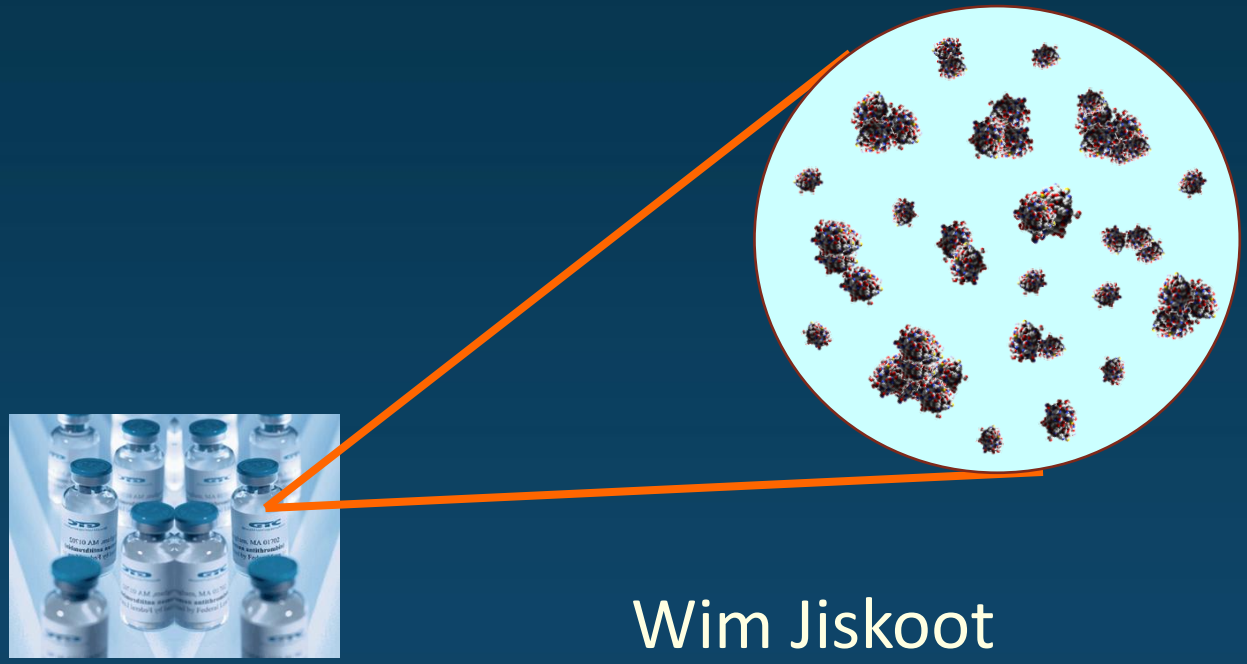
Protein Aggregates and Subvisible Particles

What are they and what is their clinical impact?



Wim Jiskoot

Division of Drug Delivery Technology
Leiden Academic Centre for Drug Research (LACDR)

EIP meeting
Munich
27 February 2013

All protein therapeutics contain (higher or lower levels of) aggregates and particles

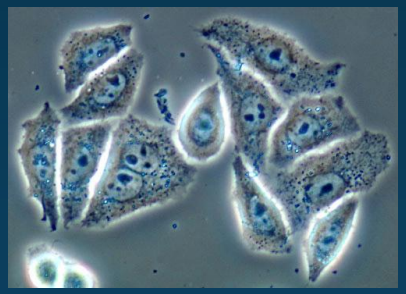
Most of these products are immunogenic

Is there a link???

When does protein aggregation occur?

Processing steps

Fermentation/expression



Purification

Formulation

Filling

Handling and storage of final product

Transport

Storage

Administration

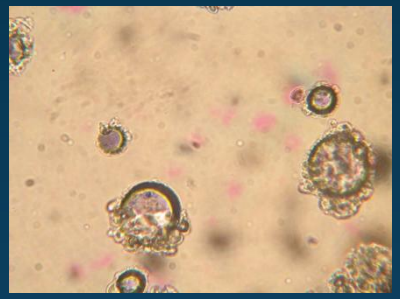
Adapted from Vasco Filipe

Some of the factors influencing protein aggregation

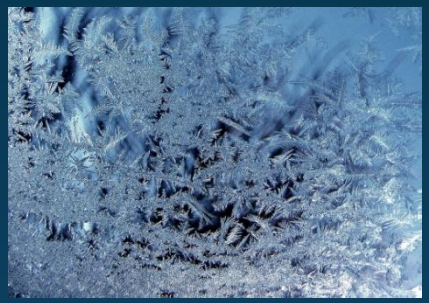
Environmental factors

Temperature

Interfaces



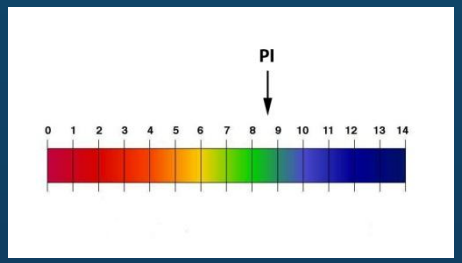
Freeze-thaw



Container

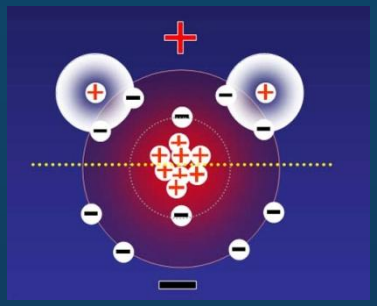
Solution factors (formulation)

pH



Excipients

Ionic strength



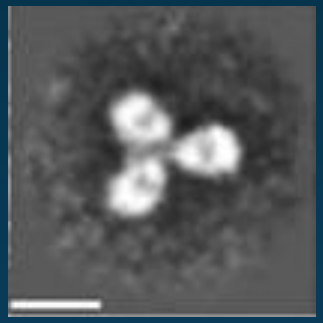
Concentration

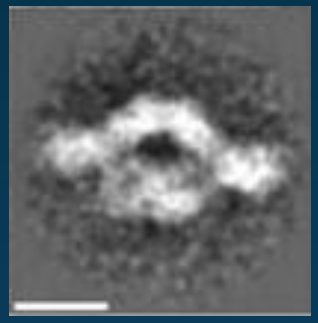
Adapted from Vasco Filipe

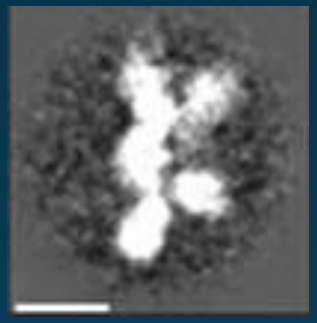
Protein aggregates: definition and categories

- Protein aggregates = assemblies of protein molecules
- Protein aggregates are heterogeneous regarding (Narhi et al., J Pharm Sci 101, 493-498):
  - Size
  - Reversibility
  - Protein conformation
  - Covalent modification
  - Morphology
- So, the question “which aggregates do matter?” is difficult to answer

Example: even a simple aggregate such as a dimer can adopt various shapes and characteristics







	monomer	process stress dimer	pH stress dimer	light stress dimer
				
Covalent Aggreg.	no	no	no	yes
Hydroph-obicity	low	low	high	heterogeneous
Relative Potency	100%	62 ± 3%	101 ± 4%	21 ± 17%

