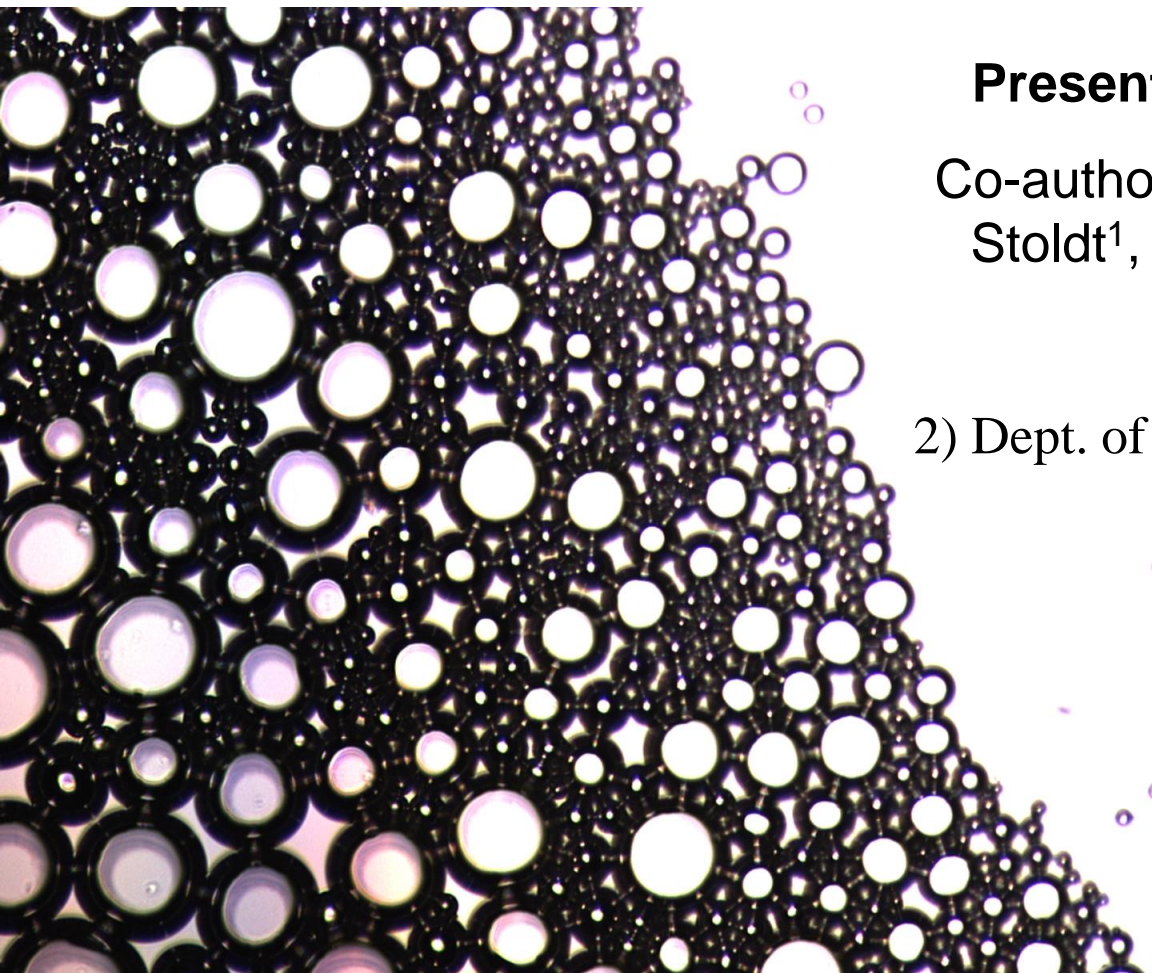




## **Conjugation Efficiency of Functionalized Microbubbles for Targeted Ultrasound-Based Molecular Imaging**



**Presentation by Steve Lammers**

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2) Dept. of Pediatrics, CU Health Sci. Cntr.

