  - Cetyltrimethylammonium Bromide (CTAB)*
- Protein
  - Immunoglobulin G **(IgG)**

=4 samples; collected data at multiple concentrations of surfactant (below, at, above CMC)

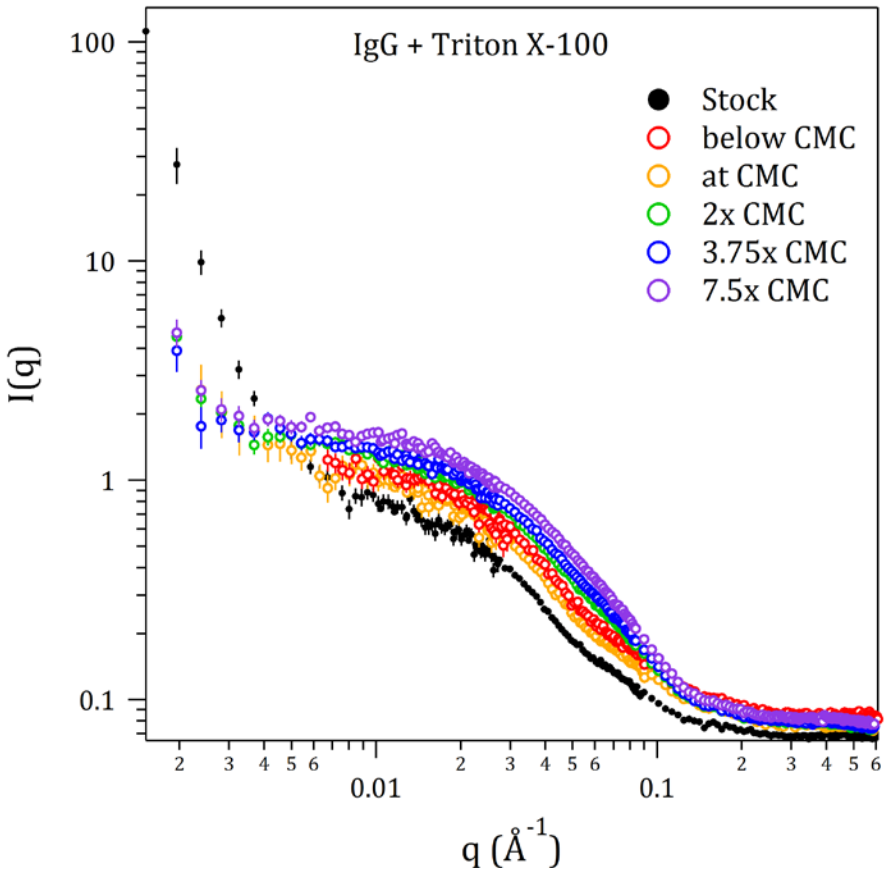


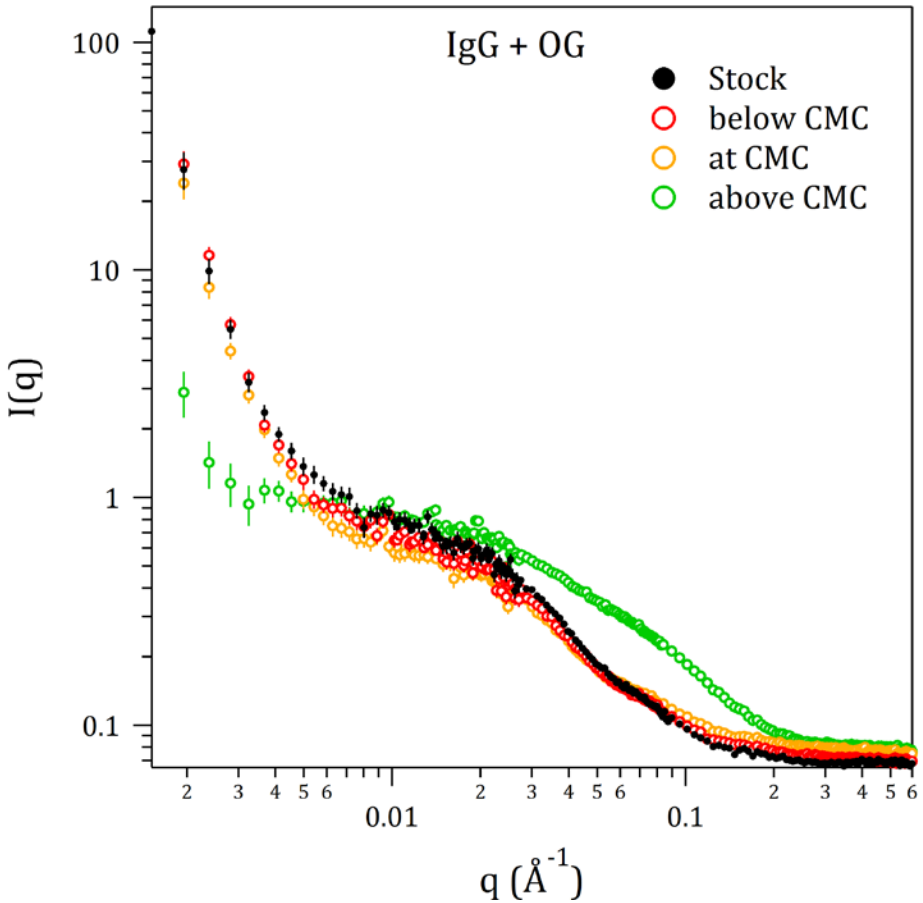
Bulk Solution Investigation:
10m SANS





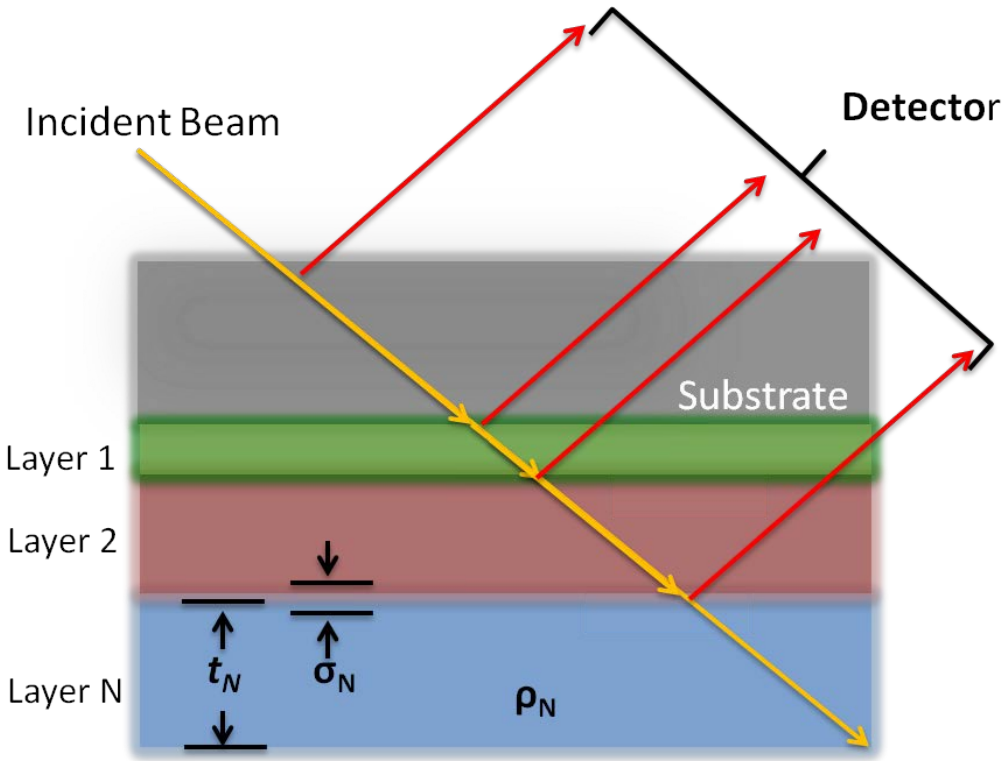
SANS Data: IgG and Two Surfactants

