Role of aggregates in therapeutic protein immunogenicity: size matters!

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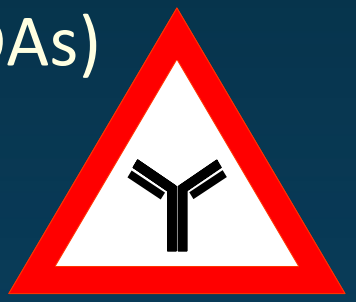
The problem: therapeutic proteins are immunogenic

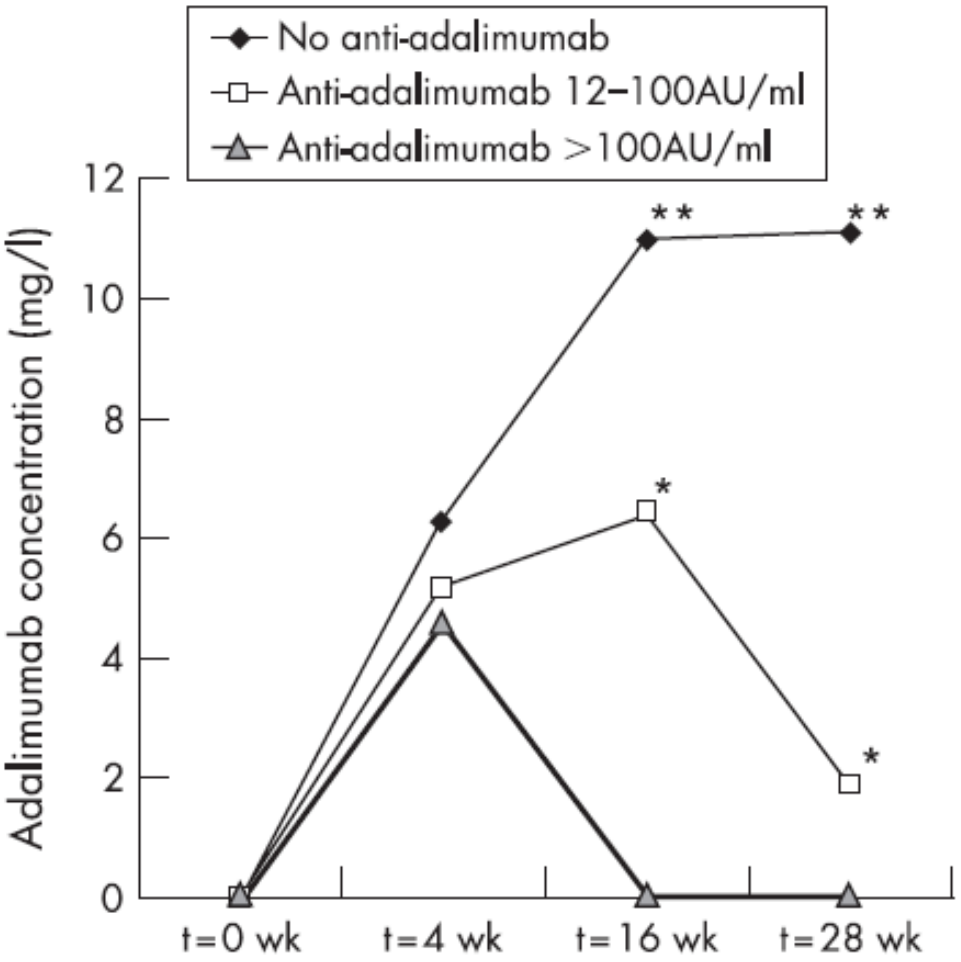
- Some patients receiving a therapeutic protein produce antibodies against it (anti-drug antibodies, ADAs)
  - Binding antibodies (accelerate drug clearance)
  - Neutralizing antibodies (block the active site)