Paul et al., Pharm Res 29: 2047–2059 (2012)

Size categories

Definitions by size (all are aggregates)

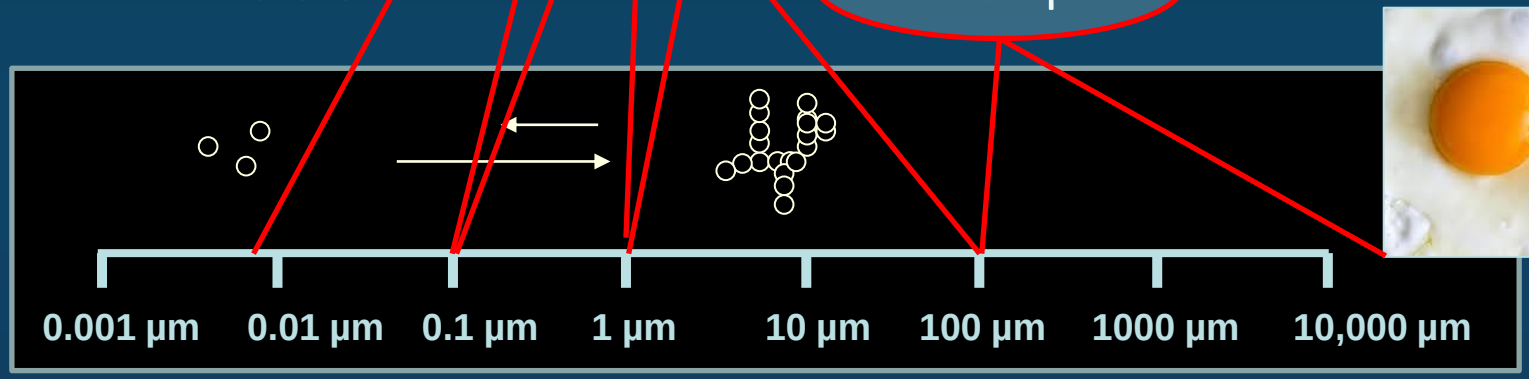
* Oligomers: < 100 nm

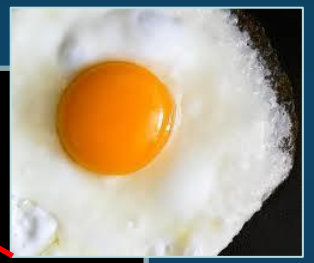
* Particles:

  - Submicron: 0.1 – 1 µm

  - Micron: 1 – 100 µm

  - Visible: ≥ 100 µm

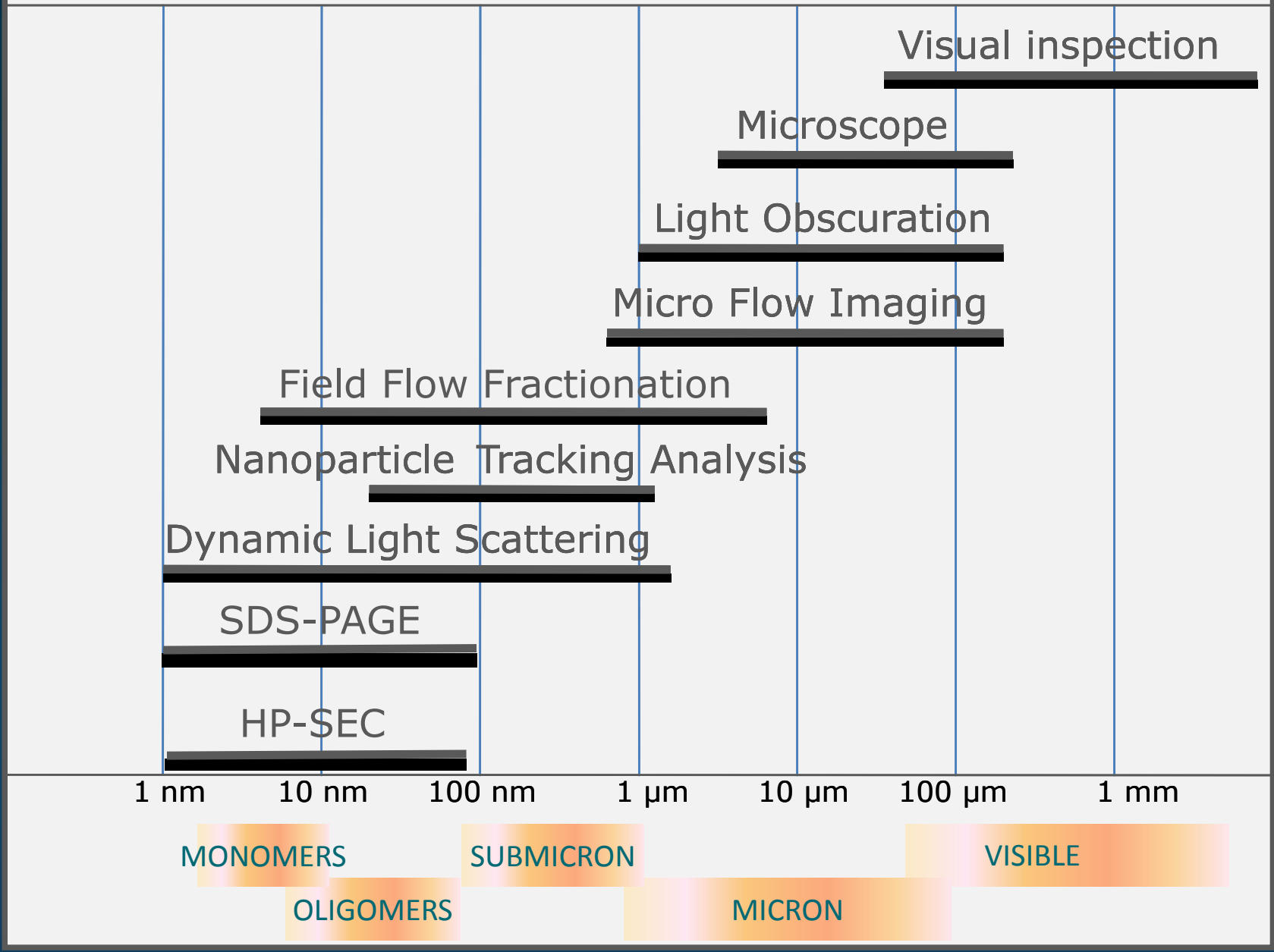




subvisible visible

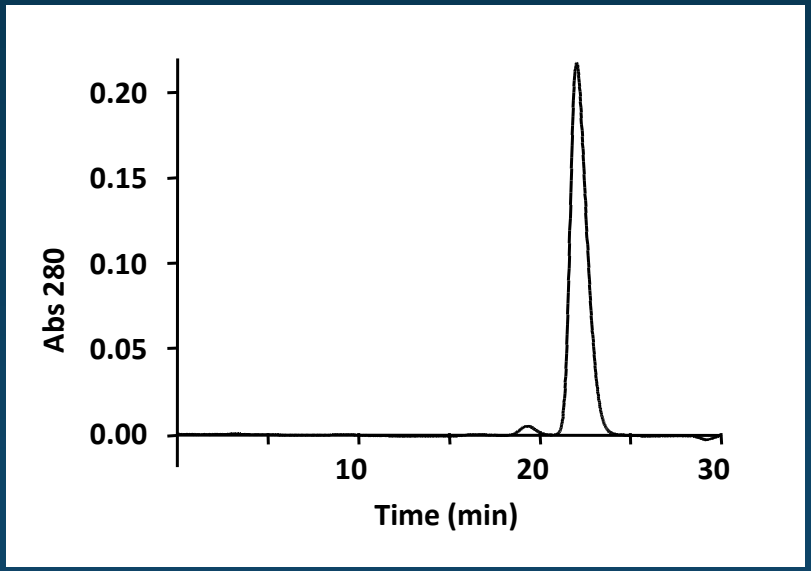
Adapted from Linda Narhi

Complementary analytics needed to cover the size range



Adapted from Michael Wiggenhorn, Coriolis

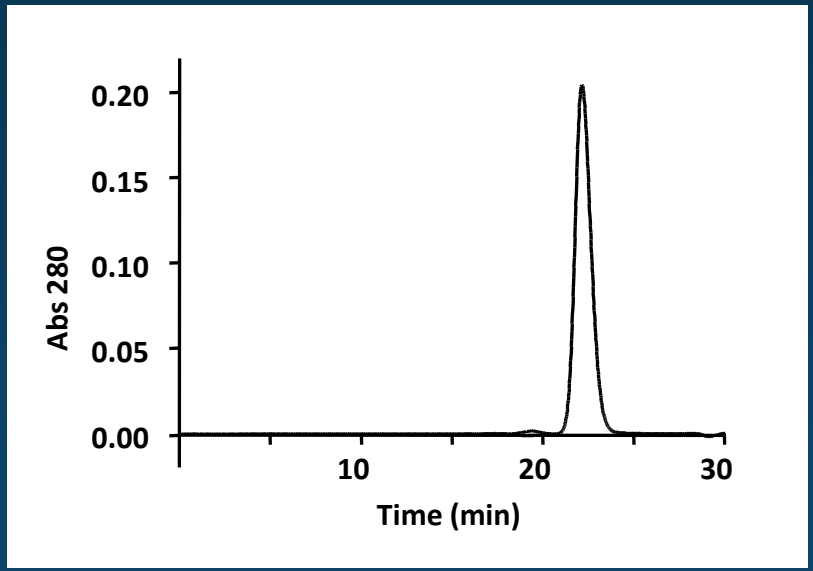
Size-exclusion chromatography results do not predict particle levels



Batch X

Optically clear

OD_{350} = 0.002



Batch Y

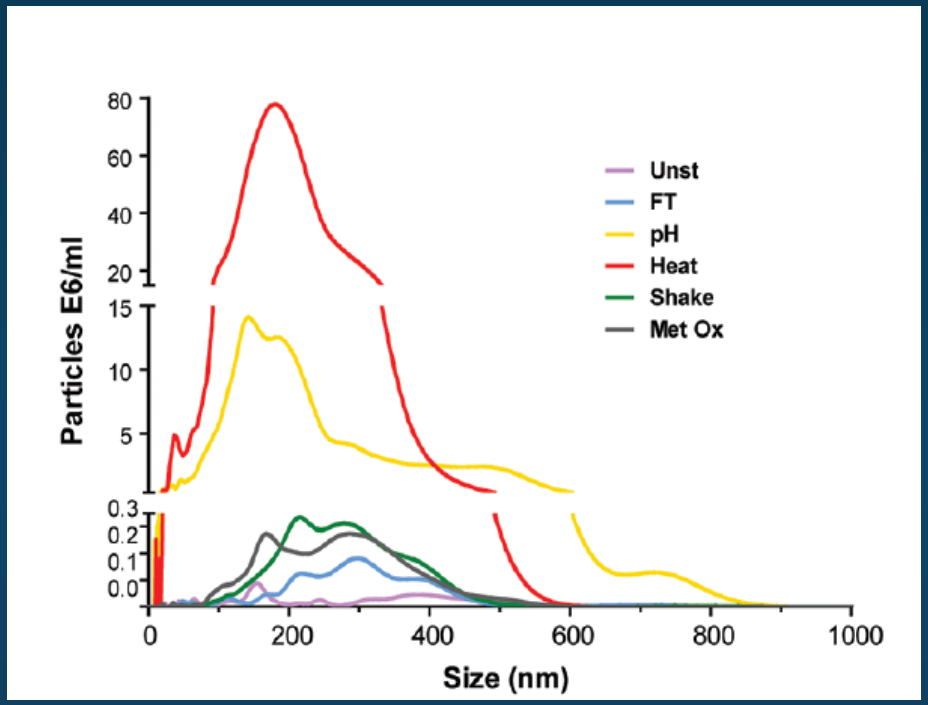
Visible aggregates

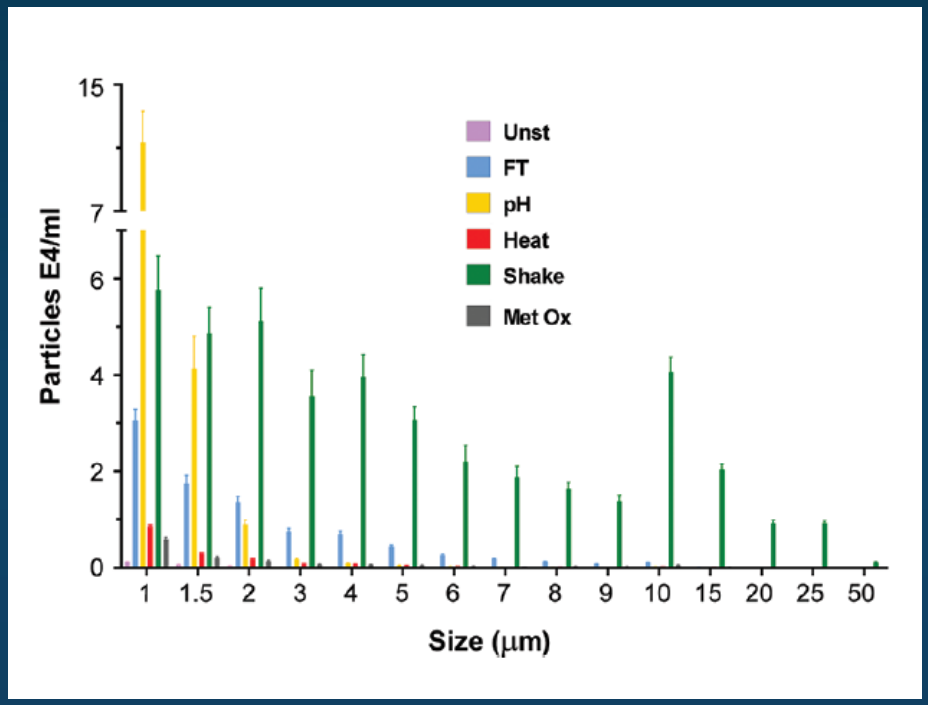
OD_{350} = 0.1

Submicron-sized particle counts do not predict micron-sized particle counts, vice versa

Nanoparticle tracking analysis (submicron particles)

Light obscuration (micron particles)