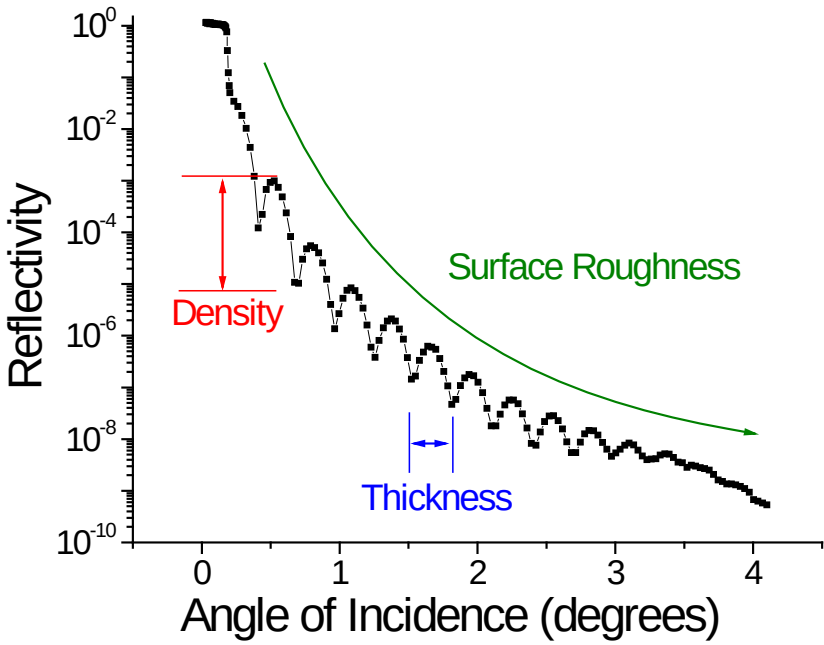




- Dampening of upturn at low q
- Some change of solution structure at medium-high q

Air/Water Interface Investigation:
Xray Reflectometry

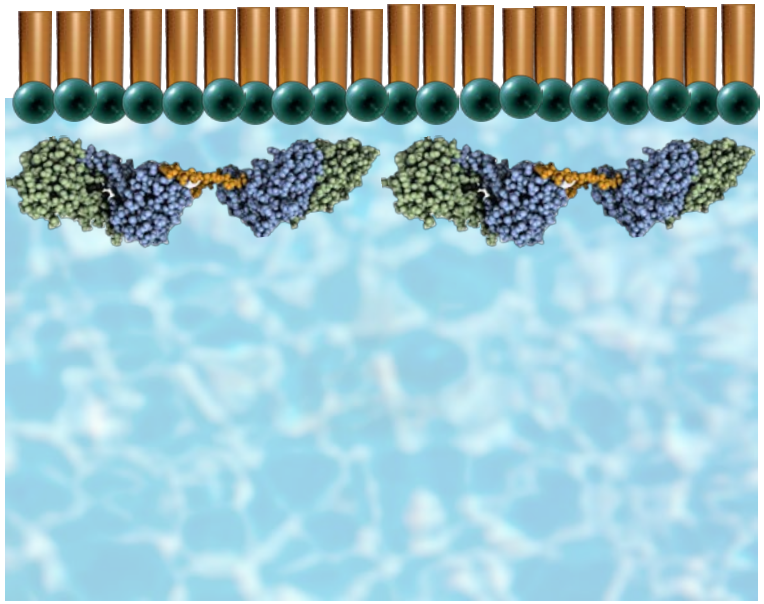


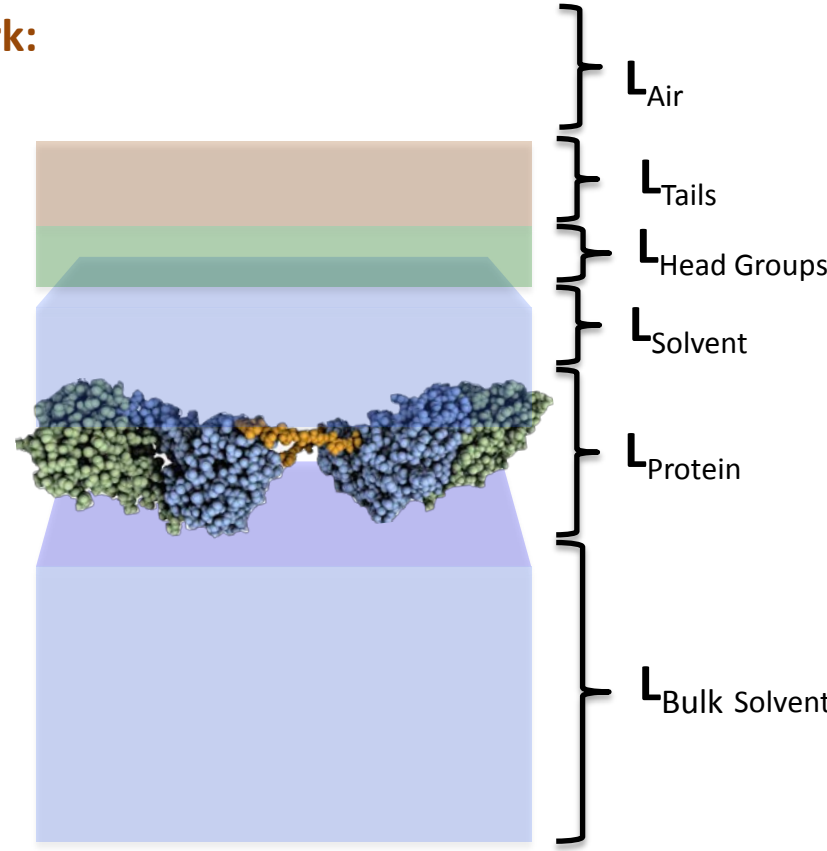


Air/Water Interface Investigation:
Xray Reflectometry

Langmuir Trough Work:

Protein Adsorption (one of the possible configurations) at air-water interface





Air/Water Interface Investigation:
Xray Reflectometry

1 mg/ml IgG

↓

+Triton X-100
(below CMC)

↓

+Triton X-100
(above CMC)

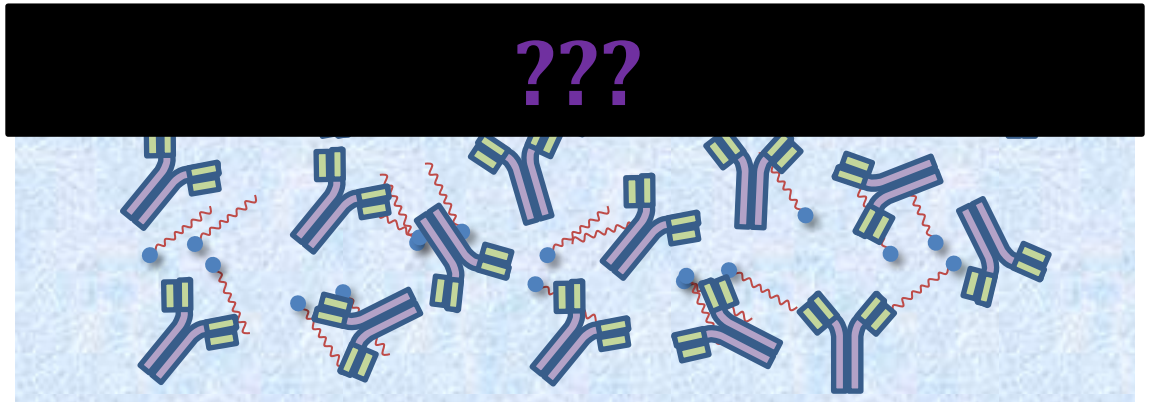
↓

+IgG
(5mg/ml)

↓

Dilate/Compress
Barrier

Competitive Adsorption



Air/Water Interface Investigation:
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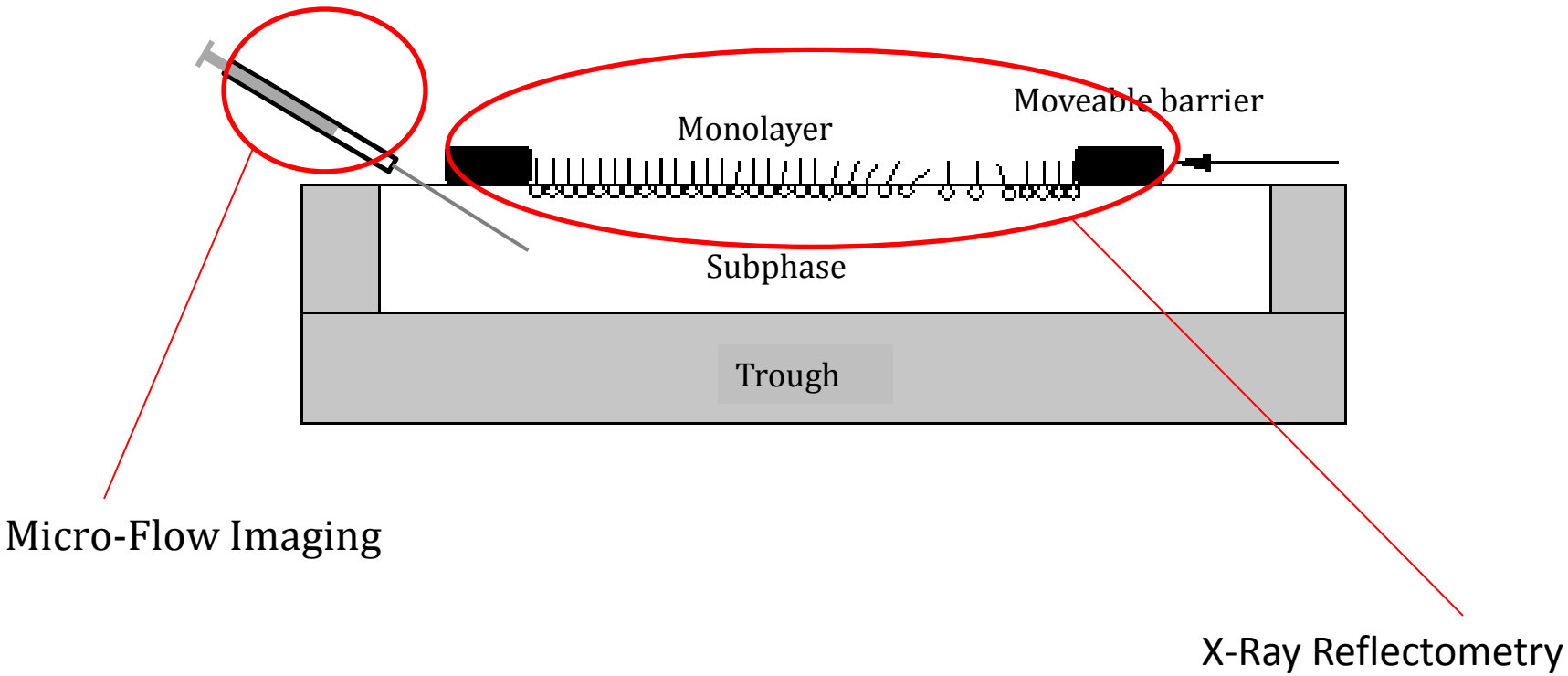

