# Effects of Silicone-Water Interfaces on Protein Structure, Aggregation, Particle Formation and Immunogenicity

John F. Carpenter, Ph.D.

University of Colorado Center for Pharmaceutical Biotechnology

john.carpenter@ucdenver.edu

# Outline

- Brief summary of causes and control of protein aggregation
- Effects of silicone oil microdroplets on protein structure and aggregation
- Effects of siliconized glass on protein aggregation and particle formation
- Effects of pumping through siliconized tubing
- Effects of silicone oil on immunogenicity
- Not silicone oil data, but brand new immunogenicity results with mouse growth hormone: SQ vs. IP vs. IV

# Acknowledgments


**University of Colorado**
Ted Randolph
Dan Schwartz
Amber Fradkin
Keith Britt
Matthew Hoehne
Maliheh Shomali
Jim Barnard
Pinaki Basu
Luke Liu
Neha Pardeshi
Merry Le
Brandon Teska
Carly Fleagle
Kimberly Hassett
Alana Gerhardt
Dilip Devineni
Pradyot Nandi
Brett Ludwig
Weijie Fang

**LMU München**
Gerhard Winter
Julia Myschik
Angelica Freitag

**Amgen, Inc.**
Sampath Krishnan
Erwin Freund
Renuka Thirumangalathu

**Pfizer**
Satish Singh
Wei Wang

**Abbott**
Michael Siedler
Zehra Kaymakcalan

**MedImmune**
Tom Leach
Jared Bee
Mariana Dimitrova

**Roche**
Hanns-Christian Mahler
Christine Wurth

**Becton Dickinson**
Javier Alarcon
Ron Pettis
Paolo Mangiagalli

**University of Kansas**
Russ Middaugh
David Volkin
Christian Schoneich

**University of Connecticut**
Michael Pikal

**NIST**
Marc Cicerone

**US FDA**
Amy Rosenberg
Jack Ragheb

**Leiden University**
Wim Jiskoot
Vasco Fillipe

# Pathway for Protein Aggregation

**M ← → M* → Soluble Agg. → Insol. Agg.**

**(Subvisible Particles???)**

- M* can be part of the native state ensemble.
- Thus, even under conditions greatly favoring the native state, aggregation can proceed at unacceptable rates.
- M* may be promoted at interfaces

# Causes of Protein Aggregation

- Even without stress, aggregates can form during storage in solution; sufficient partially unfolded molecules even in “stable” formulation

- In addition, proteins are exposed to numerous stresses (e.g., light, surfaces) during production, purification, storage, shipping and delivery to the patient.

- Often, aggregation occurs because of exposure to air-liquid or liquid-solid interfaces.
  - bubble entrainment during mixing, filling & shipping
  - solution contact with pumps, pipes, vessels, filters, columns, etc.
  - solution contact with ice

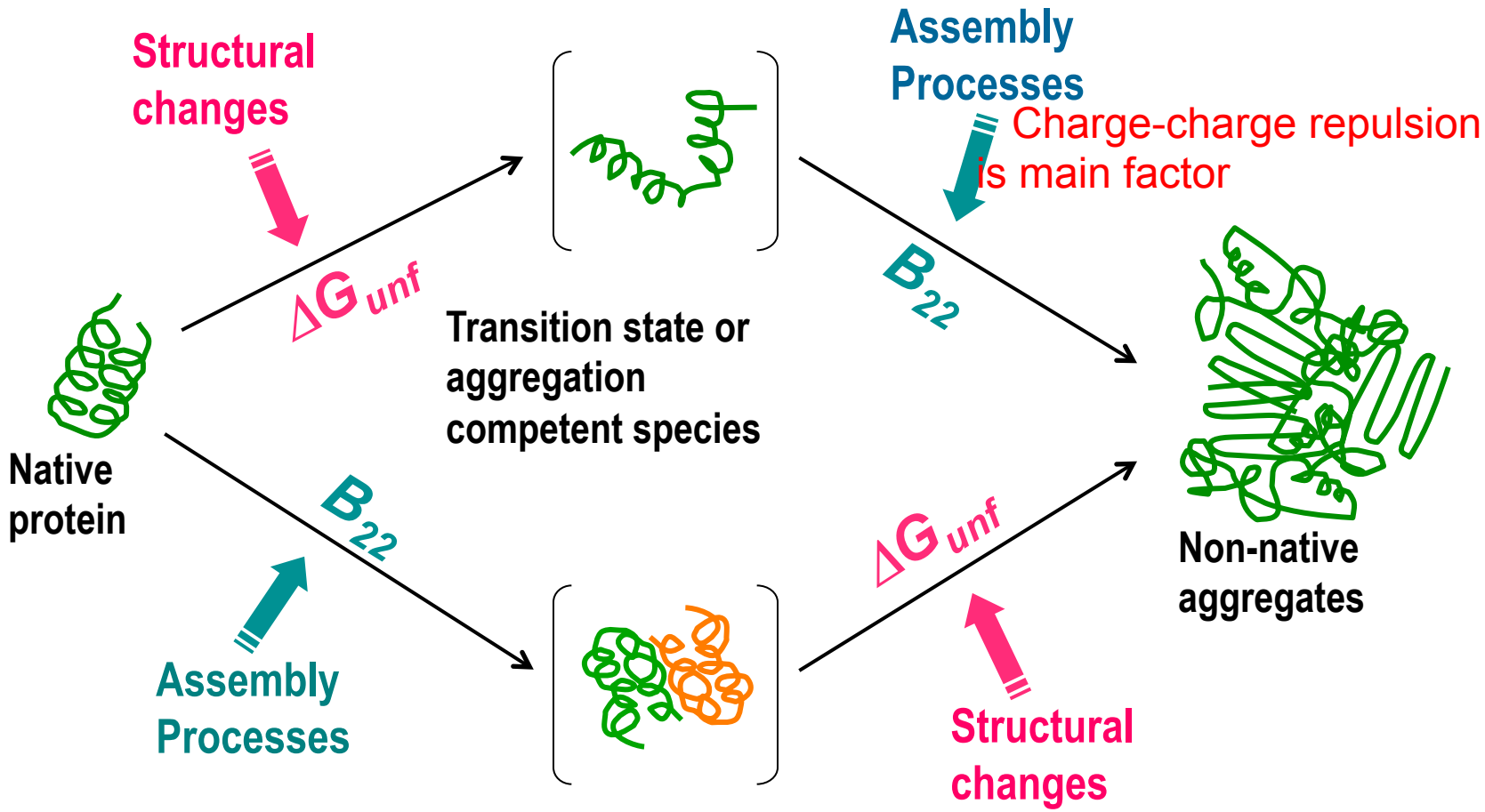
# Causes of Protein Aggregation: Surfaces

- Proteins adsorb to surfaces. May unfold on surface and/or partially unfolded molecules may more readily adsorb.

- May form aggregates on surfaces, and/or desorption of unfolded molecules may lead to aggregates in bulk solution.

- Adsorption to foreign nano- or microparticles can result in subvisible and visible protein particles.

# Control of Aggregation: Maximize Conformational and Colloidal Stability




**pH is a critical parameter.**

Chi et al., 2003. *Pharm. Res.* 20:1325

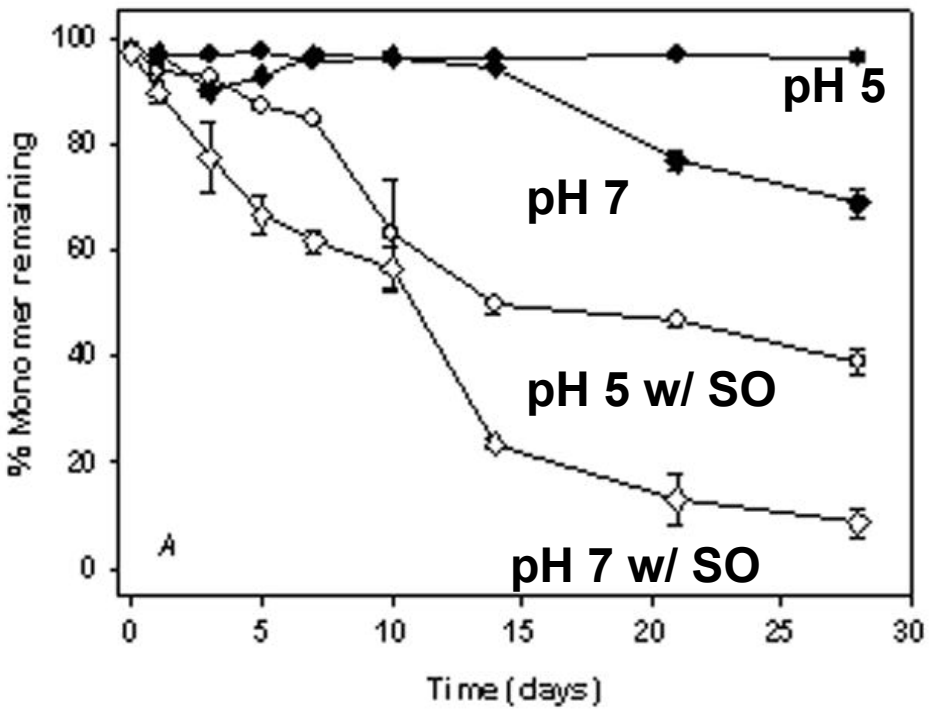
# Protein Interactions with Silicone Oil: Example of Container/Closure Incompatibility with Protein