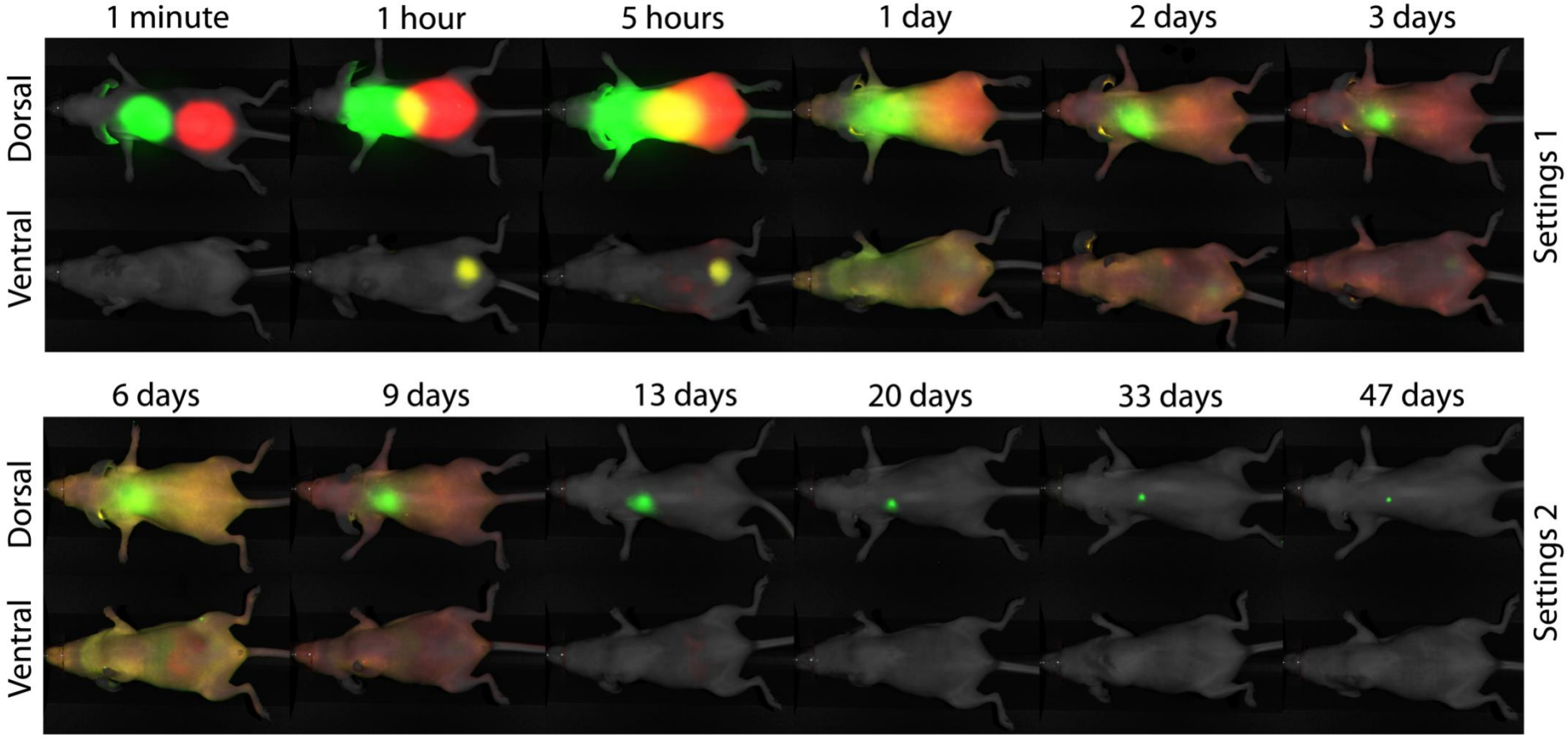




Results of monoclonal human IgG1 stressed under different conditions

Filipe et al, mAbs 4(6): 740-752 (2012)

Micron-sized IgG aggregates induced by shaking remain at SC injection site for longer than a month