Chair: Michele Grimm

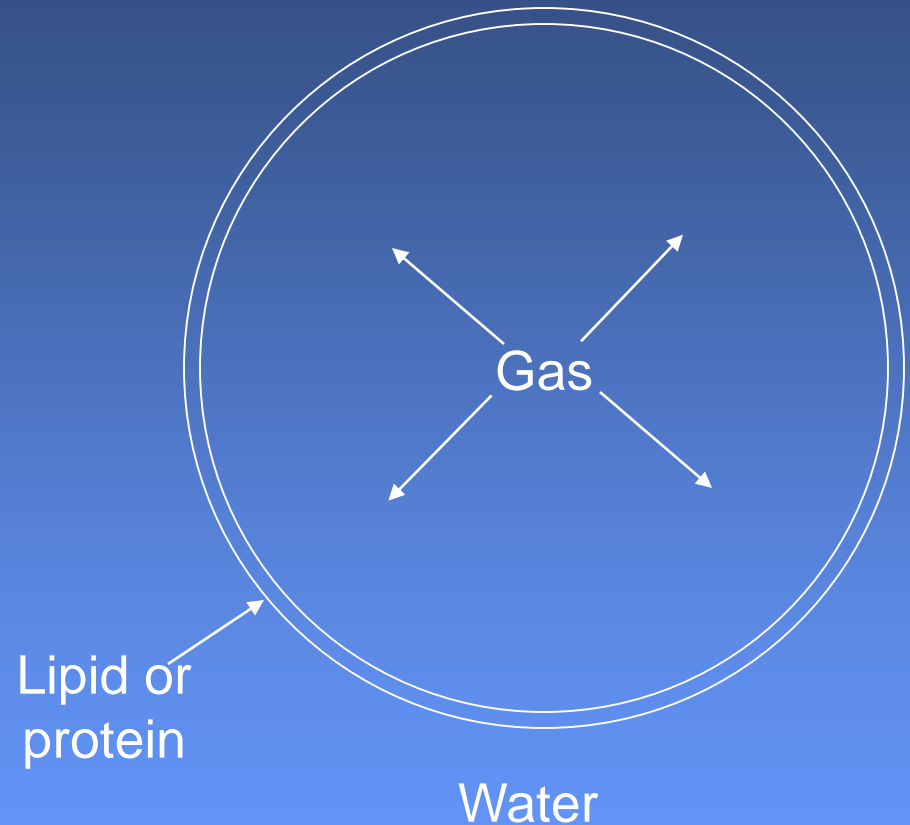
Co-Chair: Amy Lerner



# Microbubbles

## General Information:

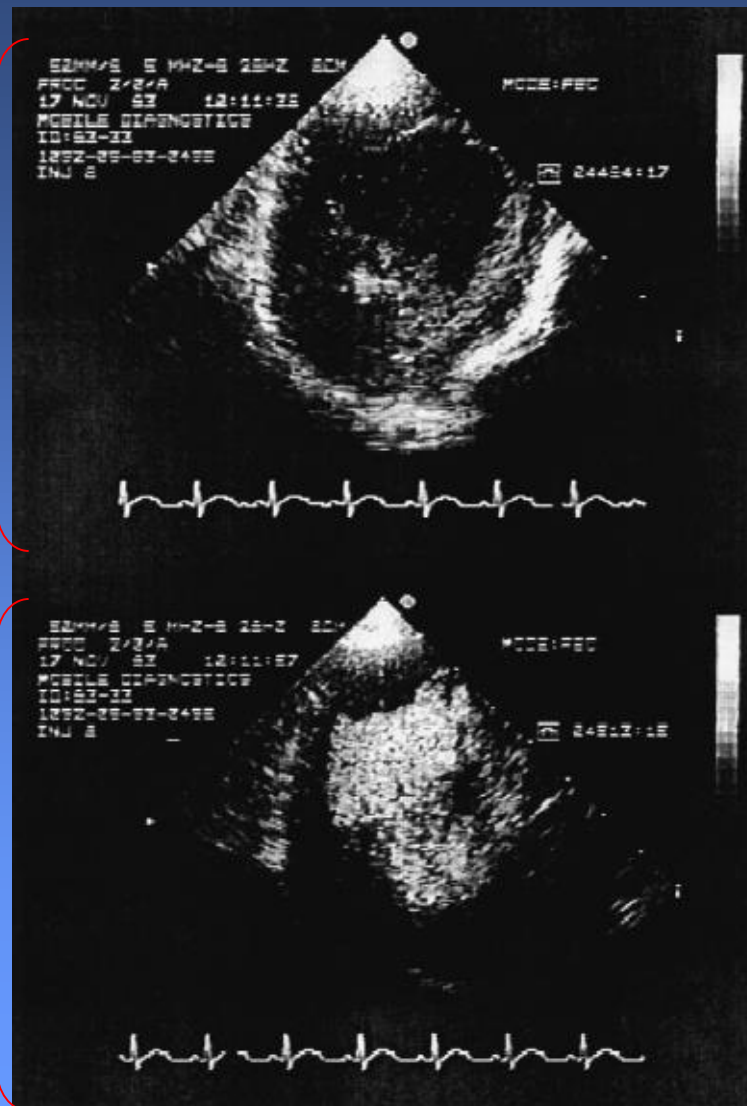
- Gas-filled vesicles with a lipid or protein shell
- Currently used to enhance imaging of organ perfusion using ultrasound
- Produce a strong signal due to a large mismatch of acoustic impedance