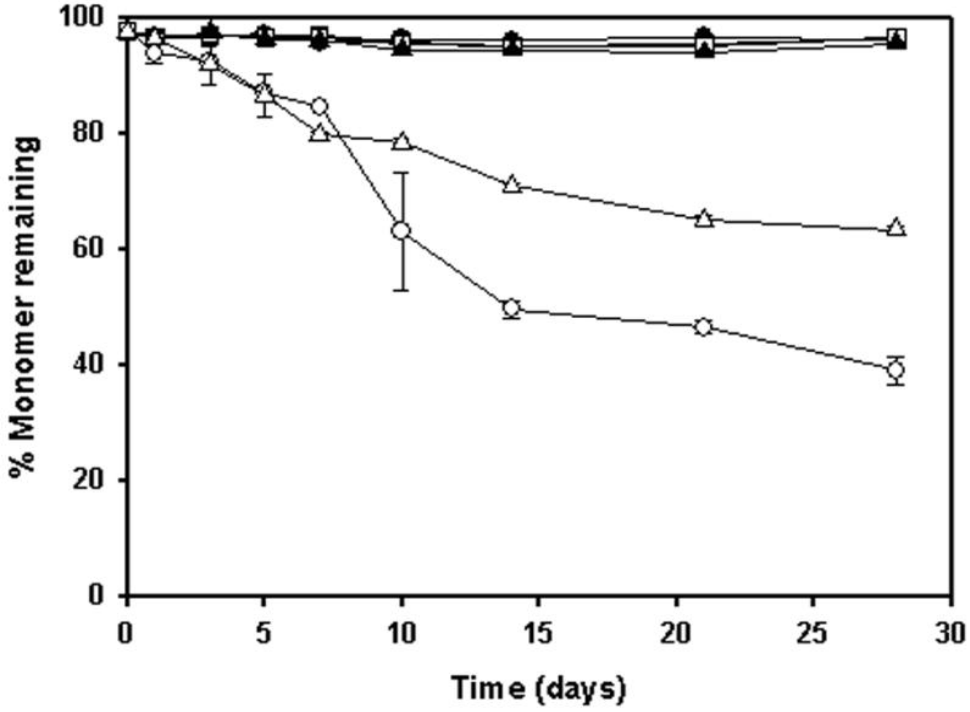
- Syringe and cartridge barrels are coated with silicone oil to facilitate smooth movement of plunger.

- Silicone oil treatment can lead to droplets of silicone oil suspended in product formulation.

- Protein adsorption to wall and droplets can result in particles with aggregated protein.

# Agitation with Silicone Oil Microdroplets Accelerates IgG Aggregation




**No aggregation observed with silicone oil without agitation.**

**Polysorbate 20 inhibits aggregation**

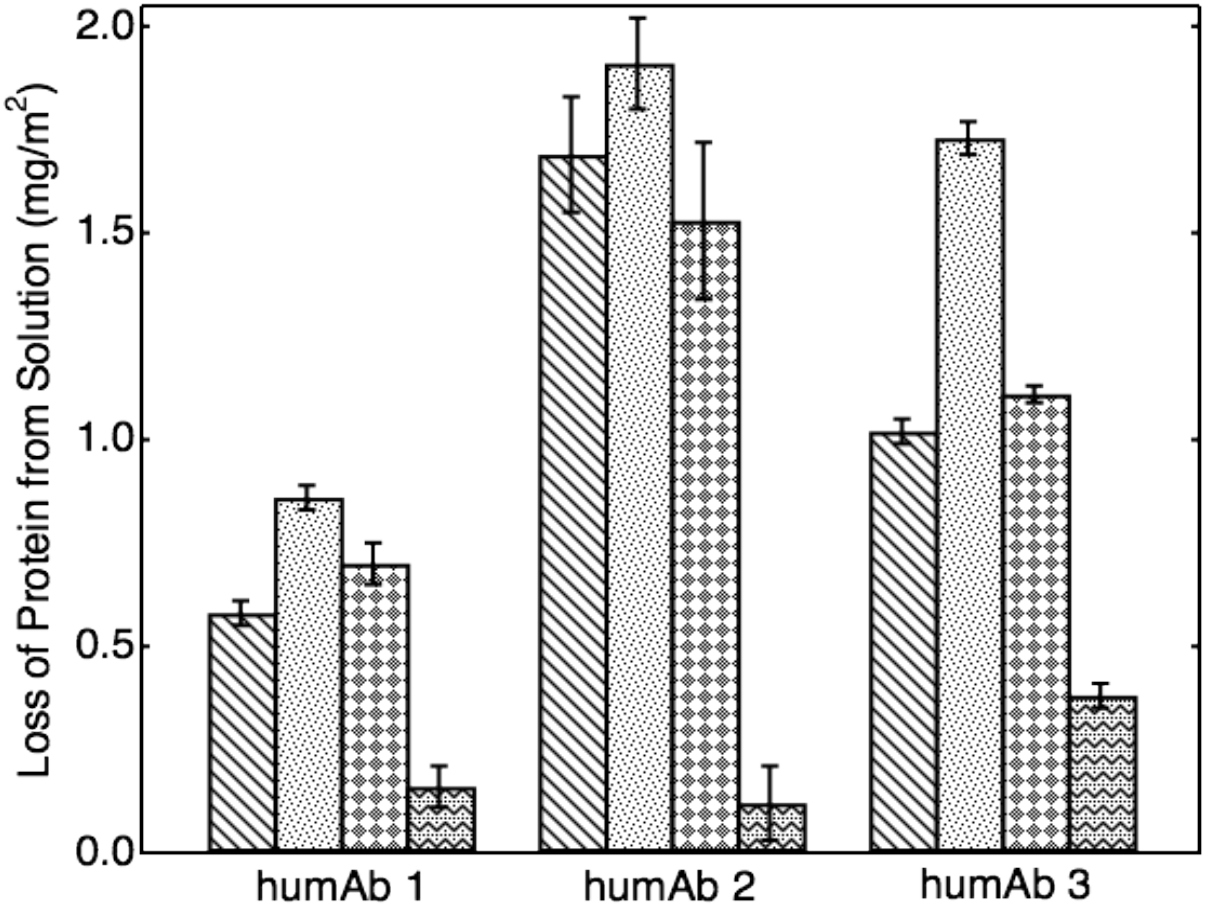
**Colloidal stability of this IgG high at pH 5 but reduced at pH 7.**



**Journal of Pharmaceutical Sciences Volume 98, 3167–3181**

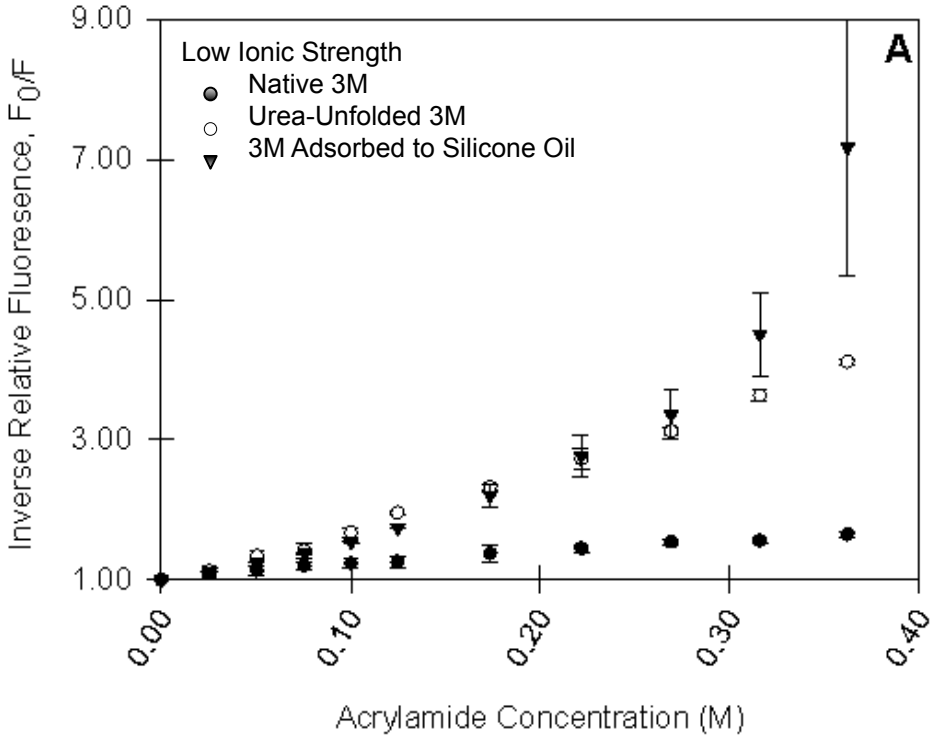
# Adsorption to Silicone Oil Reduced by Polysorbate