Unstressed IgG-680CW

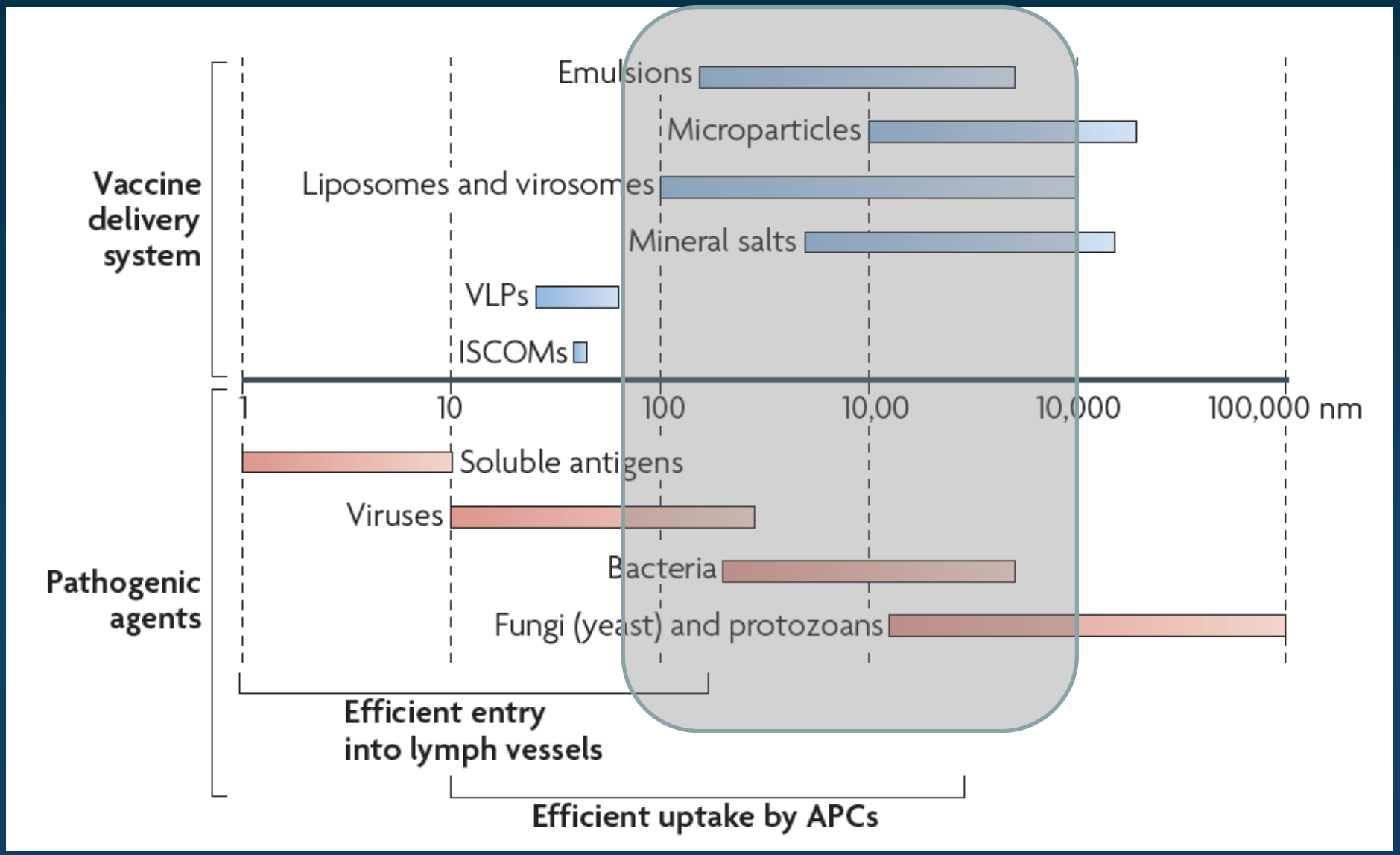
Shaken IgG-800CW

Filipe et al, manuscript in preparation

Our vaccines are based on particles in the 'gap' range

Vaccine category	Examples	Particle category
Live bacteria	Salmonella	**Micron** (~ 1 µm)
Inactivated bacteria	Whole cell pertussis	**Micron** (~ 1 µm)
Live viruses	Oral polio Measles-mumps-rubella Nasal influenza	**Submicron** (~ 30-300 nm)
Inactivated viruses	Inactivated polio vaccine Whole inactivated influenza vaccine	**< 100 nm** **Submicron** (~ 200 nm)
Virus like particles	Hepatitis B	**< 100 nm**
Split- and subunit vaccines	Influenza	**Submicron** (aggregates, ~ 300 nm)
Alum-adsorbed antigens MF59 adjuvanted antigens	Diphtheria, tetanus Influenza	**Micron** (low µm range) **Submicron** (~200 nm)

Our vaccines are based on particles in the ‘gap’ range



Bachmann & Jennings, Nature Reviews 2010

New insights from quantifying subvisible particles

- Beyond potential immunogenicity, particle sizes and levels are important product quality attributes
- Mass of protein (e.g., < 0.1%) in particles may not be detectable as loss of monomer
- Subvisible particle analysis provides very sensitive early detection of protein aggregation and new insights into aggregation pathways, manufacturing & formulation development
- Even trace levels of particles can impact subsequent stability of protein solutions (see recent papers Carpenter and colleagues)

Beyond protein particles...
Nonproteinaceous particles (often in the 'gap' range)

- Glass particles from containers
- Glass cartridges and syringes are siliconized, and free silicone oil droplets can be generated
- In syringes, there may be tungsten particles and salts from needle insertion process
- Rubber or silicone particles can come from stoppers
- Stainless steel and other particles from filling pumps
- Particles shed from filters during pre-filling sterile filtration

Protein molecules can adsorb to these particles

This will create a "vaccine"

Subvisible particles and immunogenicity

Subvisible particles are in every therapeutic protein product and most of these products are immunogenic

Is there a link???

EXAMPLES

Aggregate/particle removal can induce tolerance

Already known in the 1960s!

Administration of aggregate-free foreign protein induces immunological tolerance in animals and human patients

For instance:

Dresser, *Immunology* 5, 378 (1962)

Claman, *J Immunol* 91, 833-839 (1963)

Biro & Garcia, *Immunology* 8, 411-419 (1965)

Spiegelberg & Weigle, *Int Arch Allergy* 31, 559-567 (1967)

Cerottini et al., *J Exp Med* 130, 1093-1105 (1969)

Golub & Weigle, *J Immunol* 102, 389-396 (1969)

Weksler et al., *J Clin Invest* 49, 1589-1595 (1970)

Von Felten & Weigle, *Cellular Immunology* 18, 31-40 (1975)

Fujiwara et al., *Jpn J Microbiol* 20, 141-146 (1976)

Aggregate/particle removal can induce tolerance

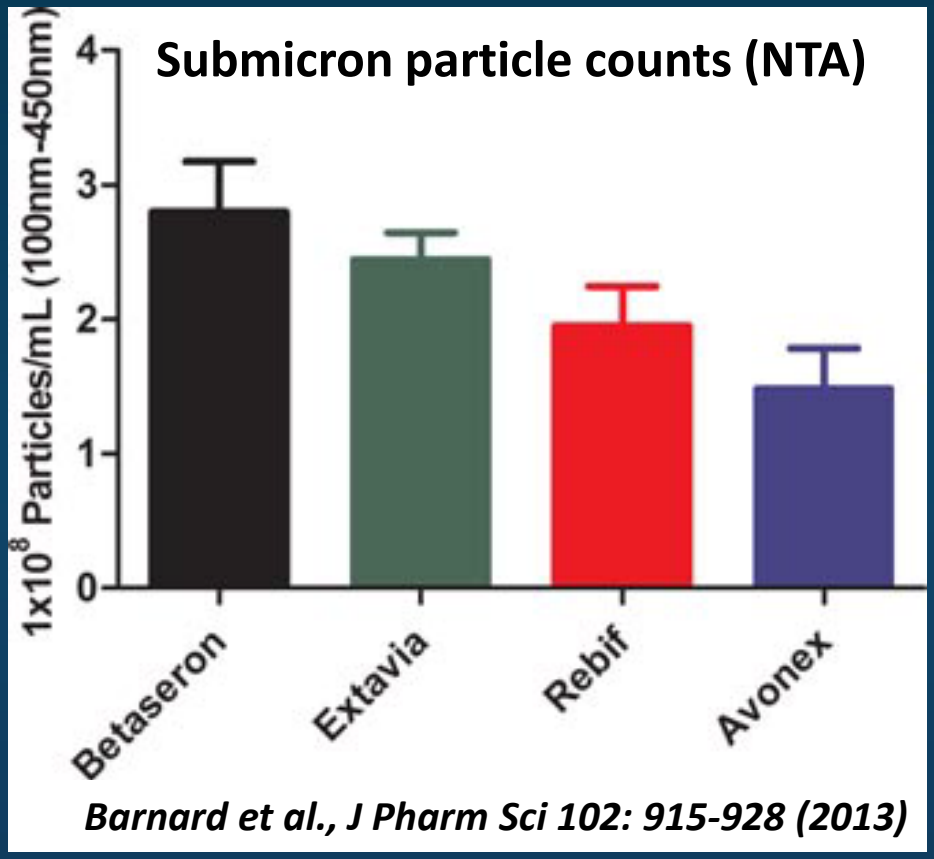
Already known in the 1960s!

- Anti-human lymphocyte IgG produced in horses
- Administration to organ transplant patients resulted in antibody response and consequently rapid drug clearance
- Administration of equine IgG in which aggregates / particles were removed by ultracentrifugation (133,500 *x g* for 1 h) resulted in no immune response and made the patients tolerant for equine IgG
- Co-medication: azathioprine + prednisolone