# Organ Perfusion

Ultrasound image of  
dog heart prior to  
microbubble injection

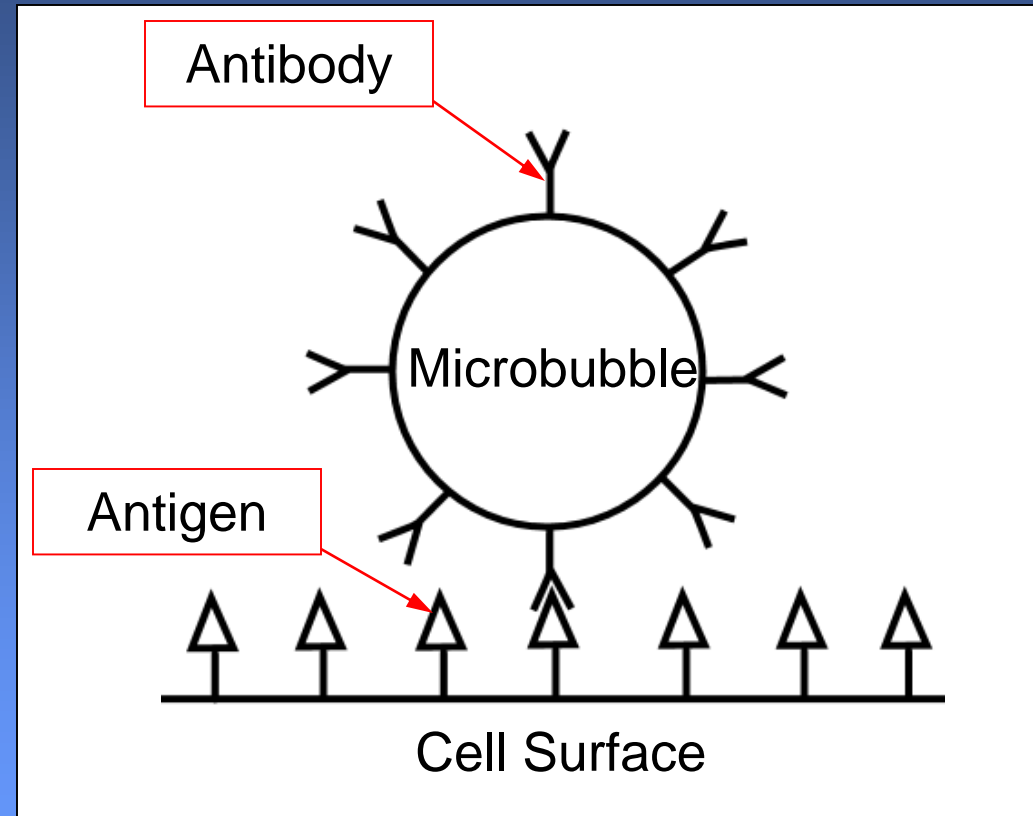


Ultrasound image of  
dog heart after  
microbubble injection



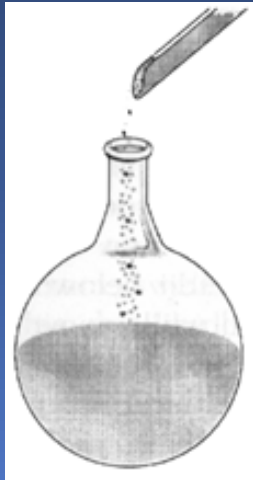
# Molecular Targeting

- The ability to target a microbubble, towards a specific molecular vector
- Allows for detection of extremely small pathogenic locals within the body
- Ultrasound equipment and operation is less expensive than MRI



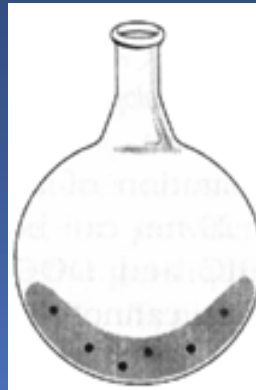


# Methods of Preparation Liposomes



Lipids are  
added / mixed

Dry  
→

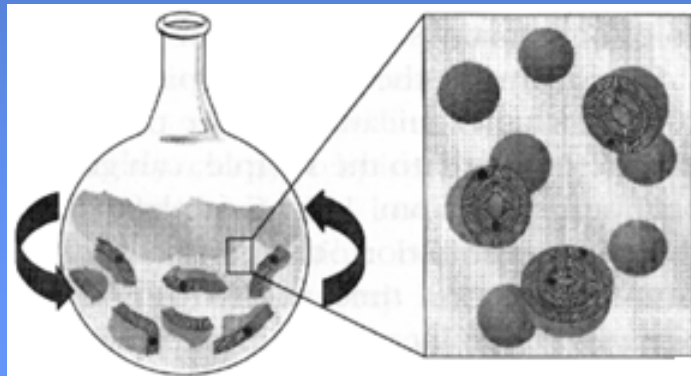


Lipid Cake

→

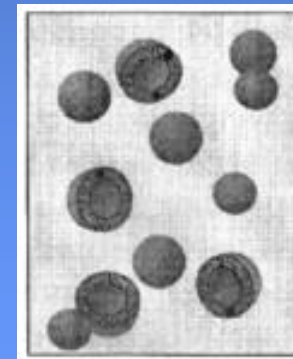


Buffer Soln.