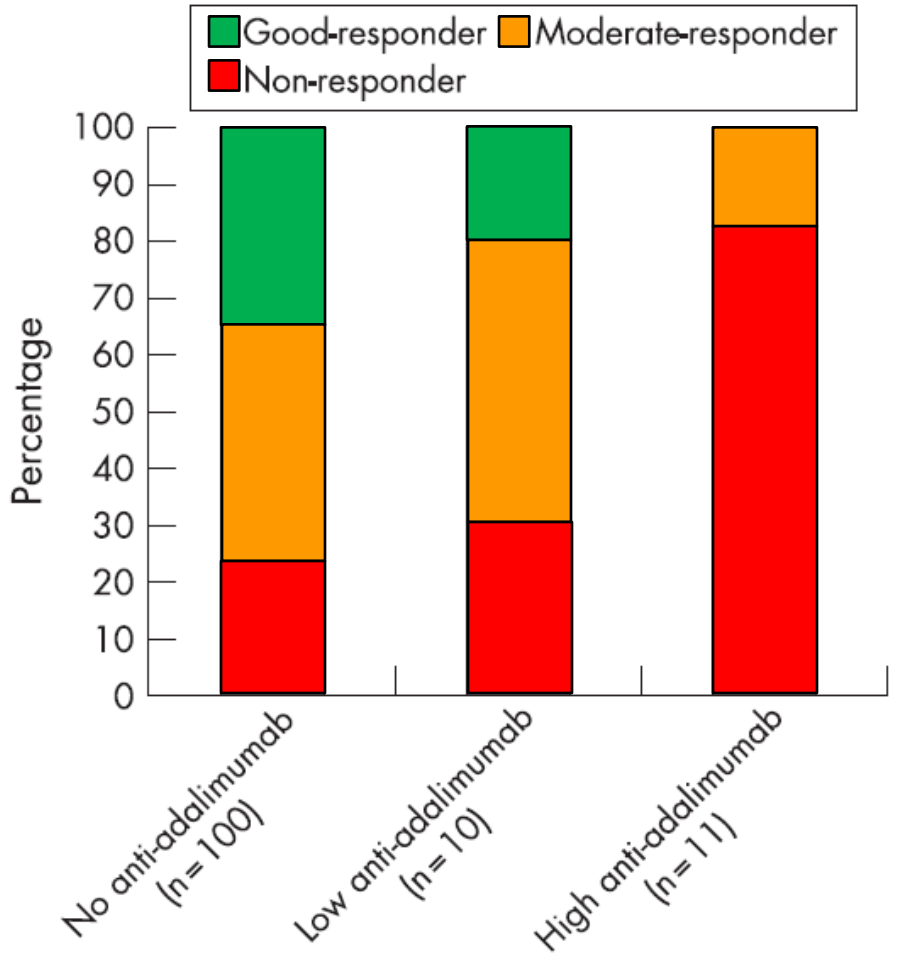


- Clinical consequences of ADAs are unpredictable
  - None (common)
  - Loss of efficacy (common)
  - Cross-reactivity with endogenous counterpart (rare)
  - General immune reactions (anaphylaxis, allergy, serum sickness)
- There are no reliable predictive tools

The problem: therapeutic proteins are immunogenic

Example: antibodies against adalimumab (Humira) reduce drug concentration in plasma and block therapeutic effect





Bartelds et al., Ann. Rheum. Dis. 66:921-926 (2007)

Unpleasant surprises still happen today…

Pfizer Discontinues Global Development of Bococizumab, Its Investigational PCSK9 Inhibitor

Tuesday, November 1, 2016 - 6:30am EDT

Pfizer Inc. announced today the discontinuation of the global clinical development program for bococizumab, its investigational Proprotein Convertase Subtilisin Kexin type 9 inhibitor (PCSK9i). The totality of clinical information now available for bococizumab, taken together with the evolving treatment and market landscape for lipid-lowering agents, indicates that bococizumab is not likely to provide value to patients, physicians, or shareholders. As a result, Pfizer has decided to discontinue the development program, including the two ongoing cardiovascular outcome studies.

With the completion of six bococizumab lipid-lowering studies, Pfizer has observed an emerging clinical profile that includes an unanticipated attenuation of low-density lipoprotein cholesterol (LDL-C) lowering over time, as well as a higher level of immunogenicity and higher rate of injection-site reactions with bococizumab than shown with the other agents in this class. The goal of treating elevated cholesterol is to reduce the occurrence of cardiovascular events such as heart attacks and stroke, which requires long-term effective and durable cholesterol-lowering.

"As a company, we understand that developing new and important medicines for patients is a critical, but difficult undertaking. Accordingly, we continually evaluate our development programs as data emerge to support prudent decisions that provide value both to the patients we serve and our shareholders," said James Rusnak, MD, PhD, Chief Development Officer, Cardiovascular and Metabolic Diseases, Pfizer Global Product Development. "We are disappointed by this outcome, but remain committed to investing in innovation,

… and can be life-threatening

The New York Times

Tuesday, May 16, 2017 | Today's Paper | Video | 70°F | Dow +0.08% ↑

When the Immune System Thwarts Lifesaving Drugs

BY GINA KOLATA

Patients often produce antibodies to the very treatments keeping them alive, sometimes to disastrous effect. The search for solutions is just beginning.

The miracle treatment that should have saved Becka Boscarino's baby boy almost killed him.

Doctors diagnosed her newborn son, Magglio, with Pompe disease, a rare and deadly genetic disorder that leads to a buildup of glycogen in the body. Left untreated, the baby would probably die before his first birthday.

There is just one treatment: a series of infusions. But after the boy received his fifth dose, he turned blue, stopped breathing and slipped into anaphylactic shock.