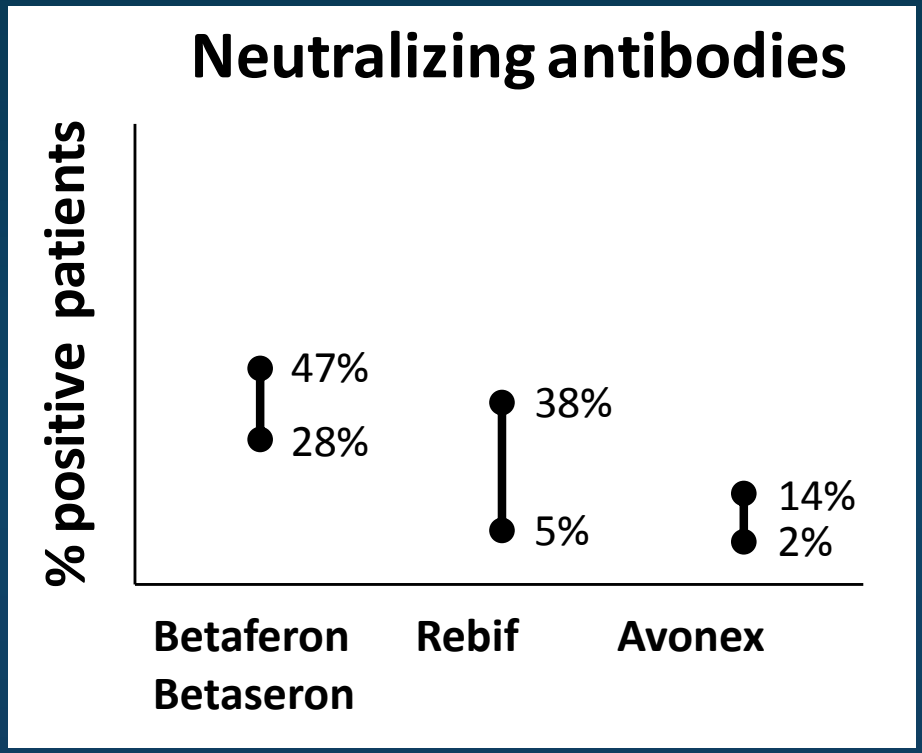
Weksler et al., 1970, *J. Clin. Invest.* 49: 1589

Commercial beta-interferon products: immunogenicity in patients

- Percentages of patients forming binding antibodies and neutralizing antibodies in various clinical studies (measured with various assays)



Barnard et al., J Pharm Sci 102: 915-928 (2013)

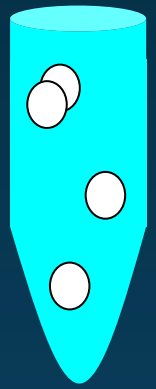


Van Beers et al., J Interferon Cytokine Res 30: 767-775 (2010)

rhIFNβ-1a with different aggregate levels

1. Bulk rhIFNβ-1a

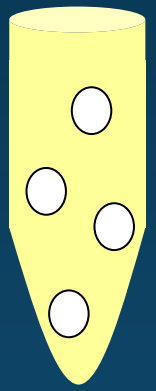
- Received as frozen bulk
- In PBS (!) pH 7.2



Medium aggregate content

2. Reformulated rhIFNβ-1a

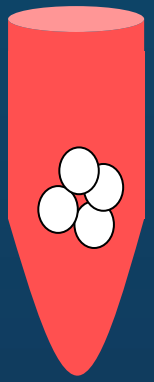
- Filtered and dialyzed bulk
- In NaAc pH 4.8
- Formulated with Tween 20 and ArgHCl



Low aggregate content

3. Stressed rhIFNβ-1a

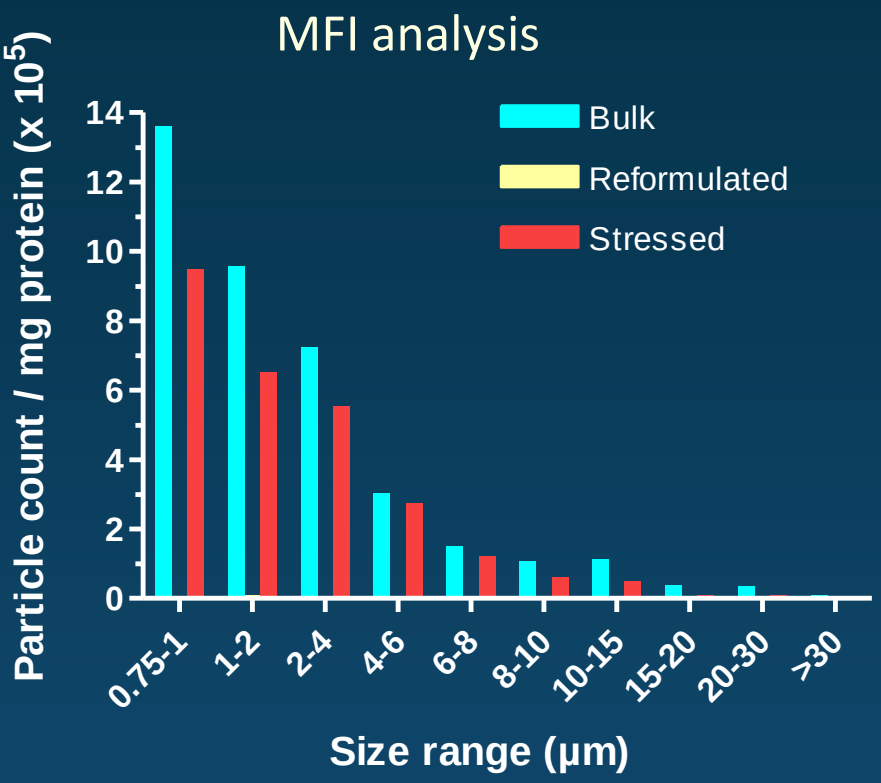
- Bulk incubated at pH 2 + 1 M NaCl
- Aggregates purified by SEC-HPLC
- In PBS pH 7.2

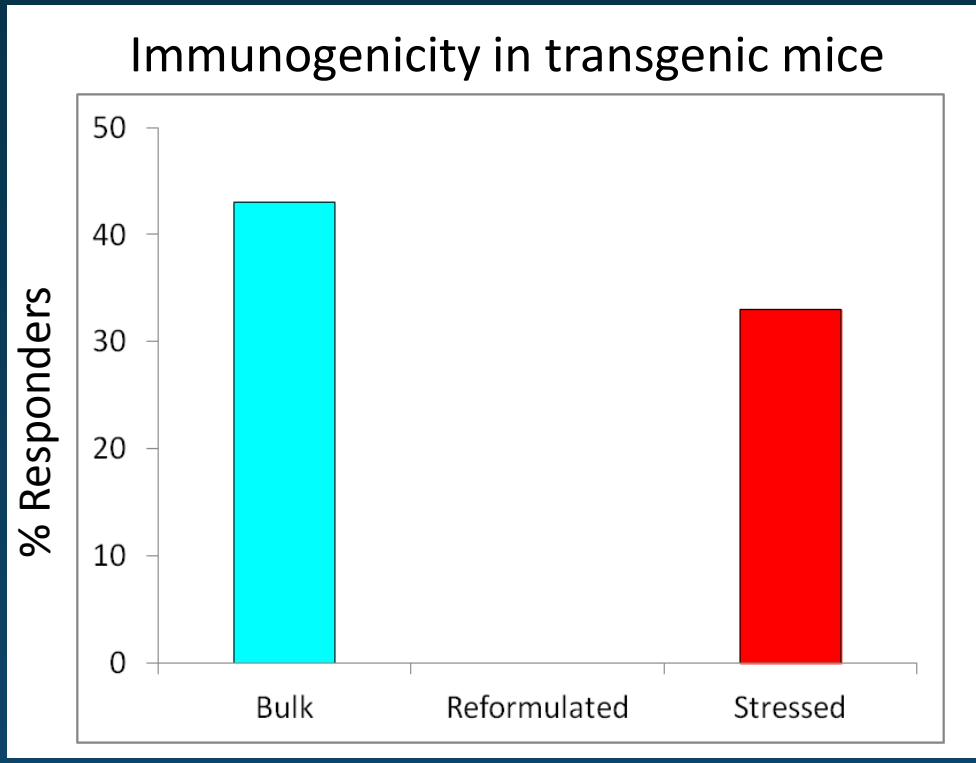


High aggregate content

van Beers et al., Pharm Res 27: 1812-1824 (2010)