Agitation: Large Multilamellar  
Vesicles

Sonication  
→  
Extrusion



Small Unilamellar Vesicles

\*Images from Avanti Polar Lipids Website





# Methods of Preparation Microbubbles

## Cavitation-Induced bubbles

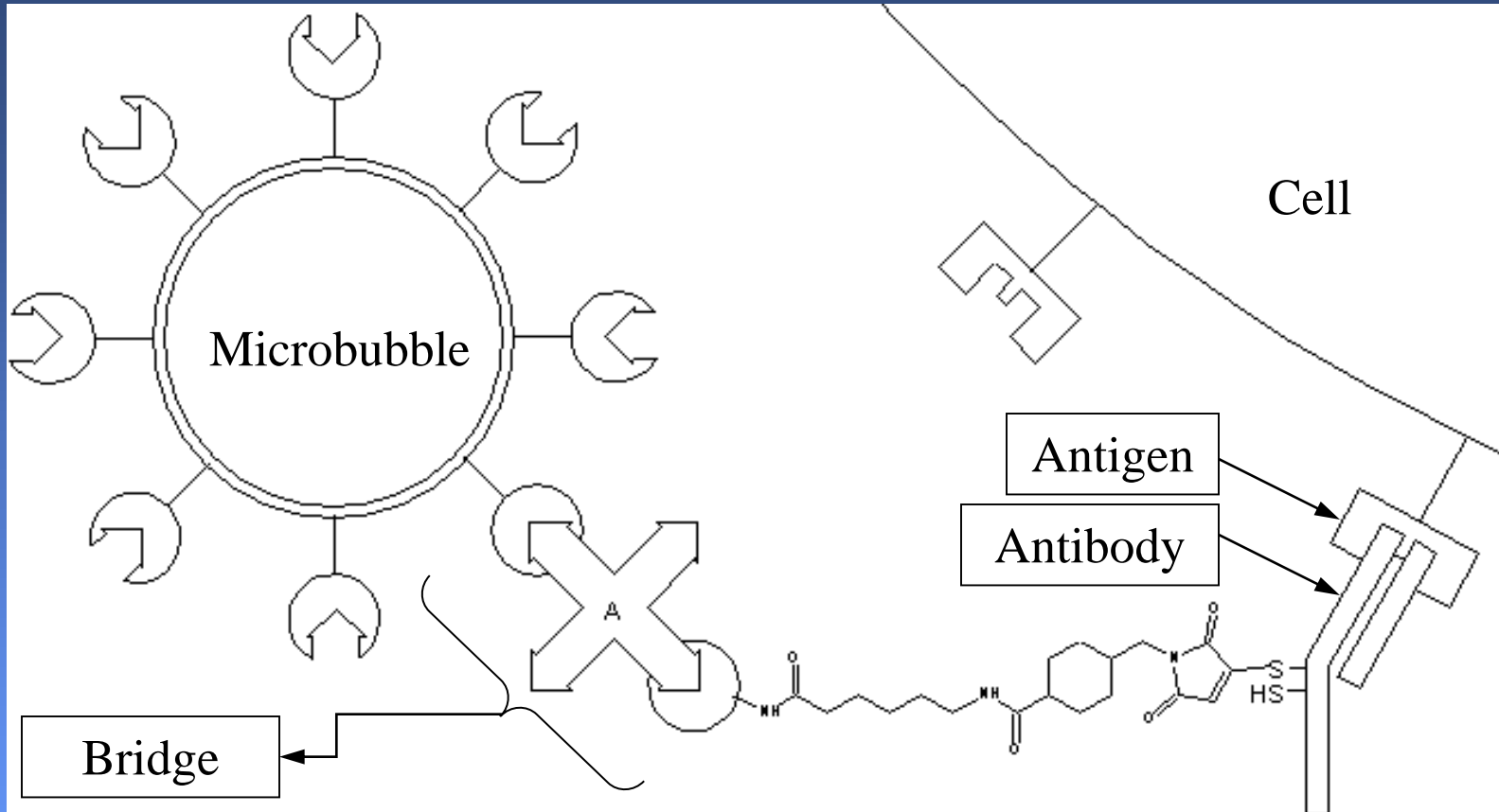
- Probe sonication disperses gas in aqueous medium containing liposomes
  - Microbubble lipid shells self-assemble at liquid-gas interface to reduce surface tension
- High temperatures may lead to protein destruction

## Shear-Activated bubbles

- Strong shear forces form microbubbles from aqueous medium containing liposomes
- Less likely to destroy or denature proteins



# Current Targeting Method



## Biotin-Streptavidin-Bridge

- Couples biotinylated antibody to biotinylated bubble
- Drawbacks: Immunogenic nature of streptavidin, reduced residence time