The problem? Eventually doctors discovered that Magglio's body was producing antibodies to the very drug saving his life.

https://www.nytimes.com/2017/05/15/health/immune-system-drugs-monoclonal-antibodies.html?emc=eta1&_r=0

Just another recent example…

Peginesatide is a 4.9-kDa peptide (two identical 21-AA chains) conjugated to a 40-kDa PEG chain

Affymax and Takeda Announce Termination of Omontys® (peginesatide) Product Collaboration and License Agreement

Takeda will withdraw the Omontys U.S. New Drug Application (NDA)

Cupertino, CA (June 13, 2014) and Osaka, Japan, (June 16, 2014) – Affymax, Inc. and Takeda Pharmaceutical Company Limited (Takeda) announced today that their Omontys® (peginesatide) product collaboration and license agreement will terminate effective September 10, 2014.

In February 2013, Affymax and Takeda voluntarily recalled all lots of Omontys and suspended promotional activities in the U.S. following postmarketing reports of serious hypersensitivity reactions including anaphylaxis, which may be life-threatening or fatal.

- Note (1): also peptides can be immunogenic!
- Note (2): PEGylated ≠ non-immunogenic!

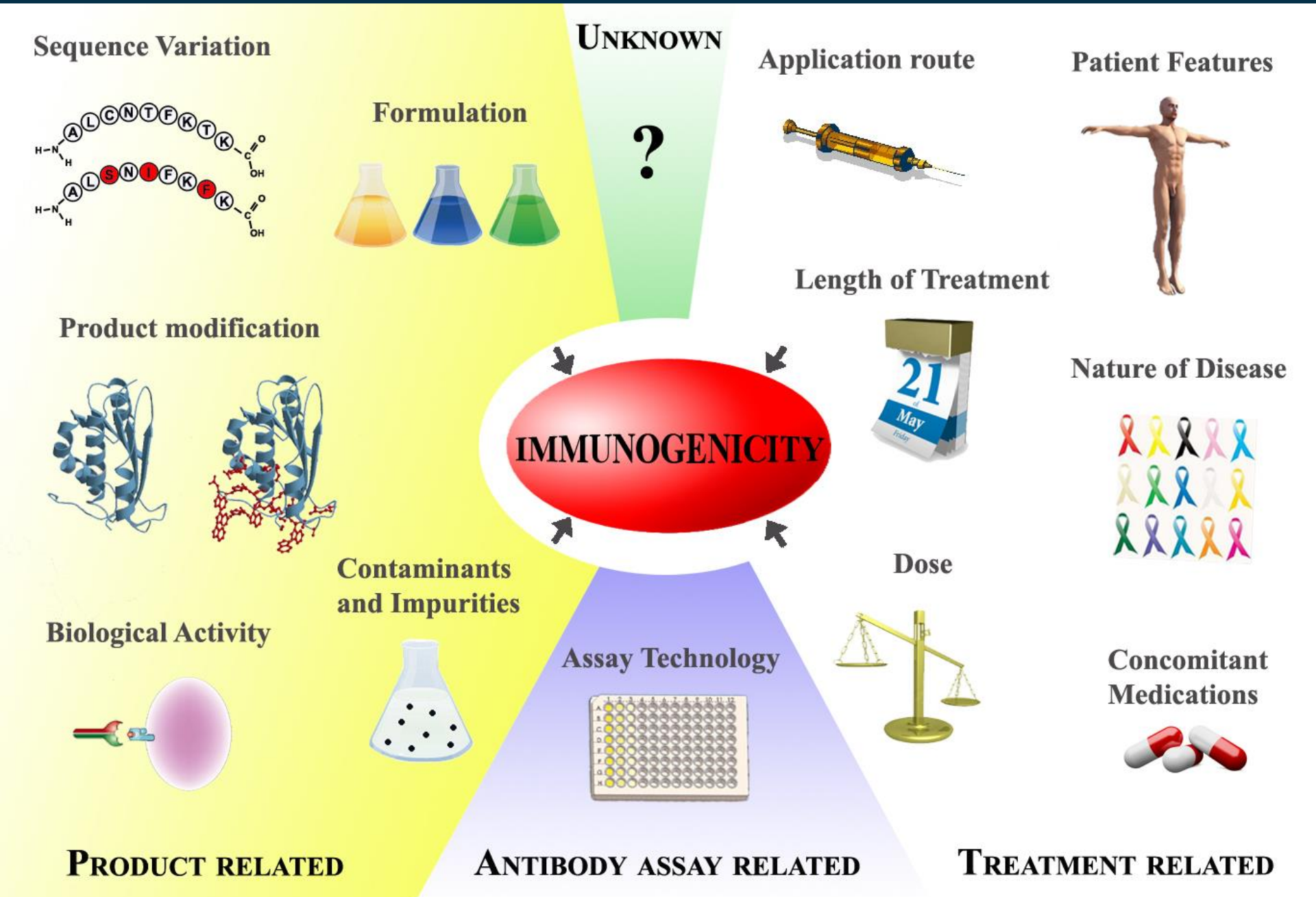
Hypersensitivity reactions were correlated with elevated SVP levels in peginesatide multi-dose vials