Subvisible particle counts and immunogenicity



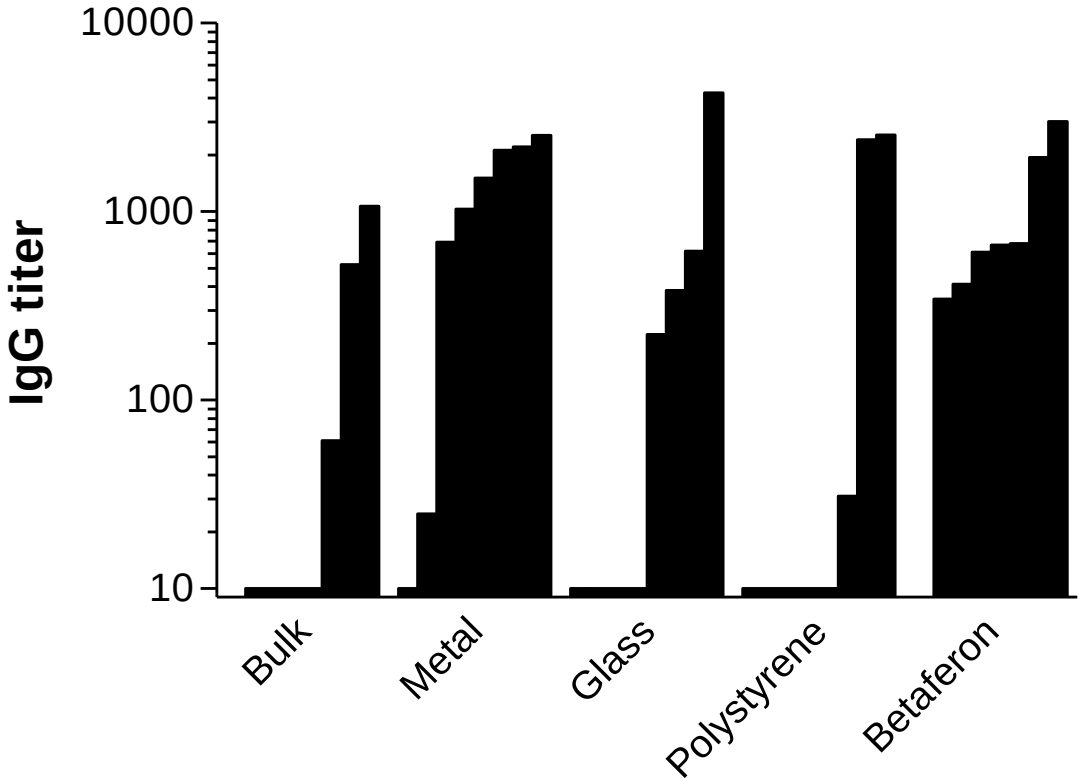


- Hardly any particles in reformulated rhIFNβ-1a
- Immunogenicity in transgenic immune tolerant mice correlates with subvisible particle counts (rather than total % aggregates)

van Beers et al., Pharm Res 27: 1812-1824 (2010)

Adsorption of rhIFNβ to metal (but not glass or polystyrene) beads enhances its immunogenicity in transgenic mice

Anti-rhIFNβ antibody titers after a 3-week injection protocol



Metal
Ø 14 µm
71% adsorbed

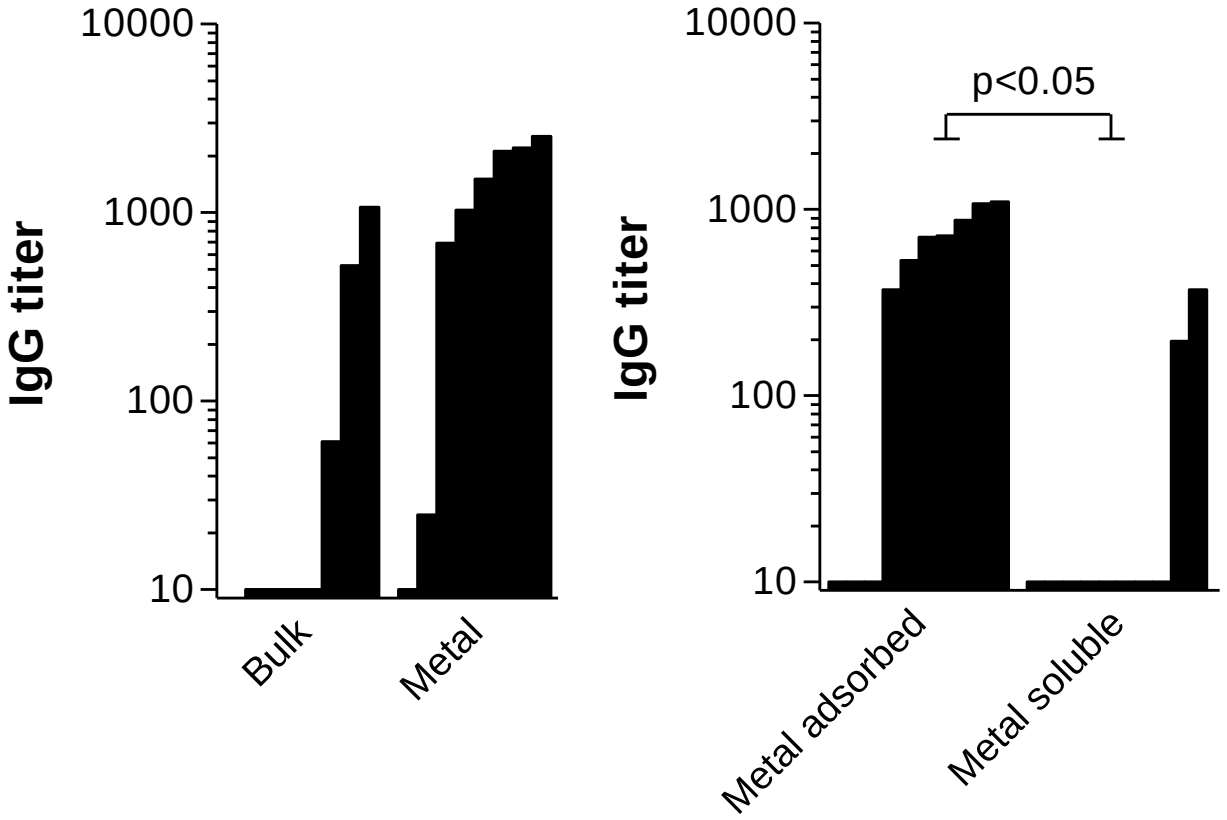
Glass
Ø 1.1 µm
78% adsorbed

Polystyrene
Ø 0.21 µm
46 % adsorbed

Van Beers et al., J Pharm Sci 101: 187-199 (2012)