# Limitations of Current Microbubbles

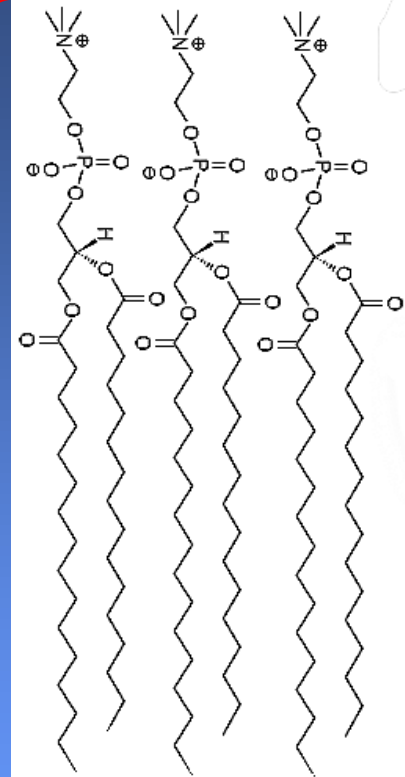
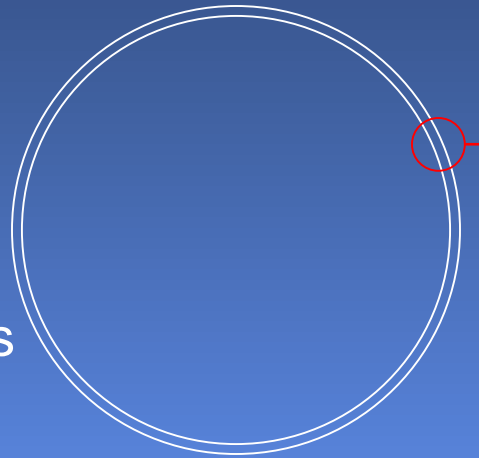
1. Use of cavitation for targeted microbubble formation
  - Does not allow for attachment of antibodies onto bubbles prior to activation
  - Requires more post-activation wash steps to target bubbles
2. Biotin-Streptavidin Bridge
  - Attaches targeting vector to bubble surface after activation
  - Streptavidin is an immunogenic protein, reduces residence time





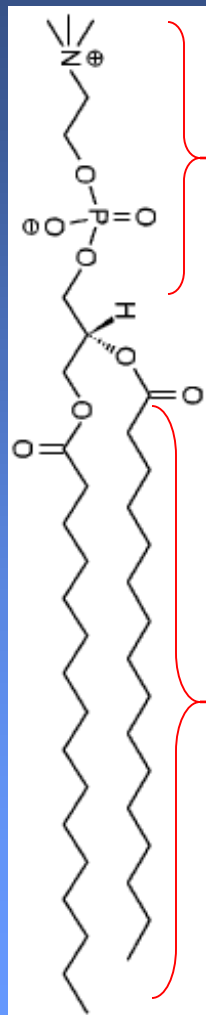
# Lipid Shell

- Monolayer consisting of several molecules
- Inhibits gas diffusion from bubble and coalescence
- Improved biocompatibility increases *in vivo* residence time
- Allows for the attachment of molecular targeting vectors onto the bubble surface





# DPPC 16:0



- [1,2-Dipalmitoyl-*sn*-Glycerol-3-Phosphocholine]
- 84 mol% of lipid shell

Electrostatic and chemically neutral.

Saturated 16-carbon-chain fatty acid tail. (Hydrophobic)



# Functional Lipids

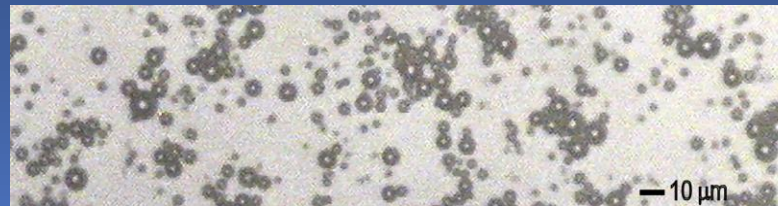
**Functional lipids are used to:**

- Prevent bubble coalescence
  - Electrostatic
  - Steric Hindrance (PEG)
- Incorporate biotin into bubble shell
- Add fluorescence into bubble shell (FITC)



# Fluorescent Tagged Microbubbles

Brightfield image of standard microbubbles, 100X.



Epi-Fluorescent image of tagged and un-tagged bubbles, 200X.