Table 1
NTA Peginesatide Median Particle Concentrations[a,b]

Hydrodynamic Diameter (nm)	SUV	MUV	p[c]
50-1000	9763	29,934	0.028
50-100	1610	1197	0.673
101-200	5176	14,426	0.022
201-300	1155	8526	0.001
301-400	286	2068	0.001
401-500	88	519	0.001
501-600	30	237	0.001
601-700	8	102	0.003
701-800	3	48	0.002
801-900	2	30	0.002
901-1000	2	18	0.008

[a] Particles/mL ($\times 10^4$).
[b] SUV and MUV were independently measured 6 (each SUV lot in duplicate) and 12 (each MUV lot in triplicate) times, respectively.
[c] Mann–Whitney test.

Factors influencing protein immunogenicity



Decades of studies suggest that aggregates and particles may contribute to immunogenicity

Since the 1960s!

Administration of particle-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)

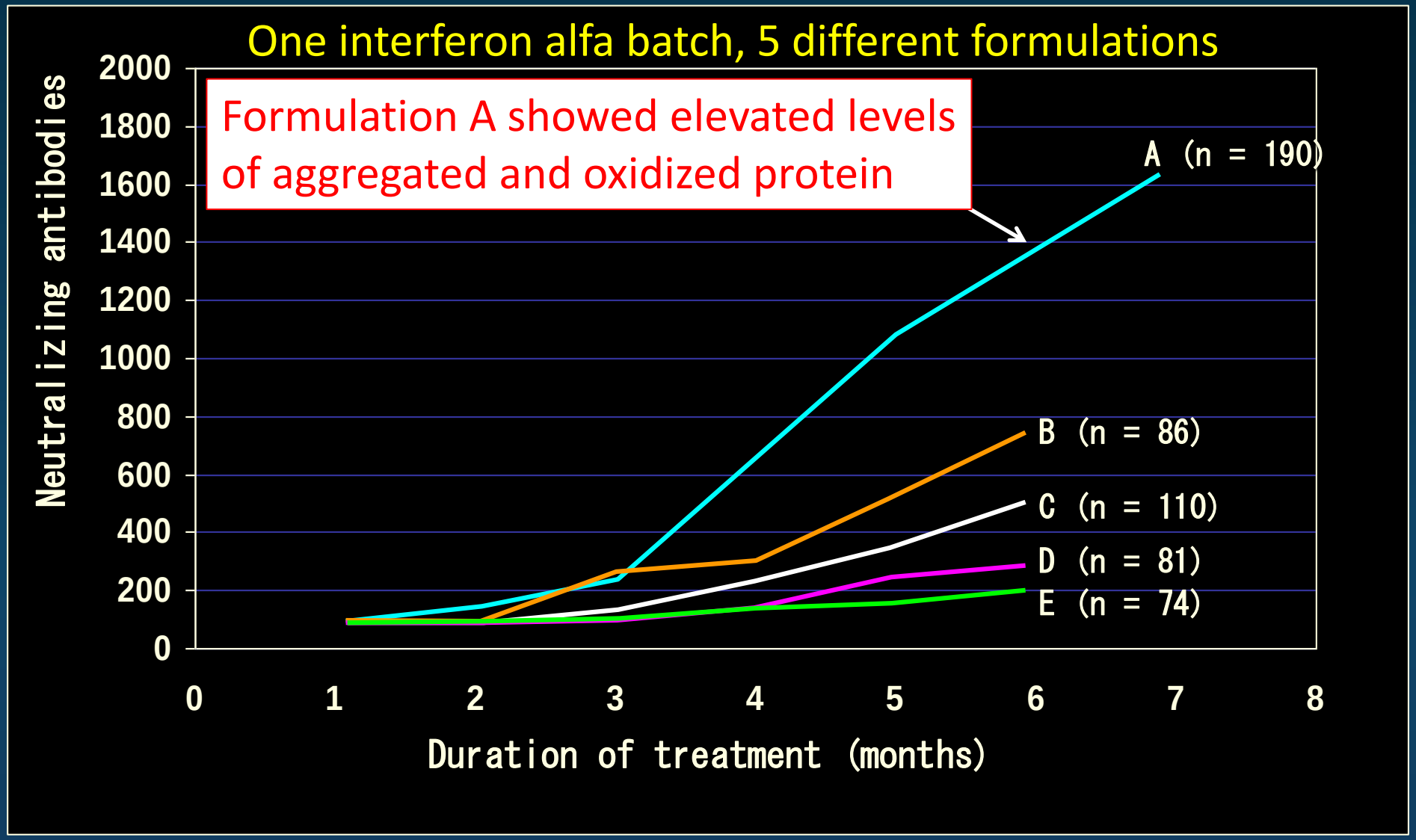
Golub & Weigle, *J Immunol* 102, 389-396 (1969)