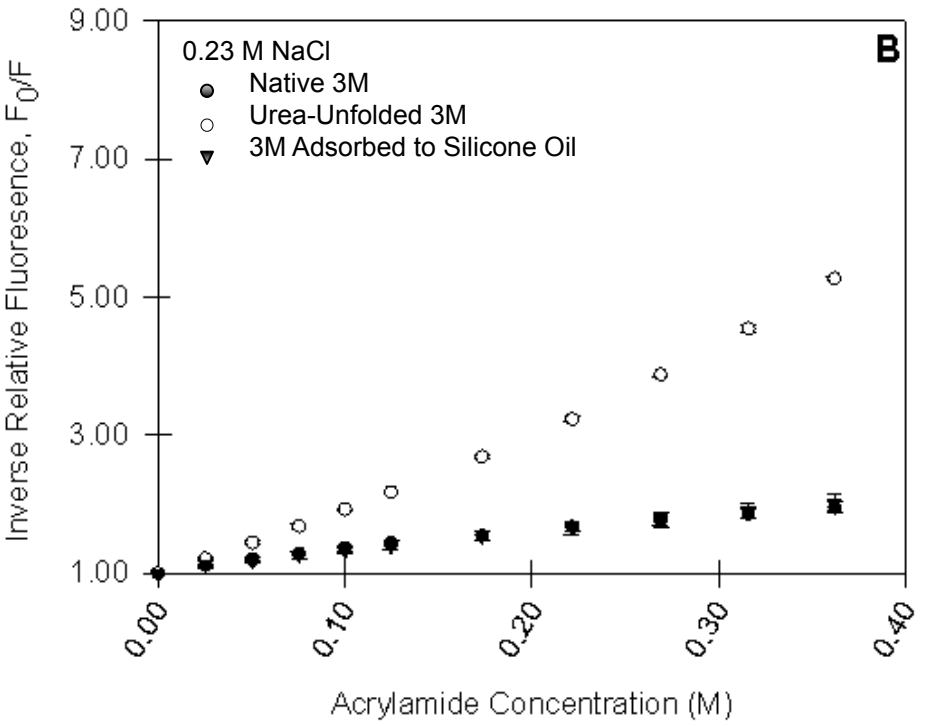
- 10 mM His pH 6.0
- 10 mM His pH 6.0, 140 mM NaCl
- 10 mM His pH 6.0, 240 mM sucrose
- 10 mM His pH 6.0, 0.03% Tween 20.




Britt et al., 2013. J. Pharm. Sci. 101:4419-4432

# Adsorption to Silicone Oil can Perturb IgG Tertiary Structure



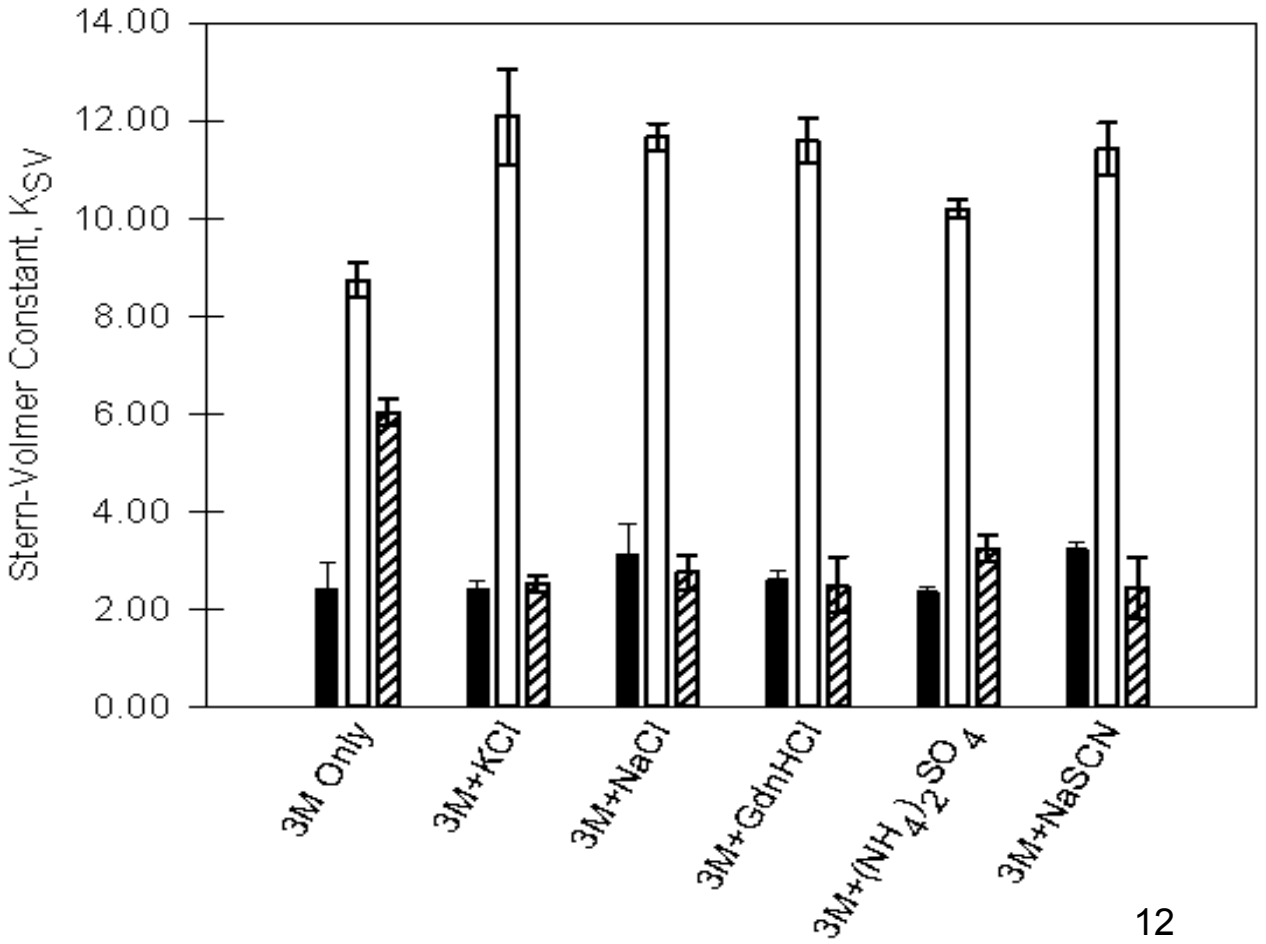




**Salt does not alter adsorption but does reduce structural perturbation.**

Gerhardt et al., 2013. J. Pharm. Sci. 102:429-440

# Tertiary Structure of Adsorbed IgG (3M) affected by Salts

- 3M is structurally perturbed at the silicone oil interface when the ionic strength is low
- Non-specific salt effects appear to minimize any structural changes in 3M due to adsorption





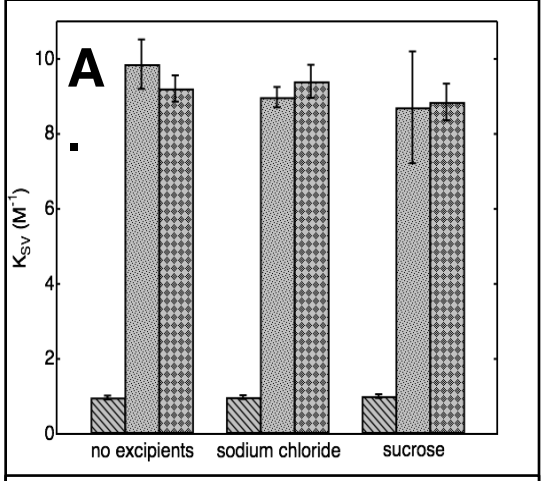

Gerhardt et al., 2013. J. Pharm. Sci. 102:429-440

# Structural Perturbation of Others mAbs

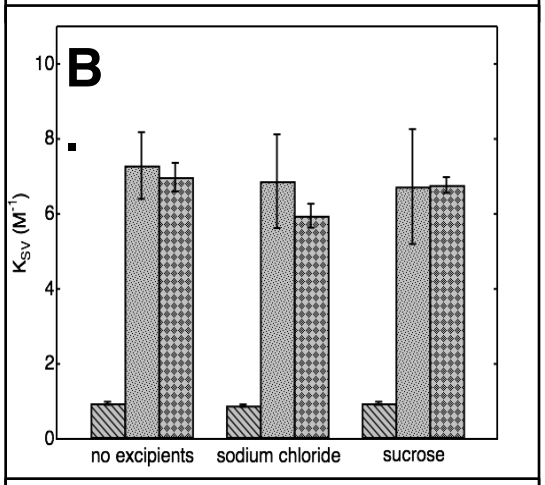
Left to Right:

- native mAb
- adsorbed to silicone oil
- unfolded in 8M urea

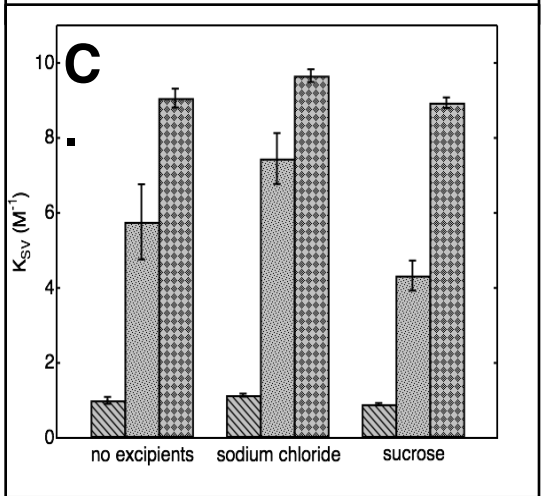
mAb structures significantly perturbed by adsorption to silicone oil microdroplets!




humAb 1




humAb 2




humAb 3

Britt et al., 2013. J. Pharm. Sci. 101:4419-4432

# The same three mAbs show minimal changes in tertiary structure upon adsorption to uncoated glass

| | Summary of Stern-Volmer Constants | | |
|---|---|---|---|
| | **Native** | **Glass** | **Unfolded** |
| humAb1 | 0.97 ± 0.05 | 1.41 ± 0.08 | 9.21 ± 0.35 |
| humAb2 | 0.95 ± 0.04 | 1.04 ± 0.02 | 6.98 ± 0.38 |
| humAb3 | 1.00 ± 0.09 | 1.08 ± 0.03 | 9.06 ± 0.25 |



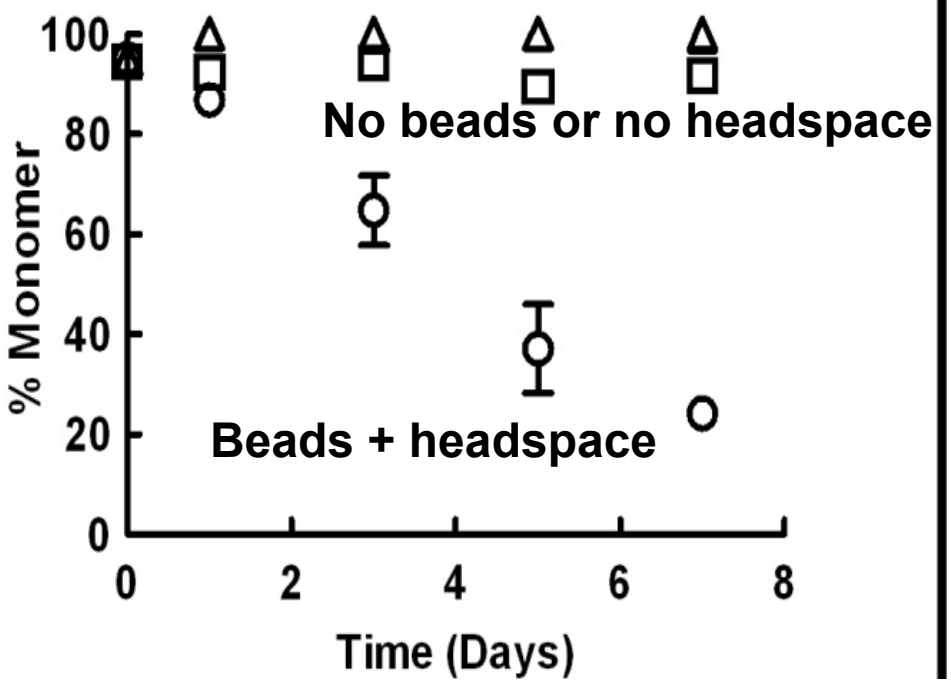
Hoehne at al., 2011. J. Pharm. Sci. 100:123-132

# Silicone Oil-Induced Particles

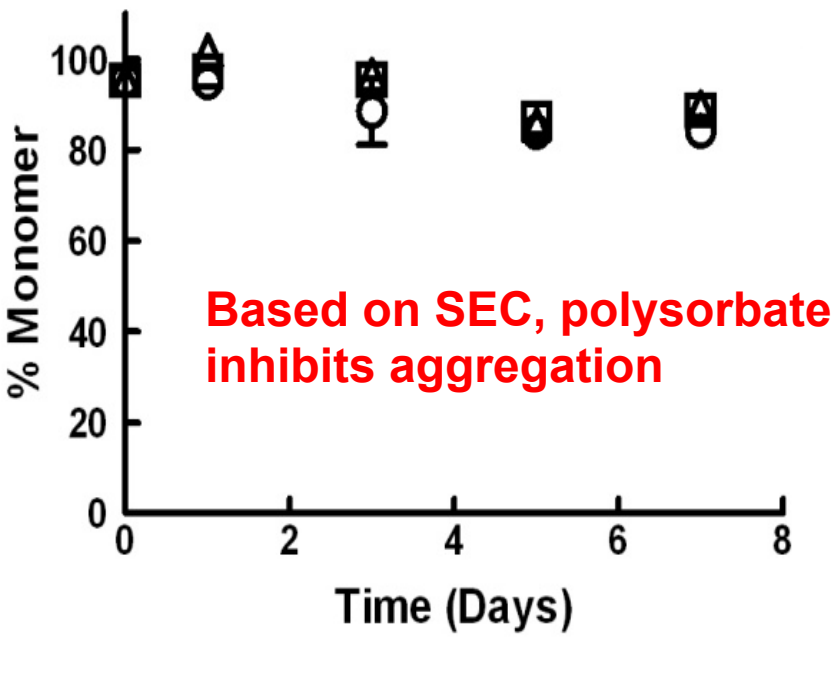
- The roles of subvisible particles in silicone oil-induced protein aggregation can be difficult to determine
- Oil droplets interfere with counting of protein particles and can be at huge excess
- With silicone-coated glass beads **(Surfasil),** both protein aggregation and particle formation can be quantified during accelerated degradation studies
- Also serves as mimic of “baked-on” silicone surface on glass from syringes, etc.

# Agitation of 1 mg/ml IgG with Siliconized Glass Beads w/ and w/o Headspace

**10 mM Sodium Acetate, pH 5**



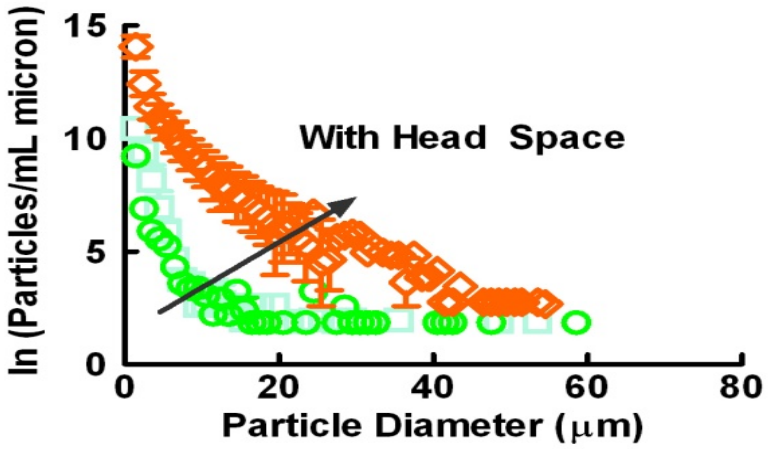

**Buffer plus 0.01% polysorbate 20**



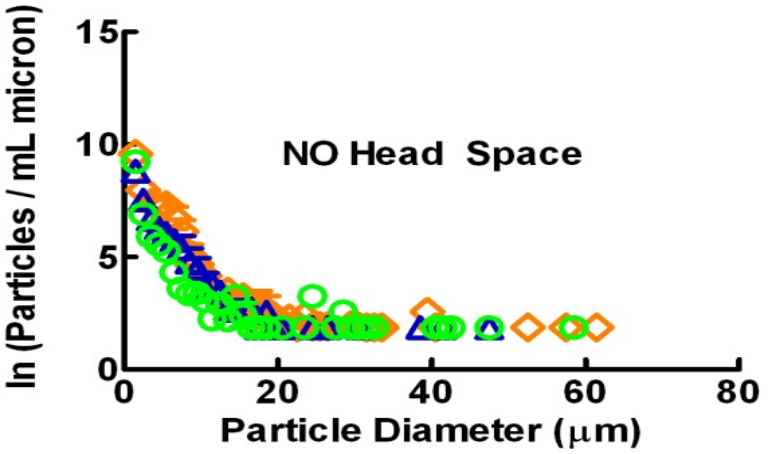

Basu et al., J. Pharm. Sci. (in press)