Microbubble tagged with quantum dots

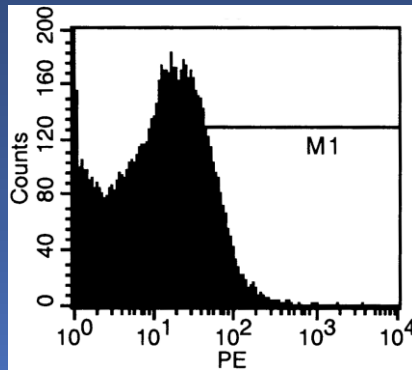
- Streptavidin-quantum dots bound to bubbles through Biotin-Cap-PE



Untagged microbubble

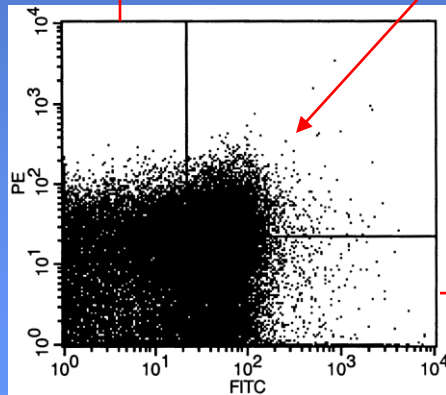


# Flow Cytometry Results



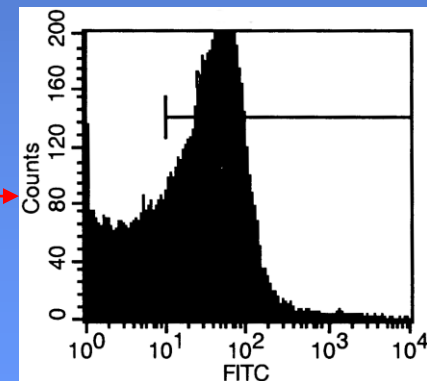
Events positive for  
Phycoerythrin (26%)

14% (43%) positive for both  
Phycoerythrin and FITC



FITC-PE Event  
Scatterplot

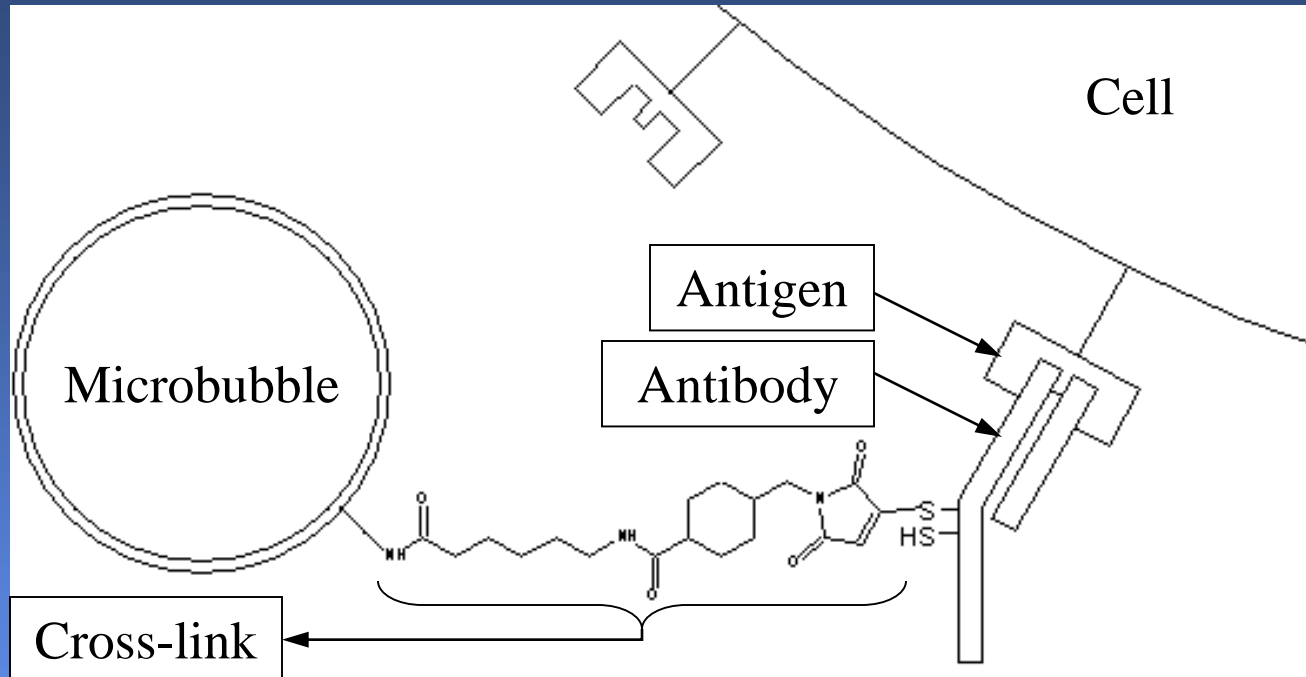
Events positive for  
FITC (61%)



The data were acquired and  
interpreted for 20,000 events each run.