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)

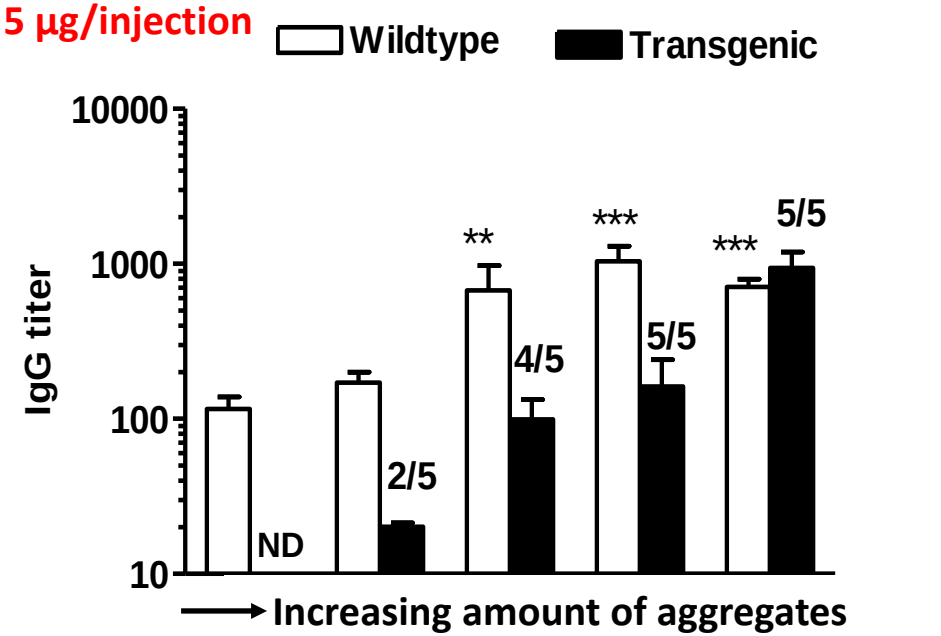
The formulation matters!



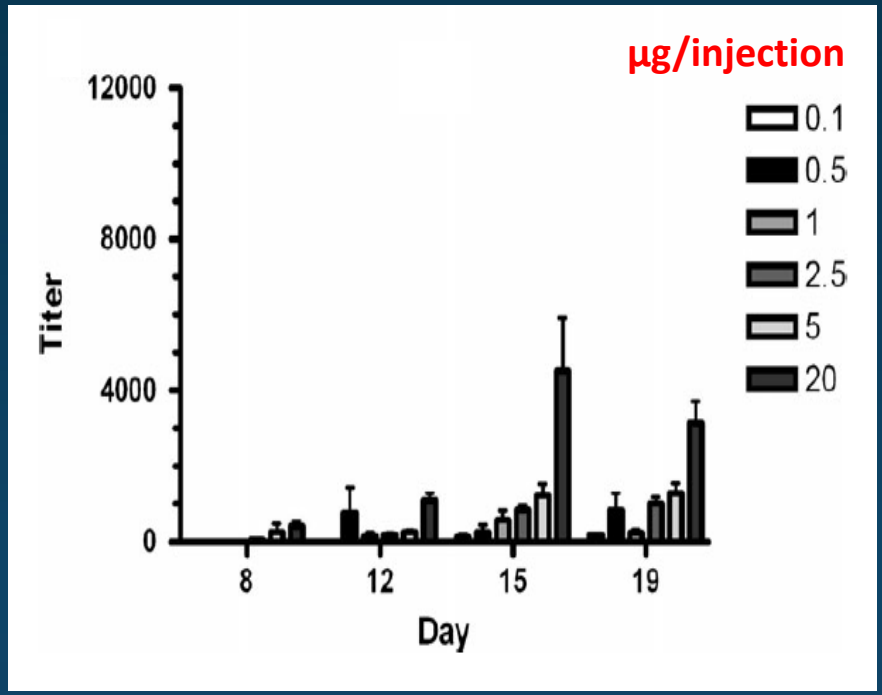
Ryff, Interferon Cytokine Res. 1997

Aggregate dose correlates with immunogenicity in immune tolerant mouse models

Anti-drug antibody titers (15 injections over 3 weeks)



Monomeric interferon α2 mixed with aggregated interferon α2



Interferon β (Betaferon) dose – antibody response study

Hermeling et al., J Pharm Sci 95: 1084-1096 (2006)
Kijanka et al., Pharm Res 30:1553–1560 (2013)