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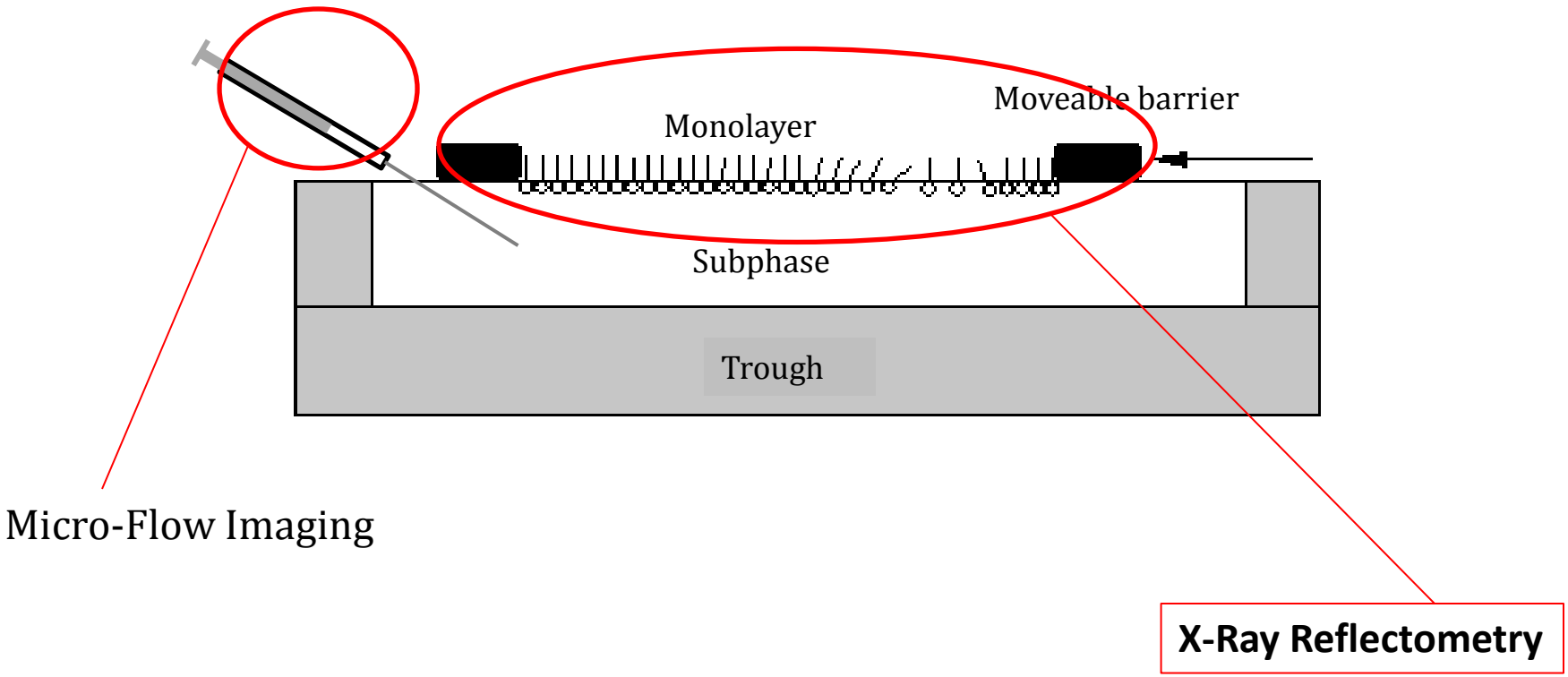
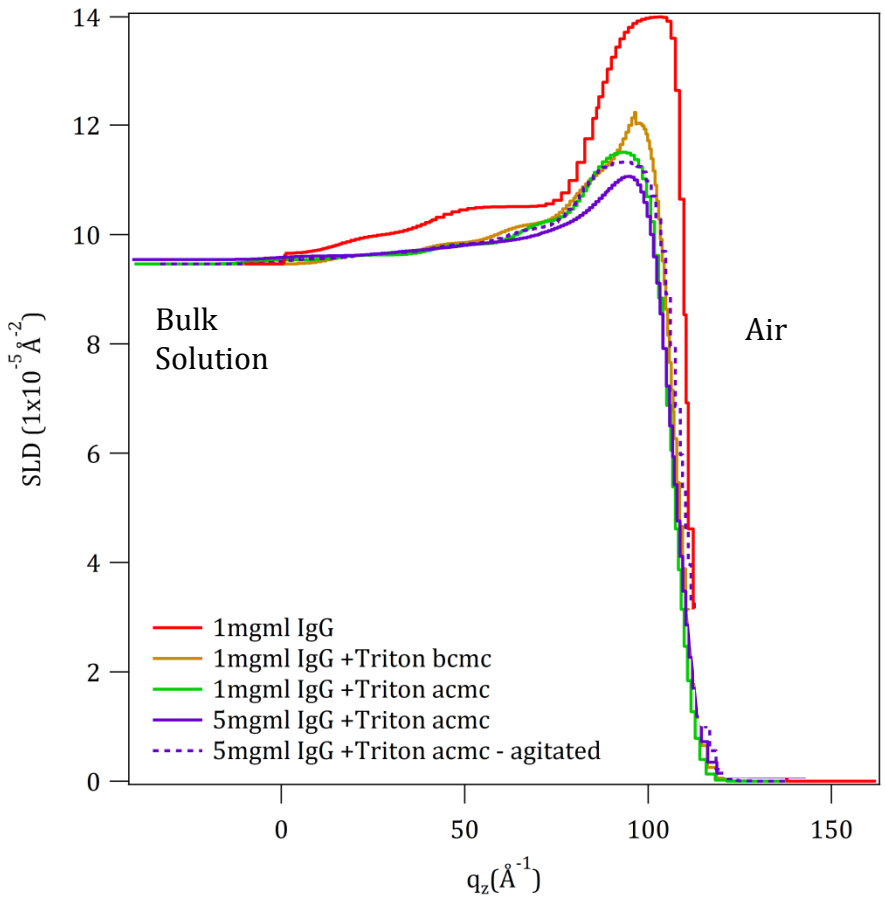
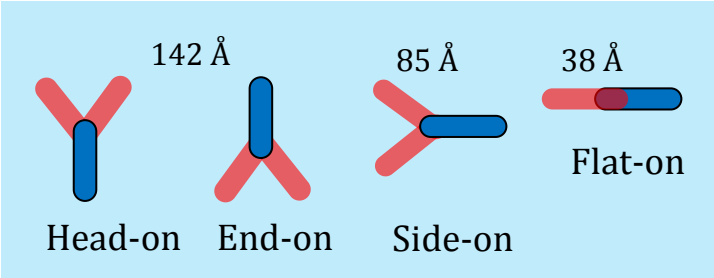
Xray Reflectometry