# Direct Conjugation



- Allows for direct coupling of antibody to microbubble through a heterobifunctional cross-link to PE
- Targeted microbubble can be produced through simple shear-activation
- Increased ability to incorporate several antibodies onto the bubble surface
- Biocompatible





# Direct Conj. Progress & Future Work

- Directly coupled Goat anti-Mouse IgG to microbubble surface via SMCC
  - Labeled IgG with FITC-Mouse antibody
  - Presence determined using epi-fluorescent microscopy
- Developing streptavidin direct conjugate
  - Quantitative relationship between DC and biotin-streptavidin bridge
    - Conjugation efficiency
    - Residence time *in vivo*



# Conclusions

1. Targeted microbubbles can be generated using shear-activation
2. Streptavidin binds with biotin-labeled microbubbles, 43% efficiency
3. IgG antibodies can be bound to microbubble surface through direct conjugation
  - Allows for conjugation prior to activation
  - Eliminates Biotin-Streptavidin bridge



# Acknowledgements

## Advisors

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## Assistance

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John Hutton (UCHSC)

Phillip Pratt (UCHSC)

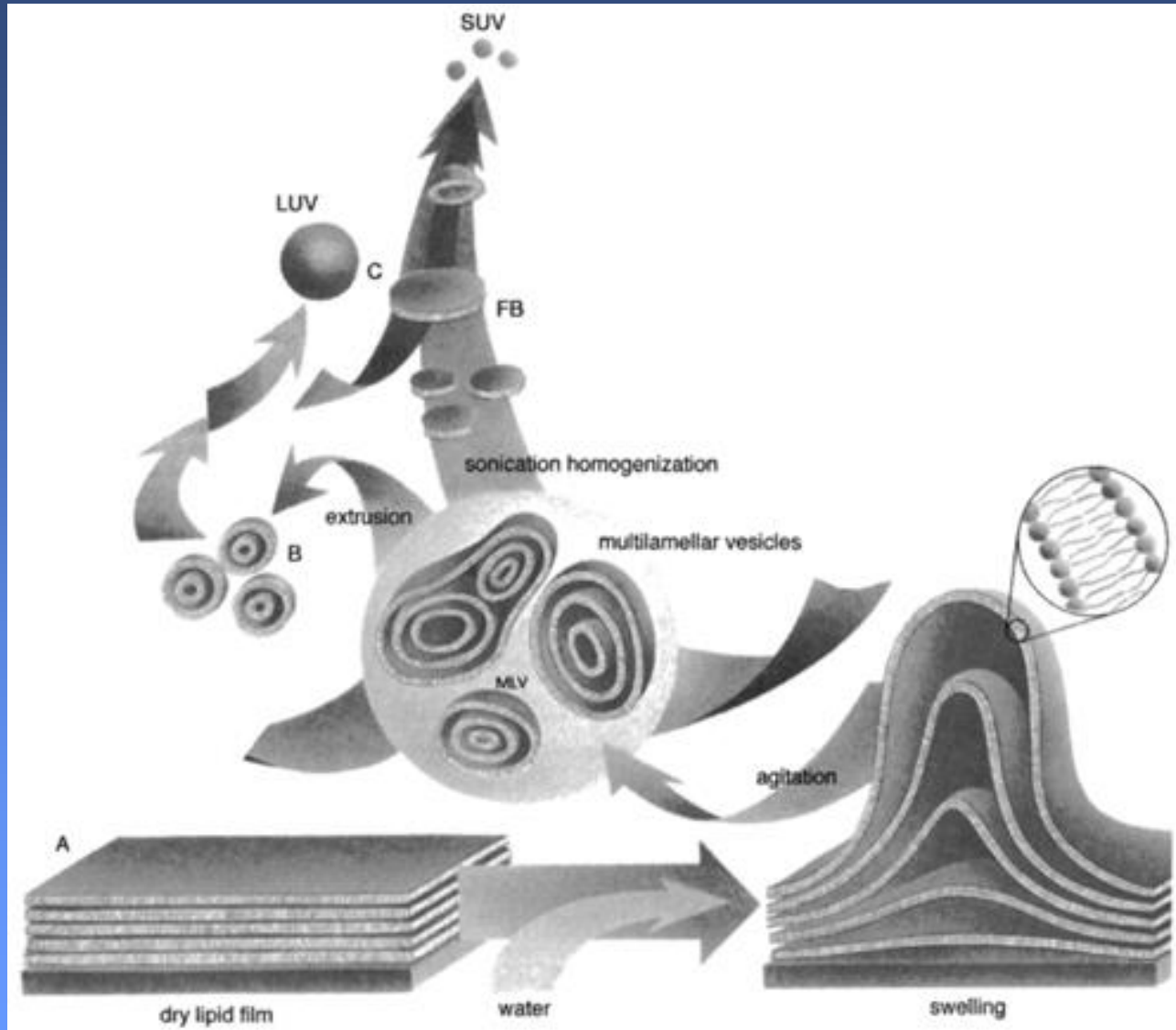
Michael Stowell (CU MCDB)