Preclinical assessment of immunogenicity

- *in silico*
- *in vitro*
- *in vivo*

- Main application: to support the assessment of relative immunogenicity risk of drug substances and/or drug products
- None of the methods predicts clinical immunogenicity risk, or enables us to assess maximal levels of aggregates/impurities that are “safe”

Journal of Pharmaceutical Sciences 105 (2016) 1567–1575

Review

Mouse Models for Assessing Protein Immunogenicity: Lessons and Challenges

Wim Jiskoot [1], Grzegorz Kijanka [1], Theodore W. Randolph [2], John F. Carpenter [3], Atanas V. Koulov [4], Hanns-Christian Mahler [4], Marisa K. Joubert [5], Vibha Jawa [6], Linda O. Narhi [5,*]

Some of the article section headings:

Protein Conformation Possibly Affects Aggregate Immunogenicity

Protein Aggregates Containing Chemically Modified Protein Are Often Immunogenic

Aggregate Size May Affect Immunogenicity

Could Non-Proteinaceous Particles Play a Role in Modulating Immunogenicity?

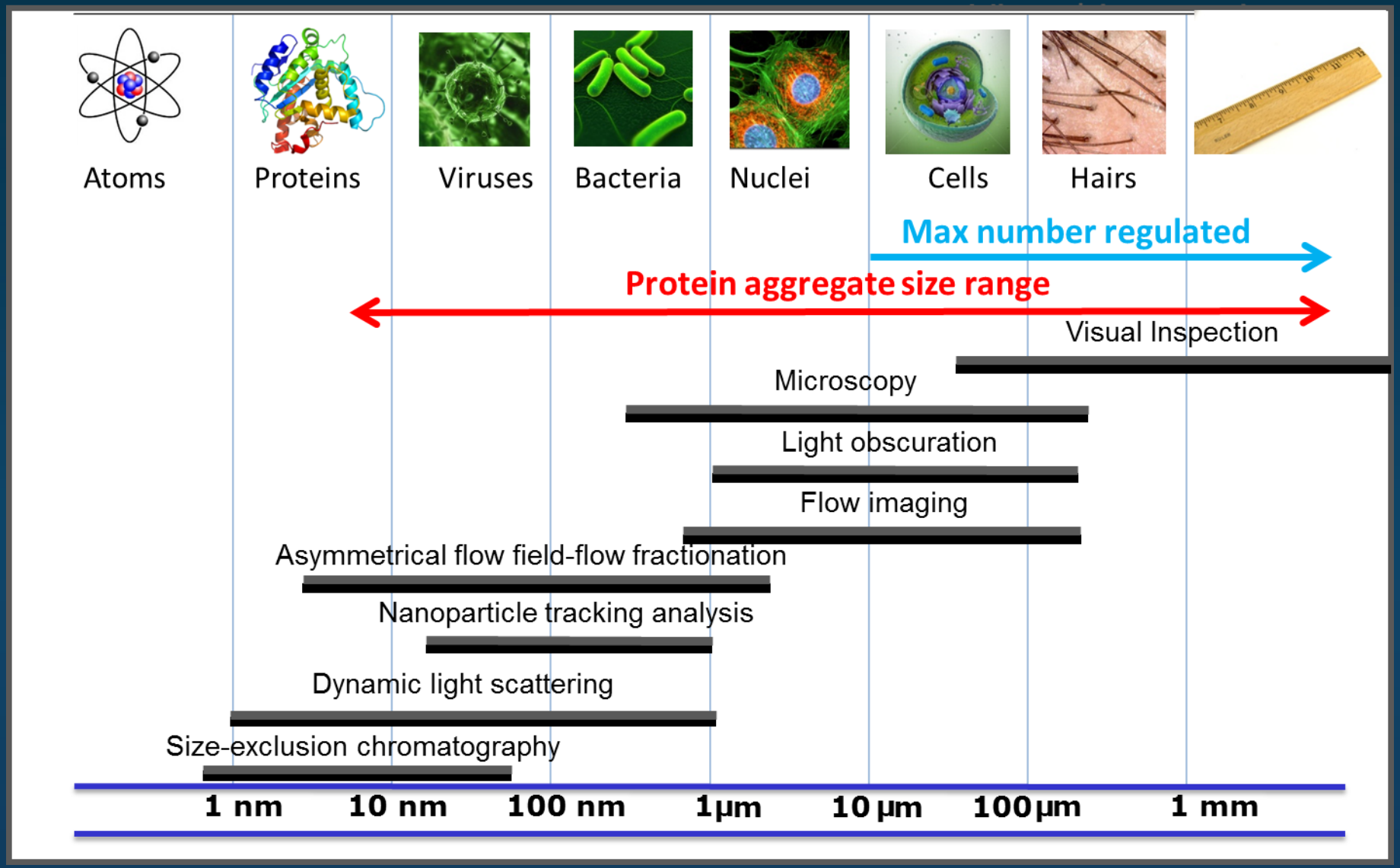
Dose and Dosing Schedule Affect Immunogenicity