Effect of Surfactant on Air-Water Interface Adsorption

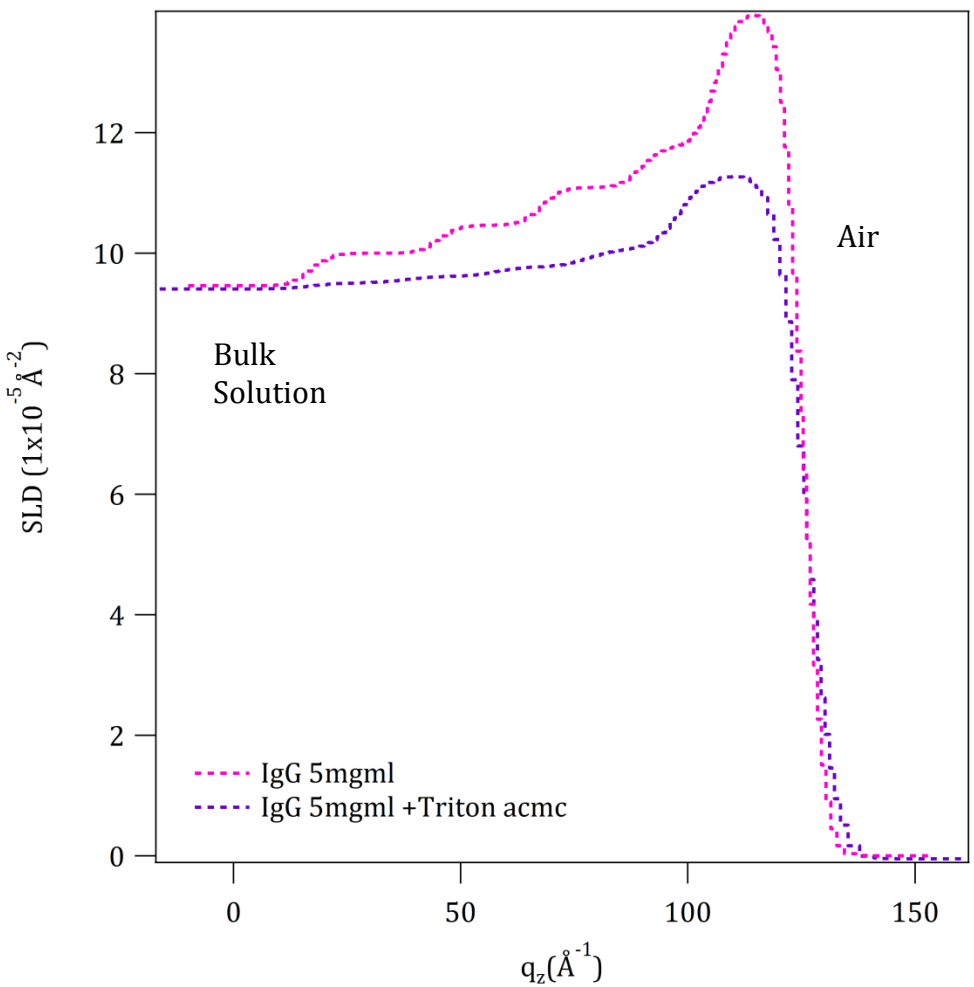


- ~50 Å of dense antibody layer (flat-on)

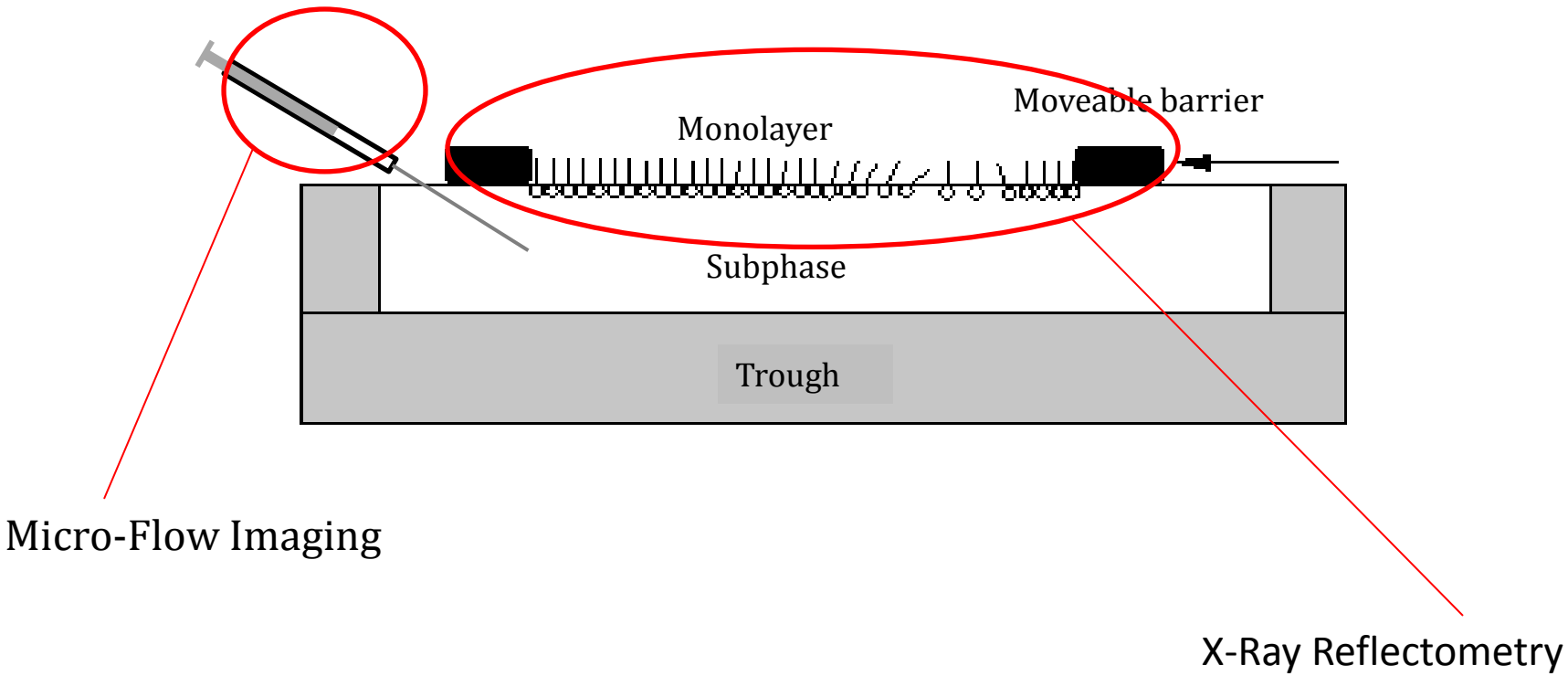


- Some diffuse layer ~60 Å
- Addition of Triton X-100 (even below CMC) yield significant competition
- Further addition of Triton X-100 yields further depletion of antibody layer
- Addition of more antibody does not negate/reverse effect of surfactant