# Particle Counts in IgG Samples

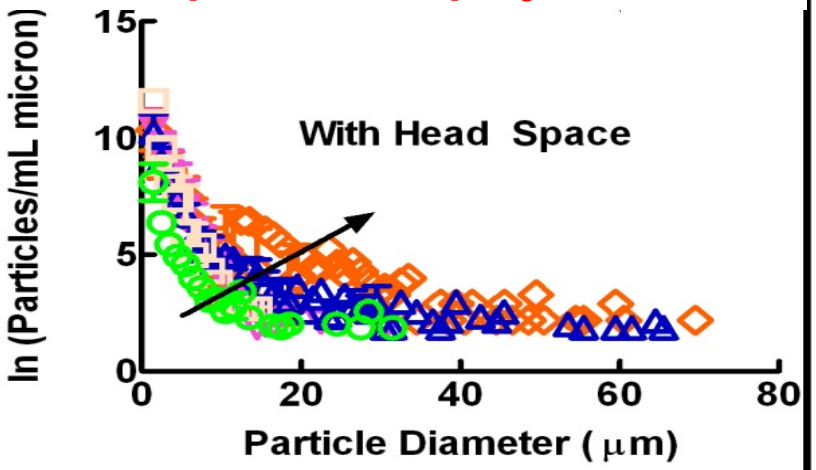
10 mM Sodium Acetate, pH 5




10 mM Sodium Acetate, pH 5

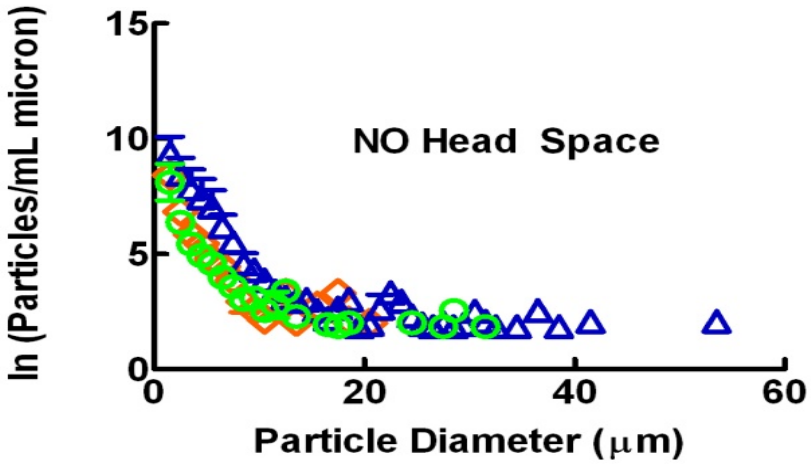



Buffer plus 0.01% polysorbate 20




Based on particles, there is low level of aggregation.

Buffer plus 0.01% polysorbate 20

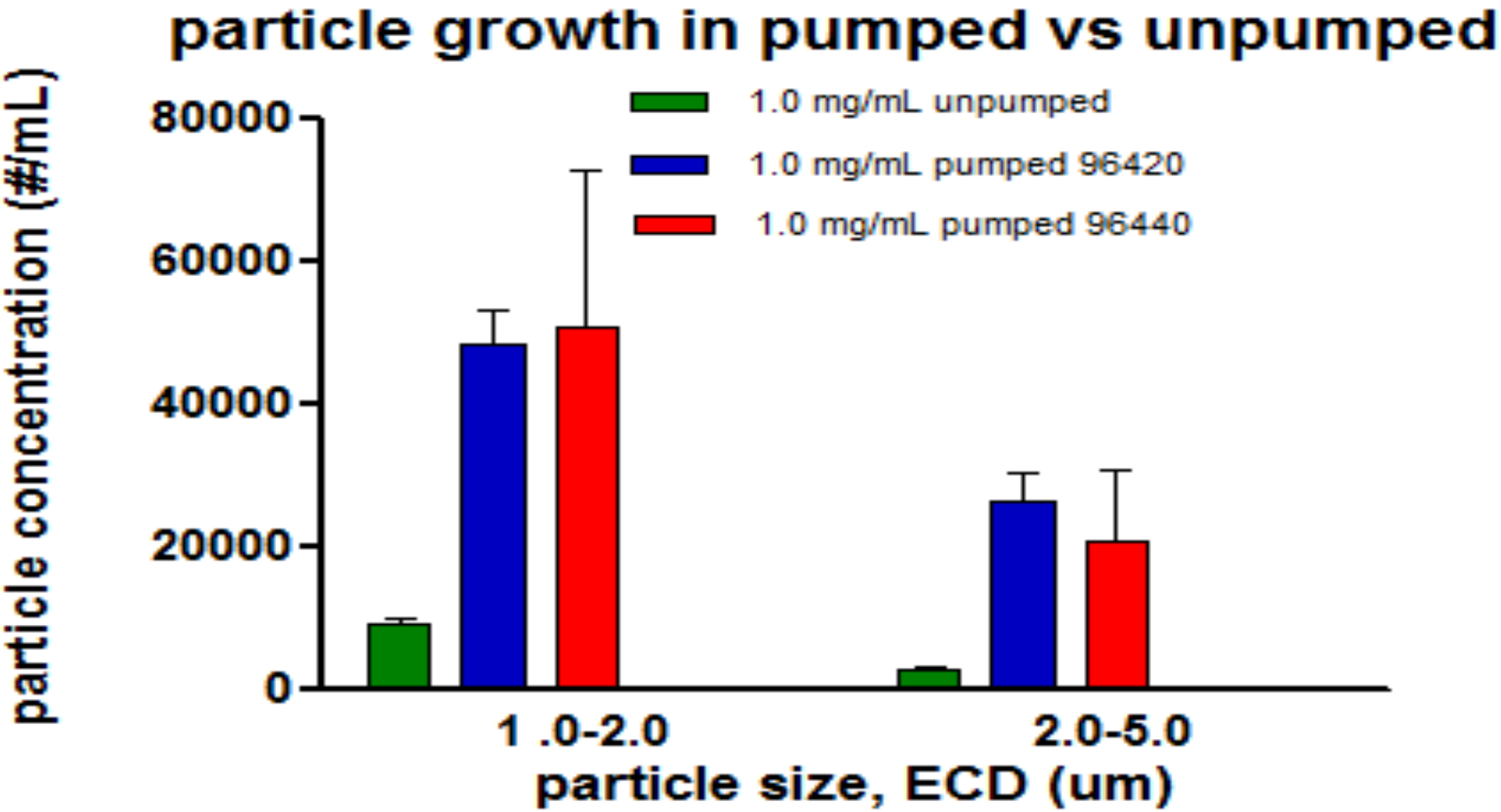


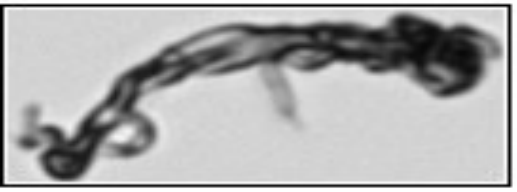

Basu et al., J. Pharm. Sci. (in press)

# Particles Generated by Peristaltic Pumping: Preliminary Study

- Peristaltic pump and **platinum-cured silicone tubing** were purchased from Cole Palmer.
- Human IVIG was dialyzed into a 10 mm phosphate buffer and used at a concentration of 1.0 mg/mL.
- Samples were pumped through 2 feet of tubing at a rate of 1.0 mL/min, controls were not pumped.
- They were analyzed using Brightwell Microflow Imaging DPI 4200, Malvern ZetaSizer Nano and HPLC size exclusion chromatography.