Administration Route Affects Immunogenicity

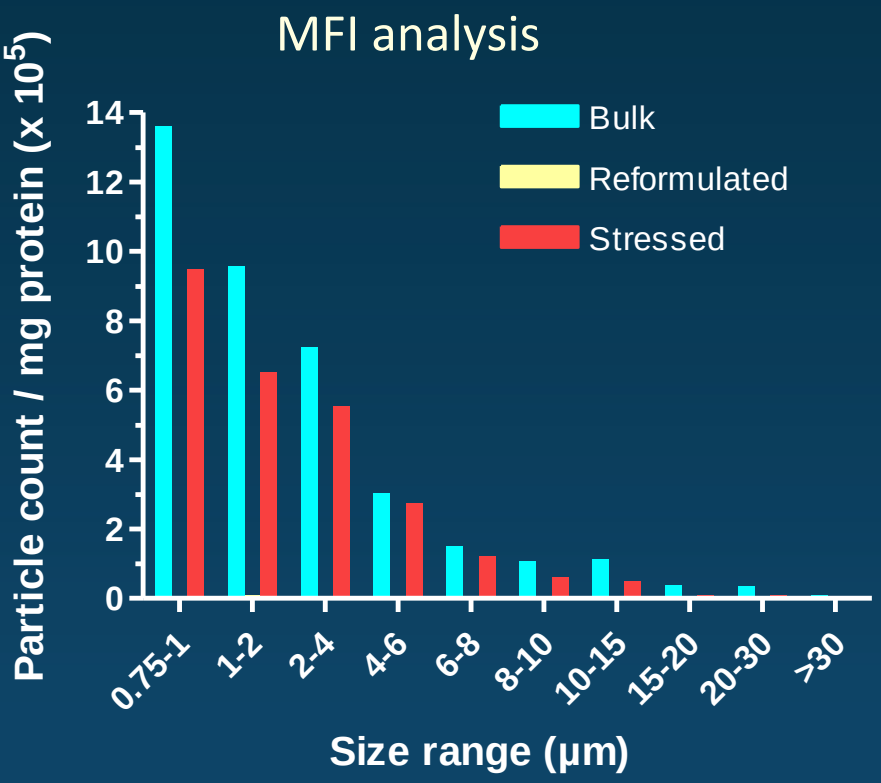
Immune Mechanisms Are Not Yet Fully Understood

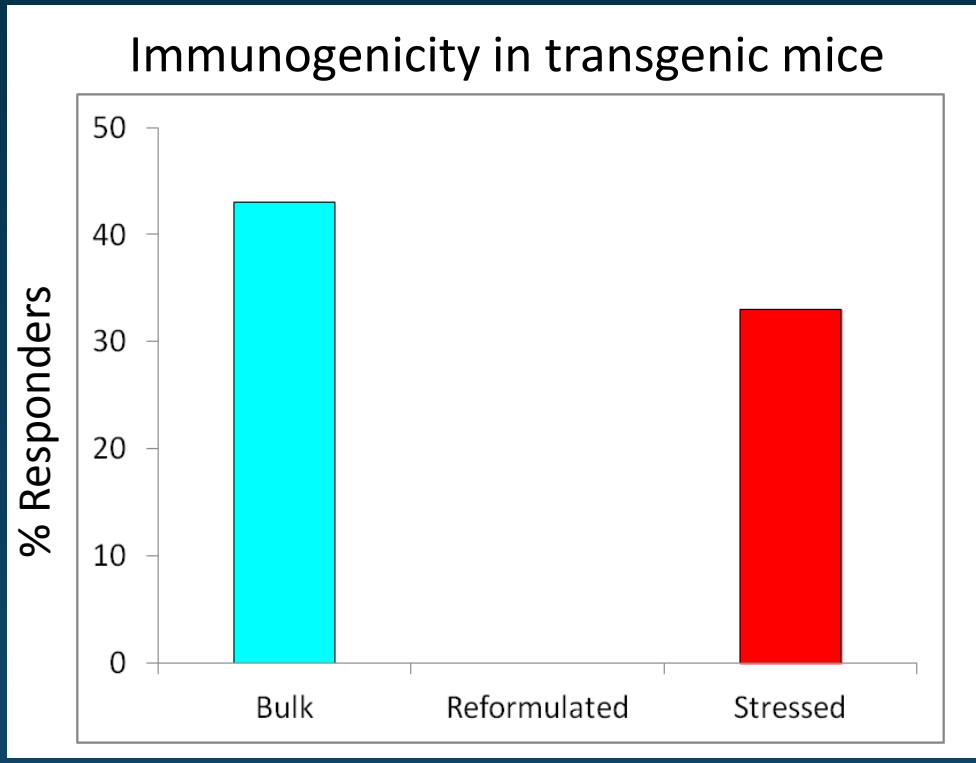
Aggregate size and immunogenicity – is there a link?