Agitation of the Air/Water Interface



Air/Water Interface Investigation:
Xray Reflectometry



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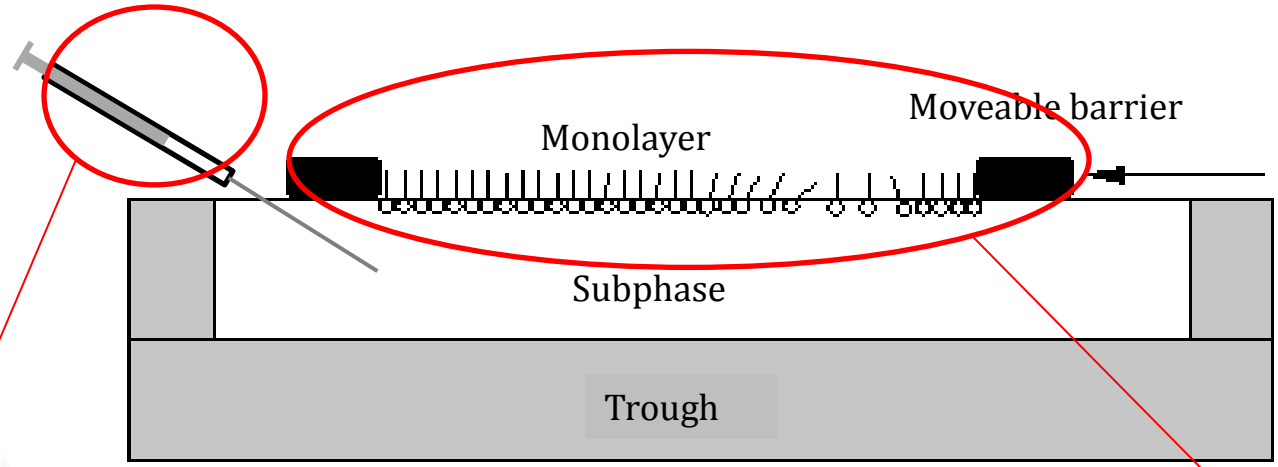
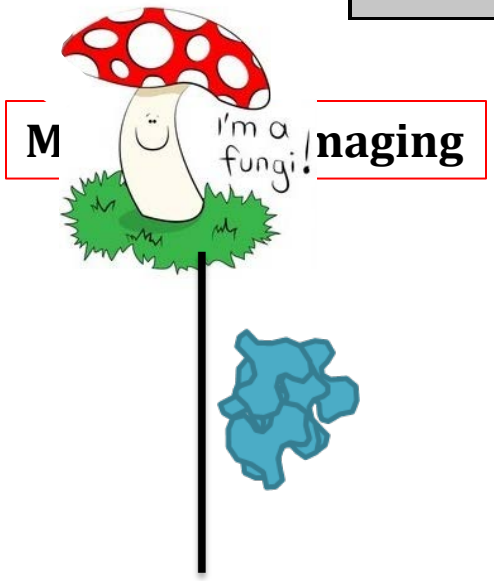
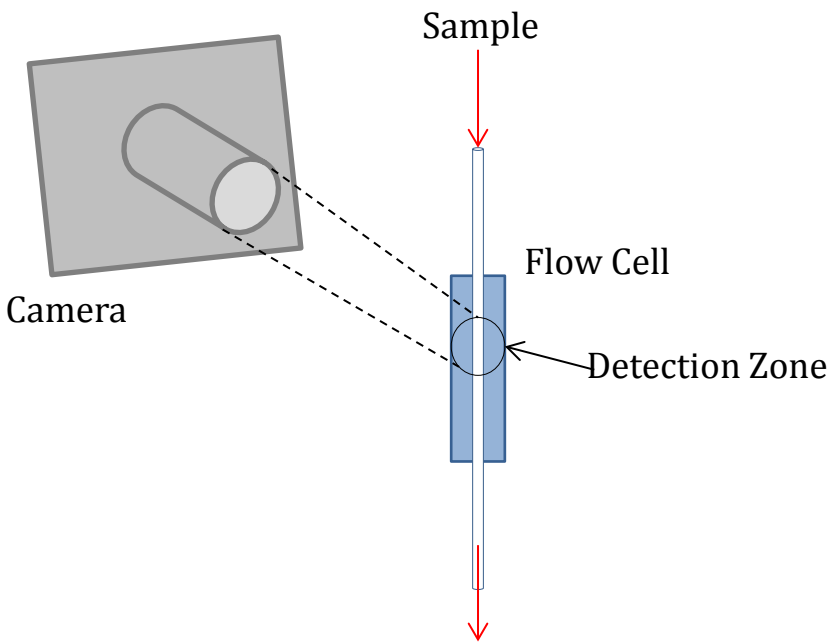




M naging

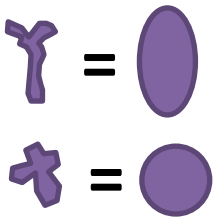
X-Ray Reflectometry

Micro-Flow Imaging

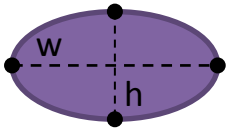


Examines:

- Size (Equivalent Circular Diameter – ECD)



- Shape (Aspect Ratio)

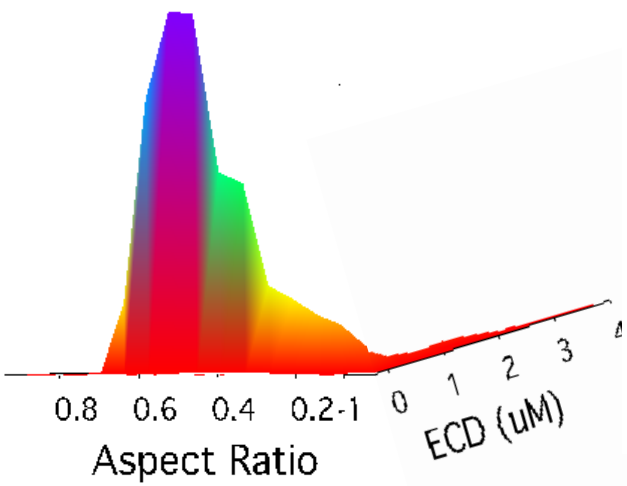


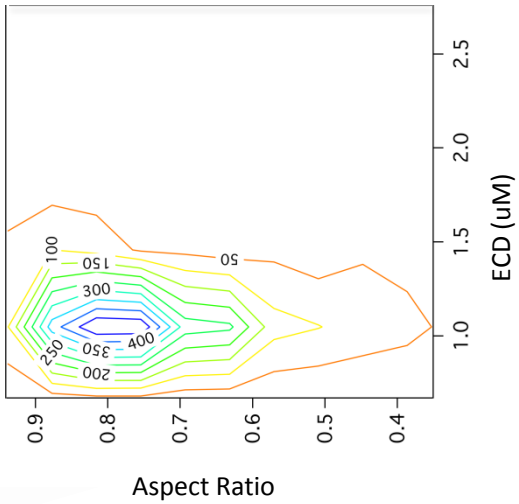
- Volume (Counts number of particles)

Solid/Water Interface Investigation:
Micro-Flow Imaging and Fluorescence Spectroscopy