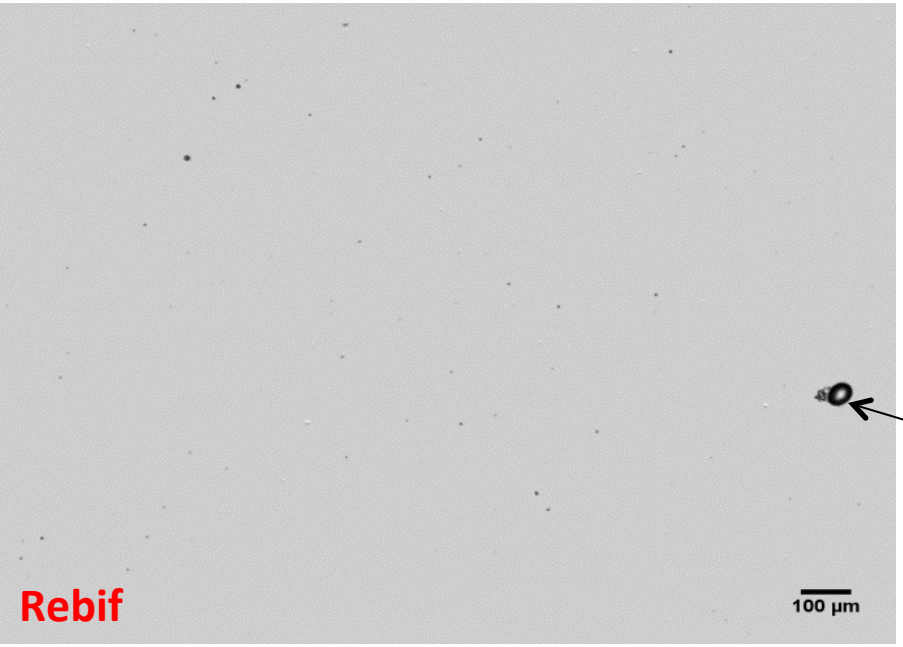
# Pumping causes particles

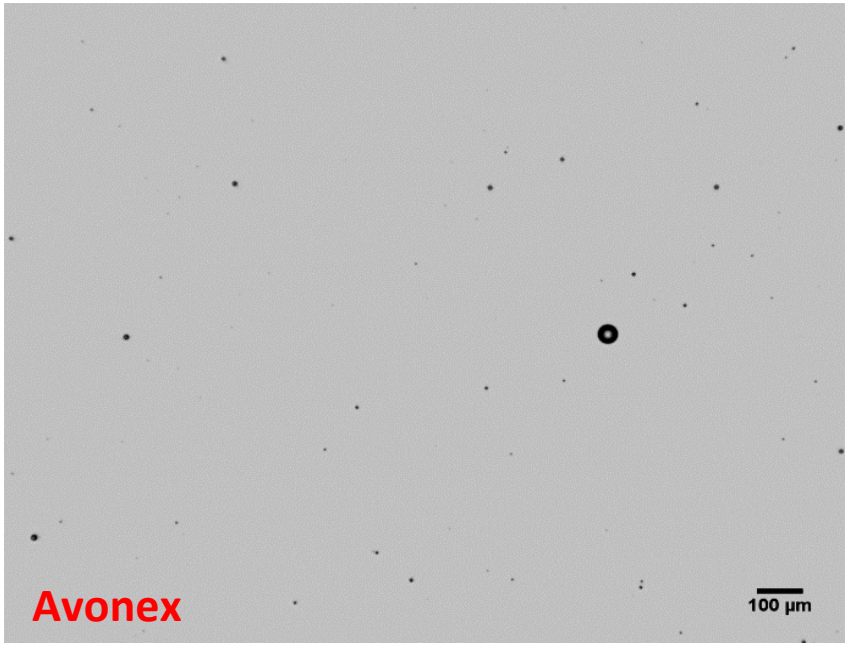


Images of typical particles

# Interferon-beta Products in Prefilled Syringes: Particle Images

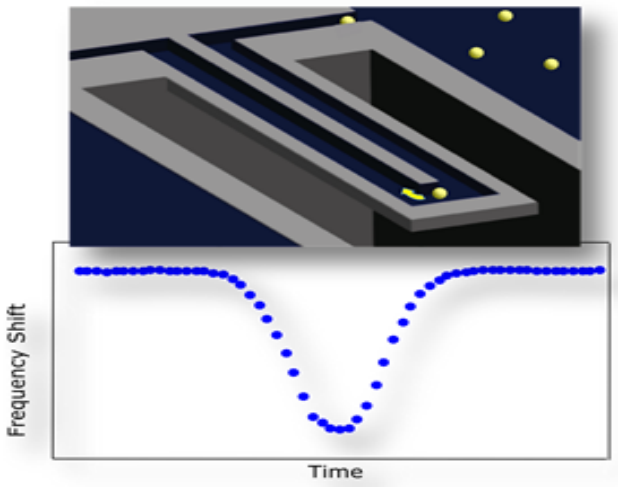


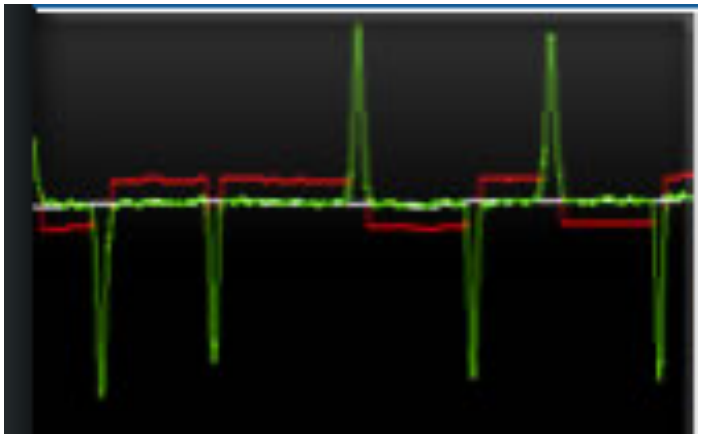

- **Rebif formulated with HSA but no surfactant.**
- **Avonex formulated with surfactant and without HSA.**
- **Rebif has much higher immunogenicity rate in patients.**

Barnard et al., 2013. J. Pharm. Sci. (in press)

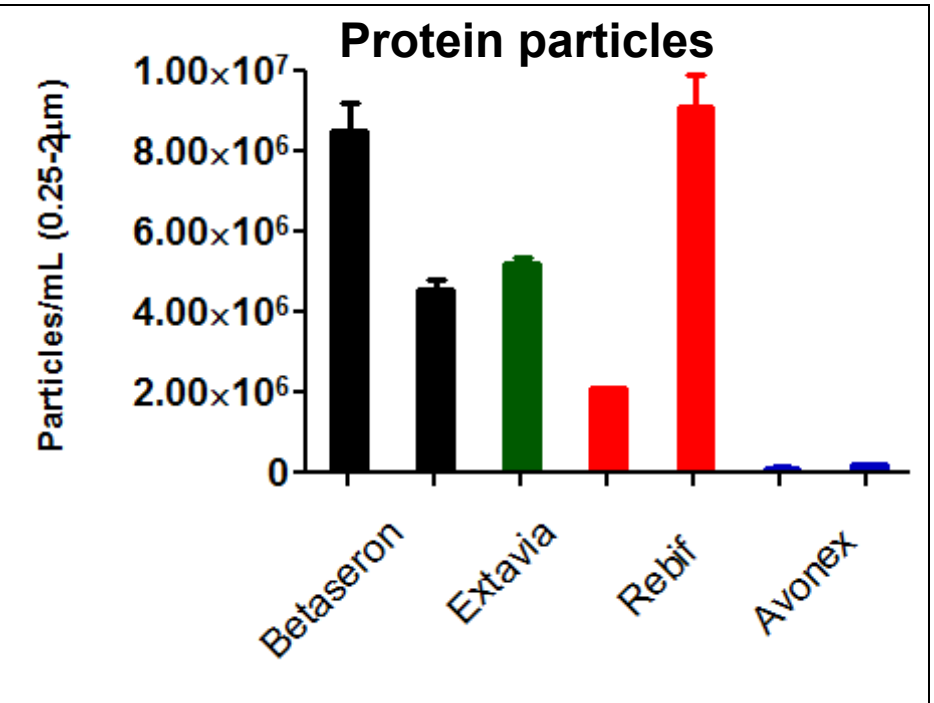
# Particle Counts in Interferon-beta Products: Archimedes