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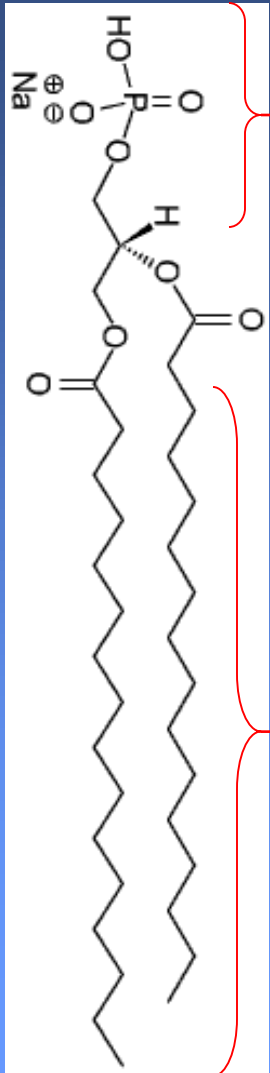
# SUV Formation



\*Image from Avanti Polar Lipids Website



# DPPA 16:0



- [1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphate]
- 10 mol% of lipid shell

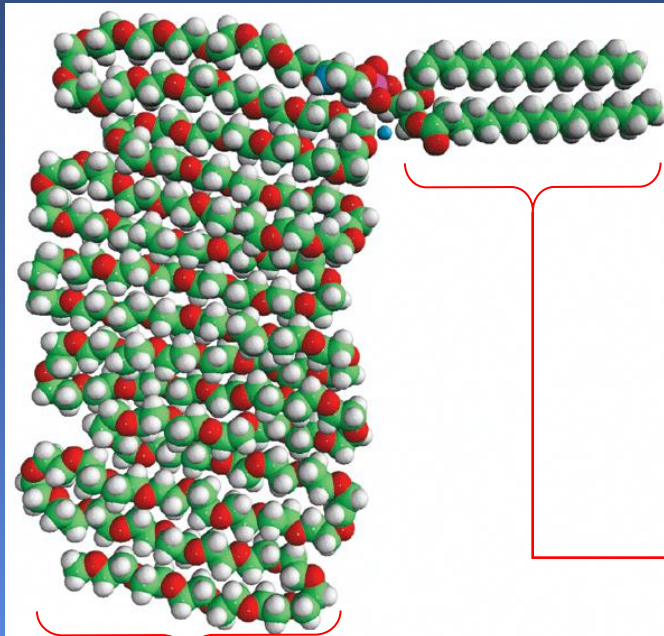
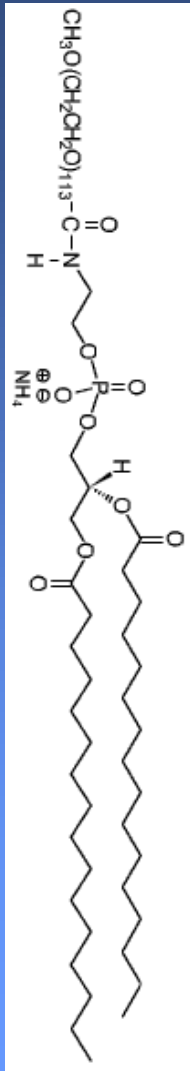
Electronegative head-group, due to ionized species.

- Prevents coalescence due to electrostatic repulsion between bubbles

Saturated 16-carbon-chain fatty acid tail.  
(Hydrophobic)



# MPEG-5000-DPPE 16:0



- 1,2-Dipalmitoyl-*sn*-Glycerol-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-5000]
- 6 mol% of lipid shell

Saturated 16-carbon-chain fatty acid tail. (Hydrophobic)

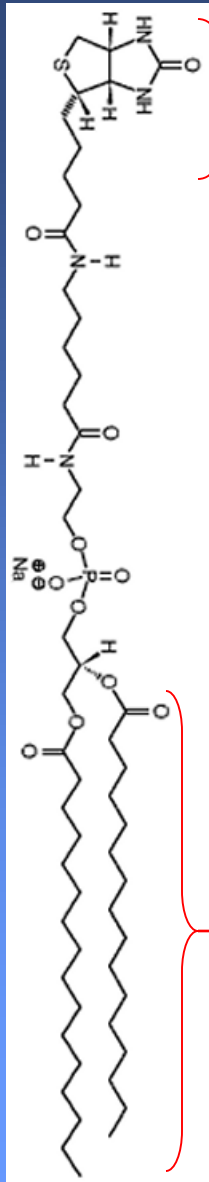
5000 MW PEG head-group.

- Soluble in water
- Prevents coalescence due to between bubbles
- Biocompatible (increases bubble residence time *in vivo*)





# N-Biotinyl Cap-PE 16:0



- 1,2-Dipalmitoyl-*sn*-Glycerol-3-Phosphoethanolamine-N- (Cap Biotinyl)
- 6 mol% of lipid shell

Biotin antigen.

- Specifically binds with Streptavidin
- Allows for bubble functionalization

Saturated 16-carbon-chain fatty acid tail. (Hydrophobic)