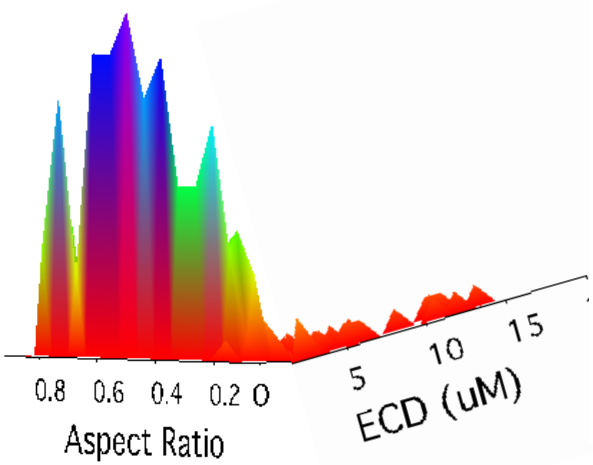
IgG pH 4.5

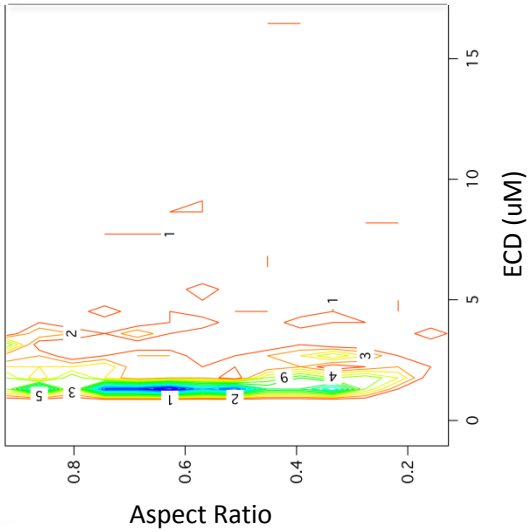
Stock Solution





Buffer Wash 2



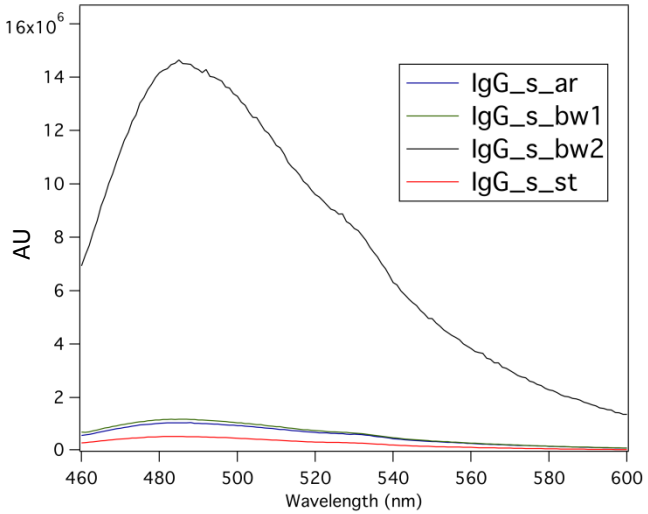


Buffer Wash 2 Images:

30.6 uM

22.4 uM

ThT Fluorescence



Discussion

- Our findings are consistent with earlier findings that surfactants affect protein aggregation and adsorption
- Suggest that surfactants may adsorb to surface of protein itself, or form structure around protein to prevent protein-protein interactions
- Surfactant outcompeted protein at air-water interface and further addition of protein did not negate this effect

Acknowledgments

Thank You to:

- Tatiana Perevozchikova
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- Katie Weigandt
- Prof. Chris Roberts

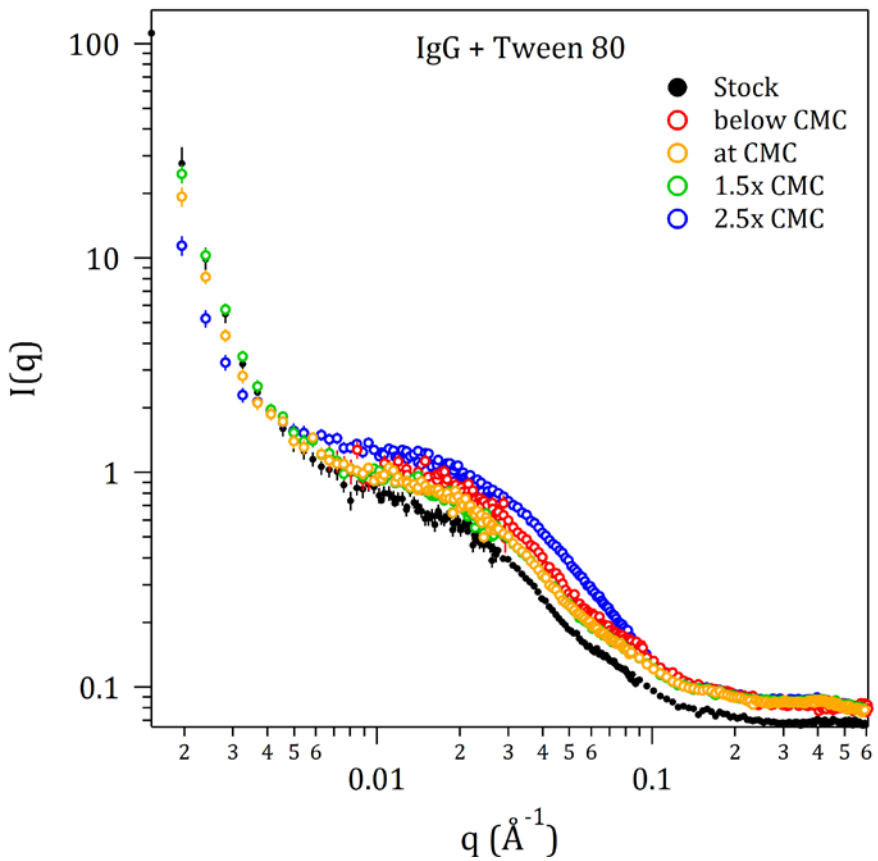
- Hirsh Nanda
- Mike Dimitrou
- Bulent Akgun

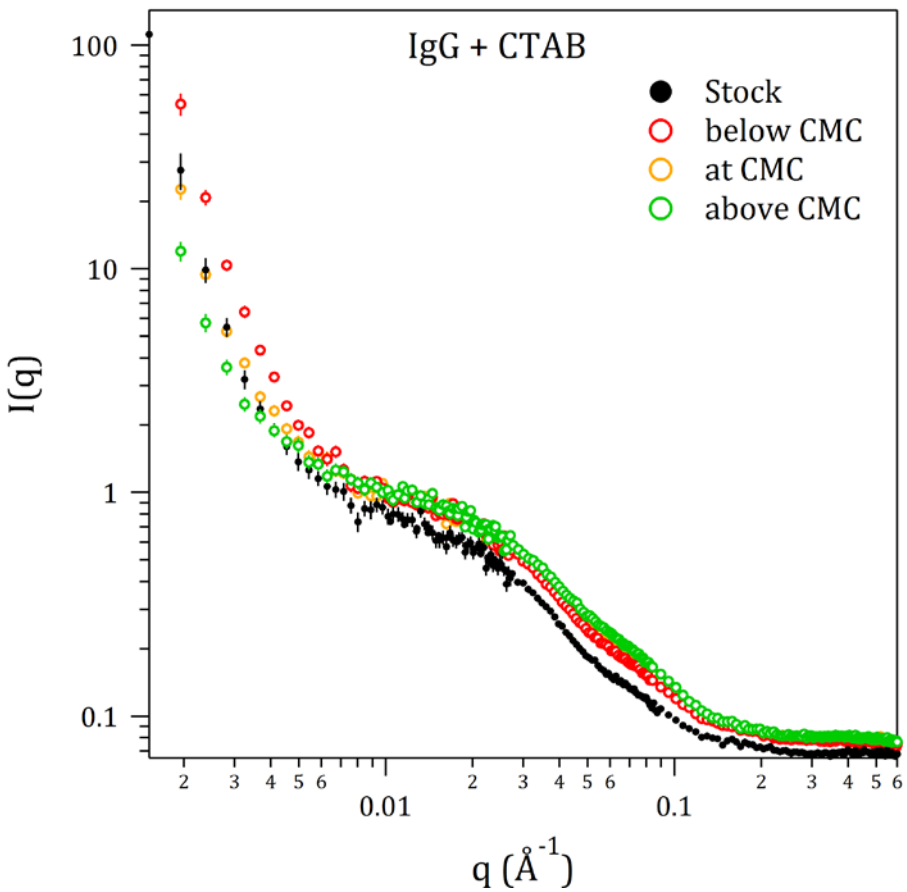
- SURF Directors
- NSF CHRNS
- NCNR

Thank you.