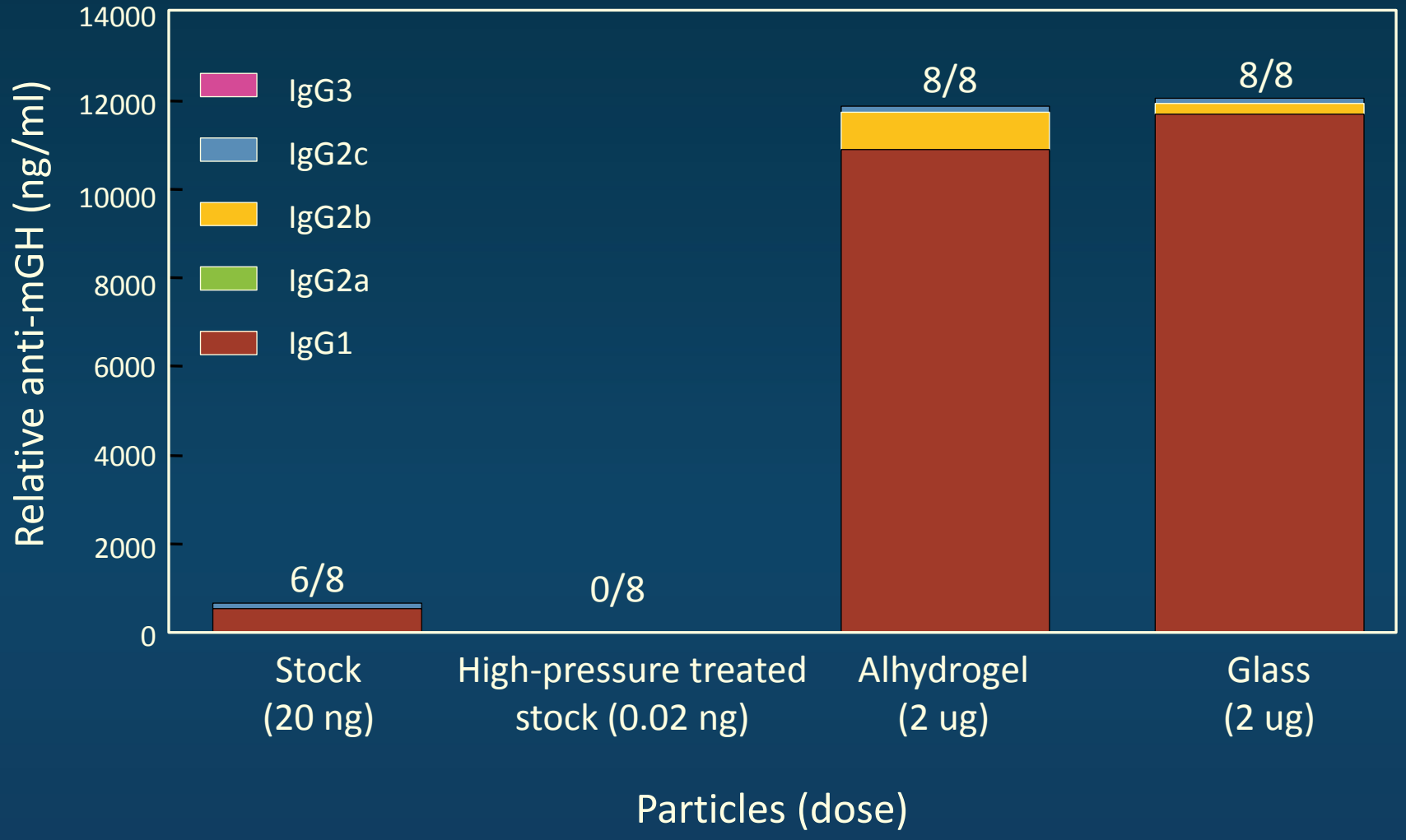
Adsorption of rhIFNβ to metal (but not glass or polystyrene) beads enhances its immunogenicity in transgenic mice

Anti-rhIFNβ antibody titers after a 3-week injection protocol



Van Beers et al., J Pharm Sci 101: 187-199 (2012)

Subvisible particles break immune tolerance in mice to murine growth hormone



Courtesy of John F Carpenter

Fradkin et al, J Pharm Sci 100, 4953-4964 (2011)

Conclusions

- Aggregates, including subvisible particles, are critical quality attributes
- Removal of aggregates & particles reduces protein immunogenicity
- Betaferon contains large amounts of aggregates & particles and is the most immunogenic rhIFNβ product
- Adsorption of protein to non-proteinaceous subvisible particles may increase immunogenicity risk
- However, no general rules: rhIFNβ adsorbed to glass particles was not very immunogenic, whereas mGH adsorbed to the same glass particles was

Conclusions

Acknowledgements

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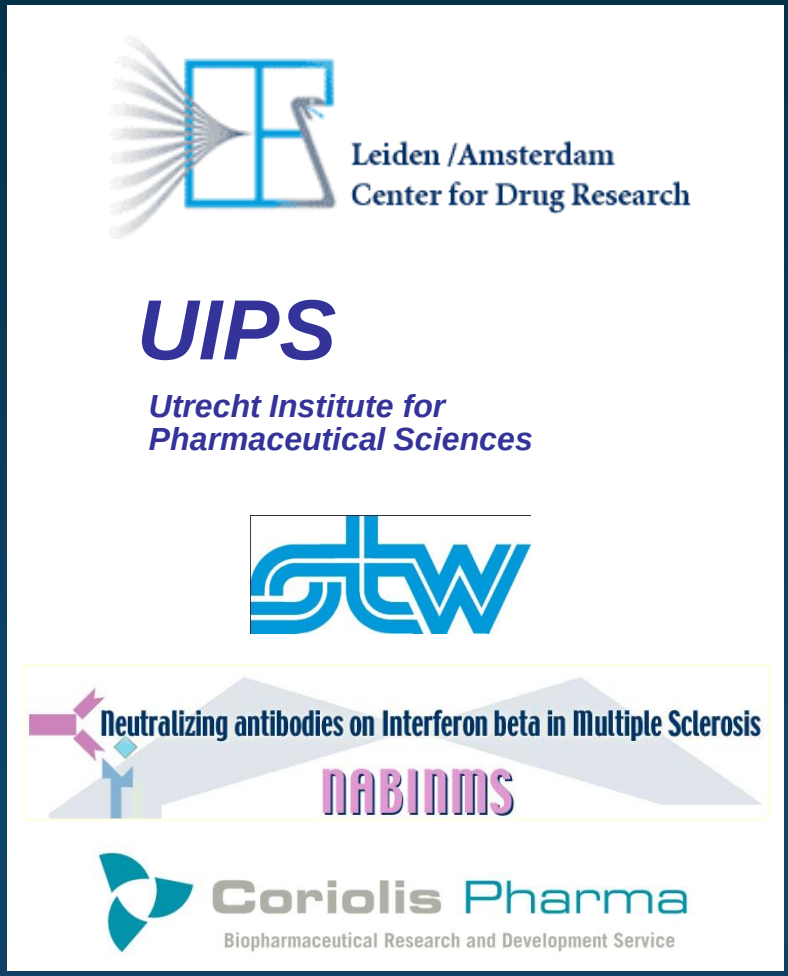
Susan Goelz
Darren Baker

Antitope

Christine Bryson
Matthew Baker



Thank you!