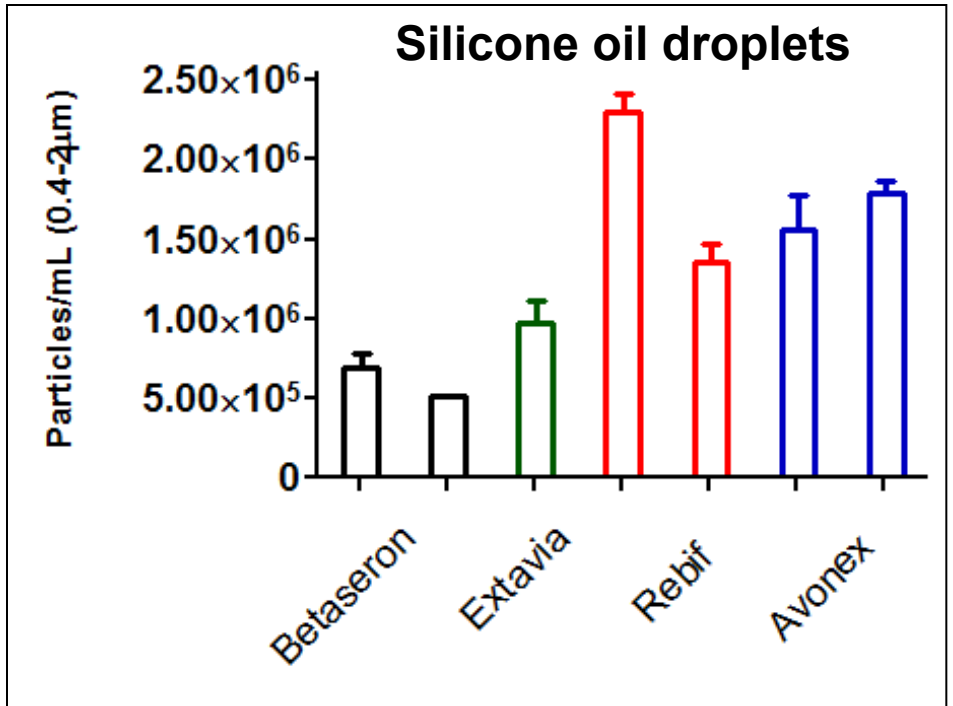



Silicone oil signal



Protein signal



# Immunogenicity of recombinant mouse growth hormone (rmGH) in mice

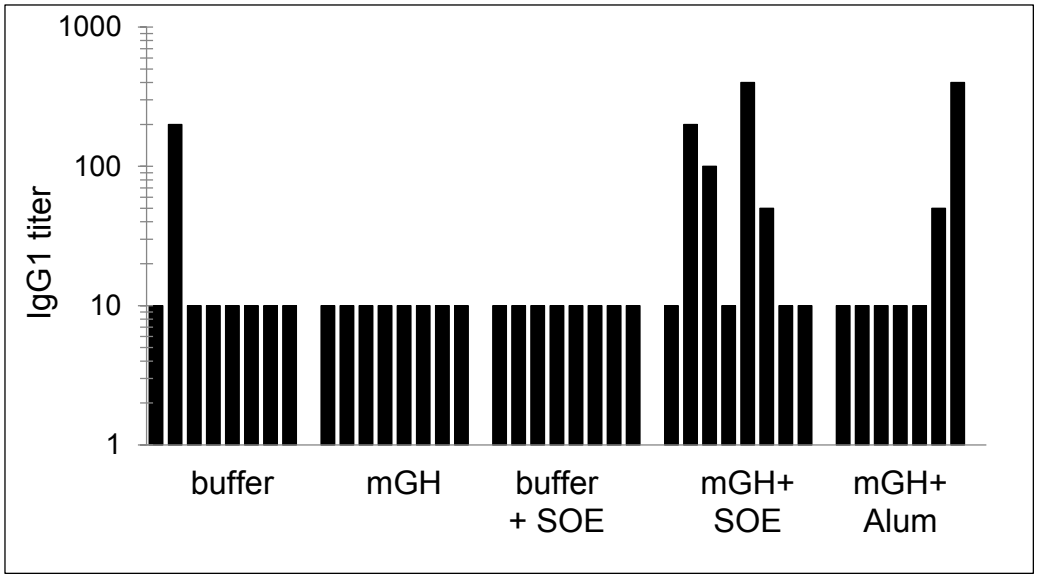
2 micrograms protein injected subcutaneously 5x a week

□ = Bleed

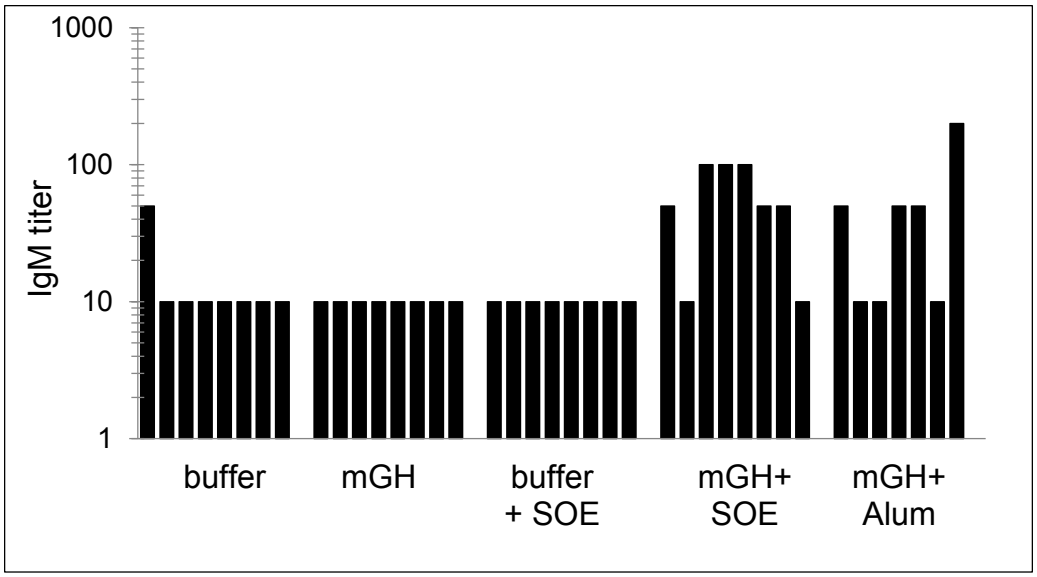
| | | | | | |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 1° |
| 8 | 9 | 10 | 11 | 12 | |
| 15 | 16 | 17 | 18 | 19 | |
| 22 | 23 | 24 | 25 | 26 | |
| 29 | 30 | 31 | 32 | 33 | |
| 36 | 37 | 38 | 39 | 40 | |
| 43 | 44 | 45 | 46 | 47 | 2° |
| 50 | 51 | 52 | 53 | 54 | |
| 57 | 58 | 59 | 60 | 61 | |

JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 100, NO. 11, NOVEMBER 2011

# Immunogenicity of rmGH Adsorbed to Silicone Oil: Preliminary Data




Mice treated with mGH adsorbed to SOE have significantly higher IgG1 titers at day 22 than other mouse groups.




Mice treated with mGH adsorbed to SOE have significantly higher IgM titers at day 43 than other mouse groups.

# Silicone Oil-Induced Protein Aggregation

- The effects of silicone oil on protein structure and aggregation are protein specific.
- Agitation and silicone-water interface synergistically promote aggregation.
- Polysorbates inhibit protein adsorption to silicone-water interface and protein aggregation, even with agitation.
- But the inhibition is not absolute; protein particles can still form.
- Silicone oil appears to promote immunogenicity

# Effects of route of administration on immunogenicity of rmGH

- Eight Female CB6F1 mice per group
- Routes of administration: SQ, IP, IV (20 μg dose in 0.1 ml)
- Protein treatments:
  - Freeze-thawed: rmGH frozen and thawed once, then diluted to 0.2 mg/ml
  - Ultra-centrifuged: rmGH frozen and thawed once, centrifuged at 110K g for 1hr at 4°C, then diluted to 0.2 mg/ml
  - Prepared the day of injections
    - Aggregates and particle populations quantified via resonant mass measurement, flow imaging, particle tracking analysis and SEC
- Anti-rmGH IgG isotype antibodies were monitored by ELISA

Merry Le, et al., unpublished data

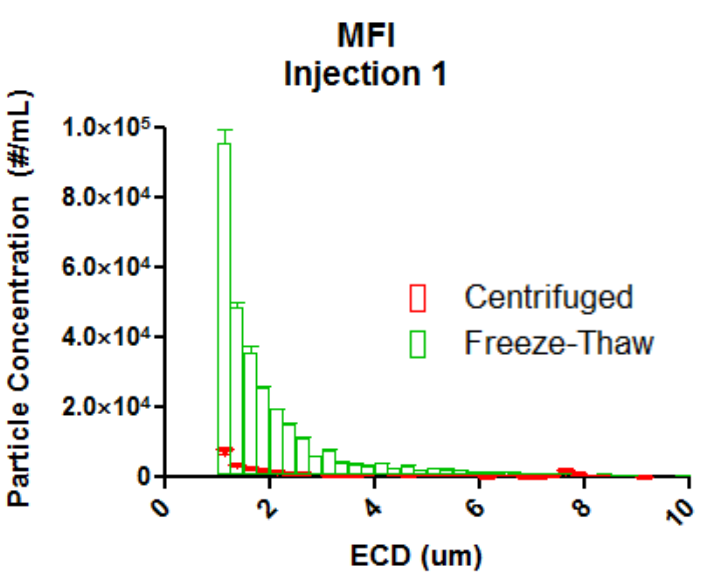
# Injection and Bleed Schedules

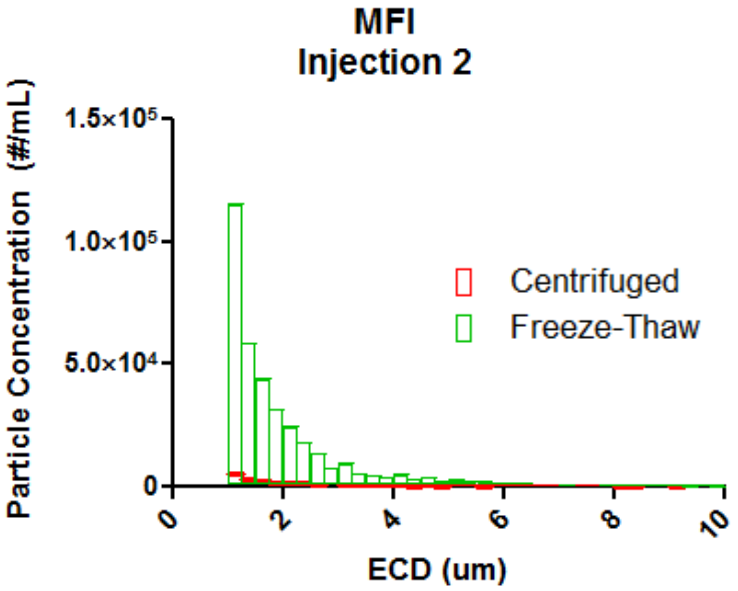
| | | | | | |
|---|---|---|---|---|---|
| Week 1 | | |  |  | |
| Week 2 | | | | | |
| Week 3 | | | | | |
| Week 4 | | |  |  | |
| Week 5 | | | | | |
| Week 6 | | |  | | |