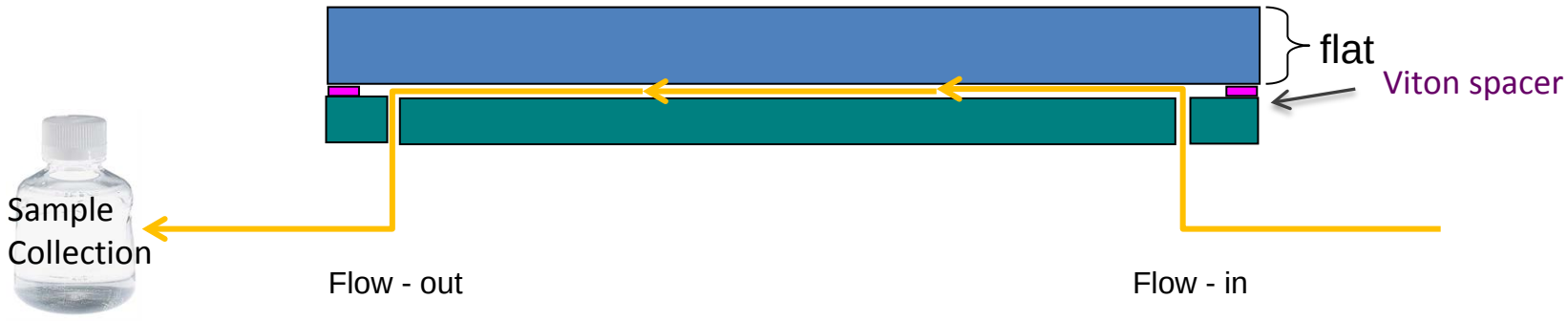
Questions?

SANS Data: IgG and Two Surfactants

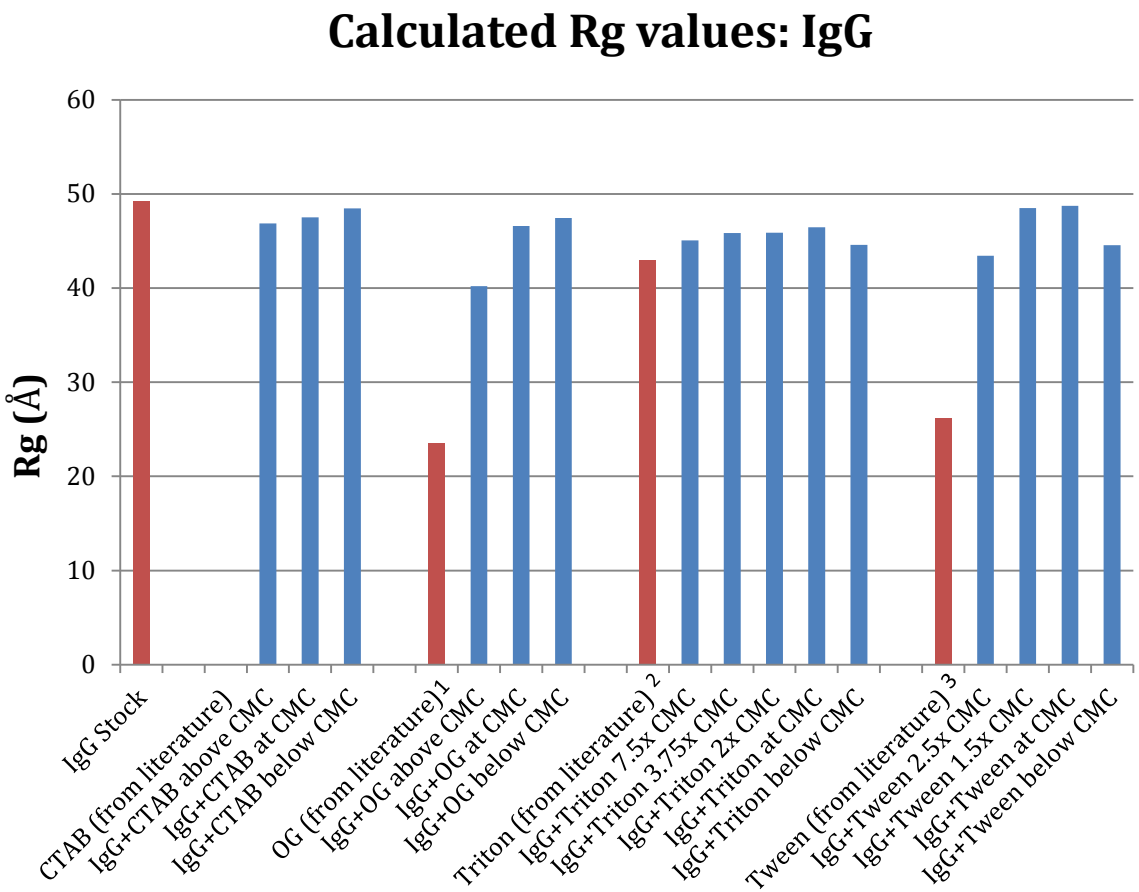




Solid/Water Interface Investigation:
Micro-Flow Imaging and Fluorescence



Sample	Rg (Å)
IgG Stock	49.256
CTAB (from literature)	
IgG+CTAB above CMC	46.865
IgG+CTAB at CMC	47.504
IgG+CTAB below CMC	48.471
OG (from literature)	23.5
IgG+OG above CMC	40.209
IgG+OG at CMC	46.6
IgG+OG below CMC	47.448
Triton (from literature)	43
IgG+Triton 7.5x CMC	45.079
IgG+Triton 3.75x CMC	45.859
IgG+Triton 2x CMC	45.865
IgG+Triton at CMC	46.459
IgG+Triton below CMC	44.591
Tween (from literature)	26.2
IgG+Tween 2.5x CMC	43.446
IgG+Tween 1.5x CMC	48.496
IgG+Tween at CMC	48.732
IgG+Tween below CMC	44.57



[1] Oliver RC, Lipfert J, Fox DA, lo RH, Doniach S, et al. (2013) Dependence of Micelle Size and Shape on PLoS ONE 8(5): e62488. doi: 10.1371/journal.pone.0062488

[2] Streletzky K, Phillies GDJ. (1994) Temperature Dependence of Triton X-100 Micelle Size and Hydration. Langmuir 11: 42-47

[3] Amani A, York P, de Waard H, Anwar J. (2011) Molecular dynamics simulation of a polysorbate 80 micelle in water. Soft Matter 7: 2900-2908