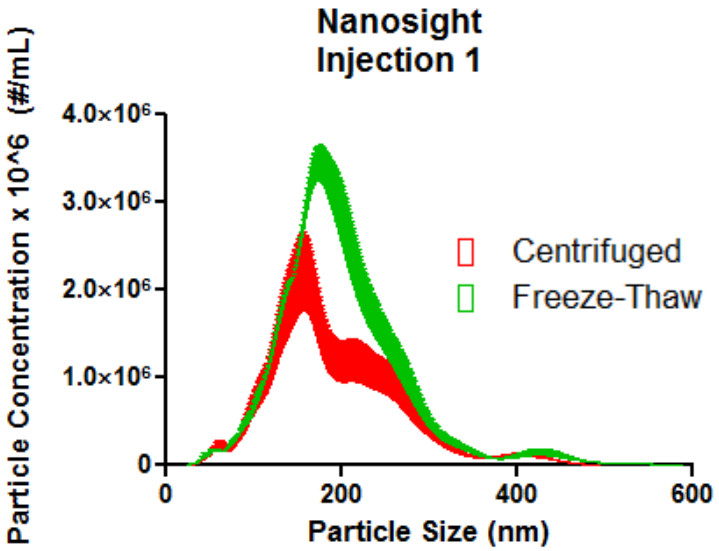
 Injection

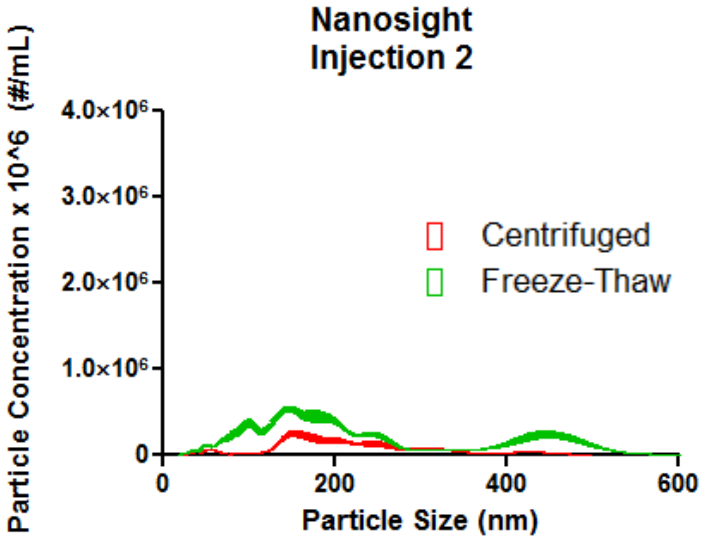
 Submandibular Blood Draw

# Particles in rmGH Samples

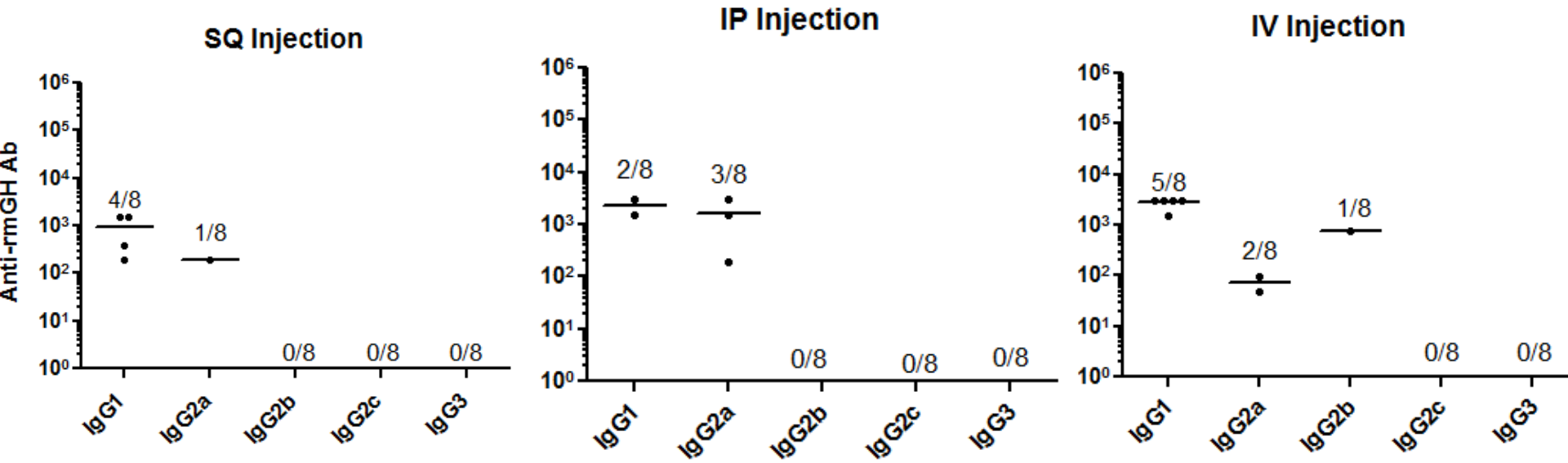


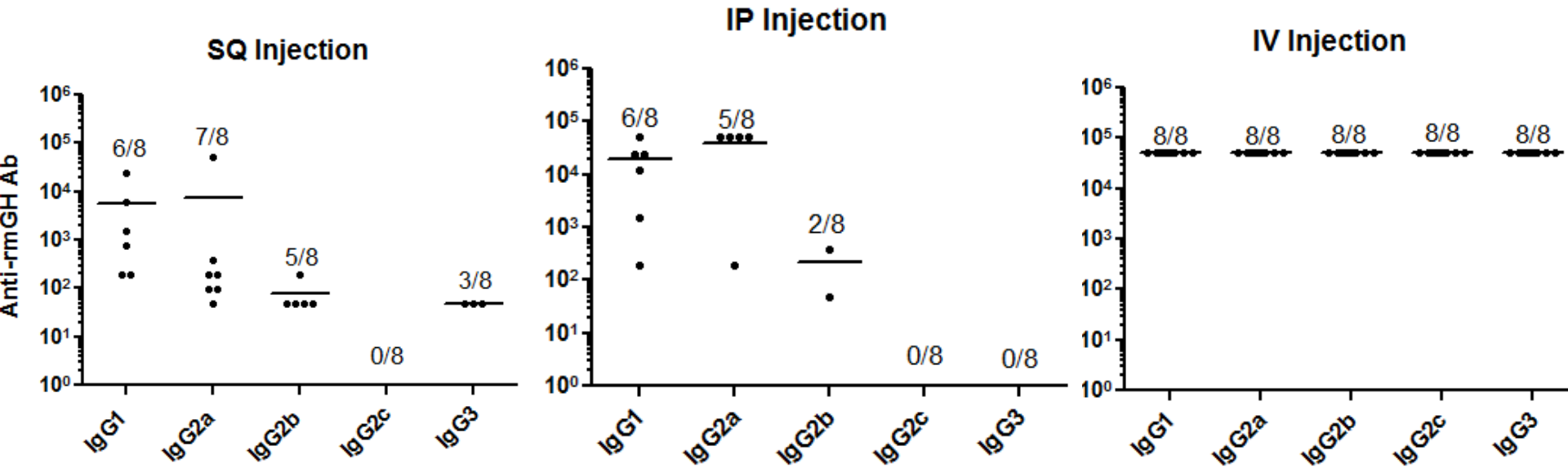



# Ultra-centrifuged rmGH: Results for Week 4 (2 weeks after 1st injection)




The fraction above the titer represents the number of mice of the total that elicited a positive response

# Ultra-centrifuged rmGH: Results for Week 6 (2 weeks after 2nd injection)




**IV administration much more immunogenic than SQ.**

**Similar results observed with freeze-thawed samples that were not ultracentrifuged.**

The fraction above the titer represents the number of mice of the total that elicited a positive response

# 2013 Colorado Protein Stability Conference

## July 9-11

## Breckenridge, Colorado


john.carpenter@ucdenver.edu