Aggregate size range: 6 orders of magnitude!



Subvisible particle counts and rhIFNβ immunogenicity

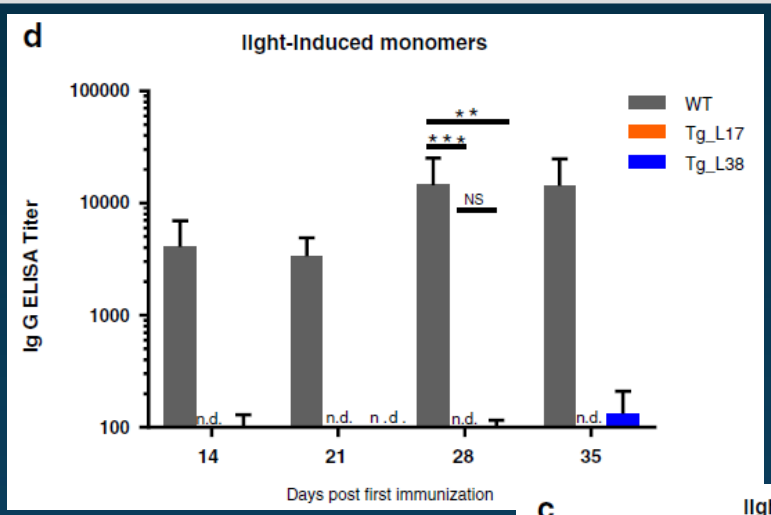


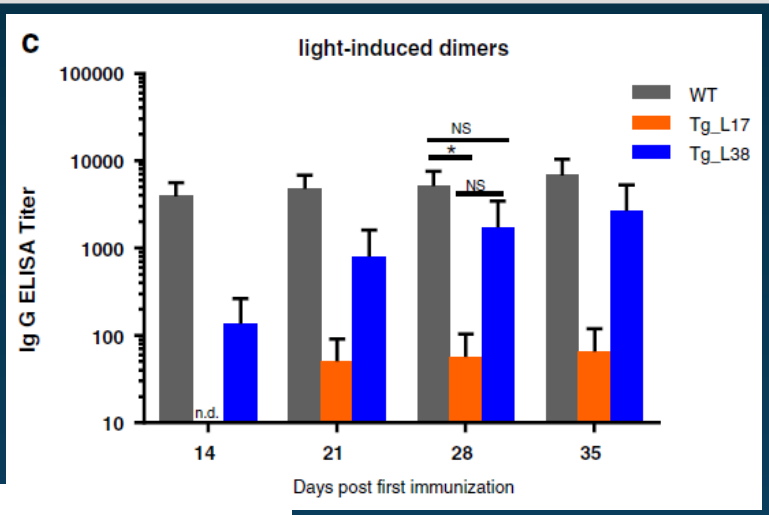


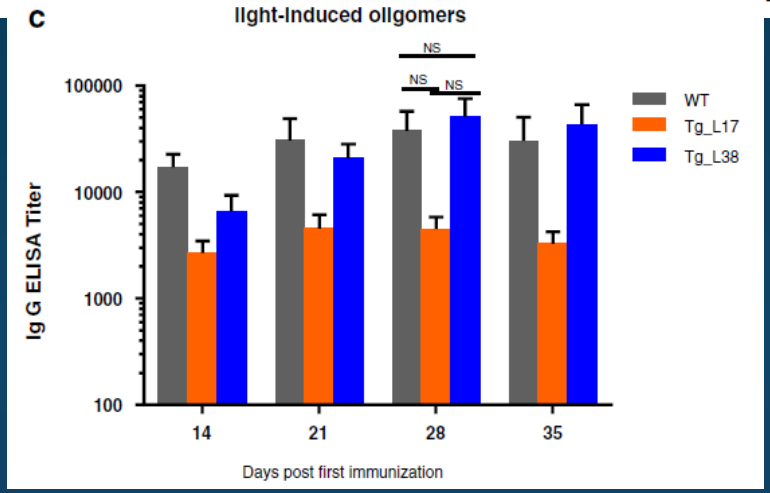
- Virtually particle-free rhIFNβ-1a is non-immunogenic
- Immunogenicity in transgenic immune tolerant mice correlates with subvisible particle counts (rather than total % aggregates)

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

Aggregate size and monoclonal IgG1 immunogenicity







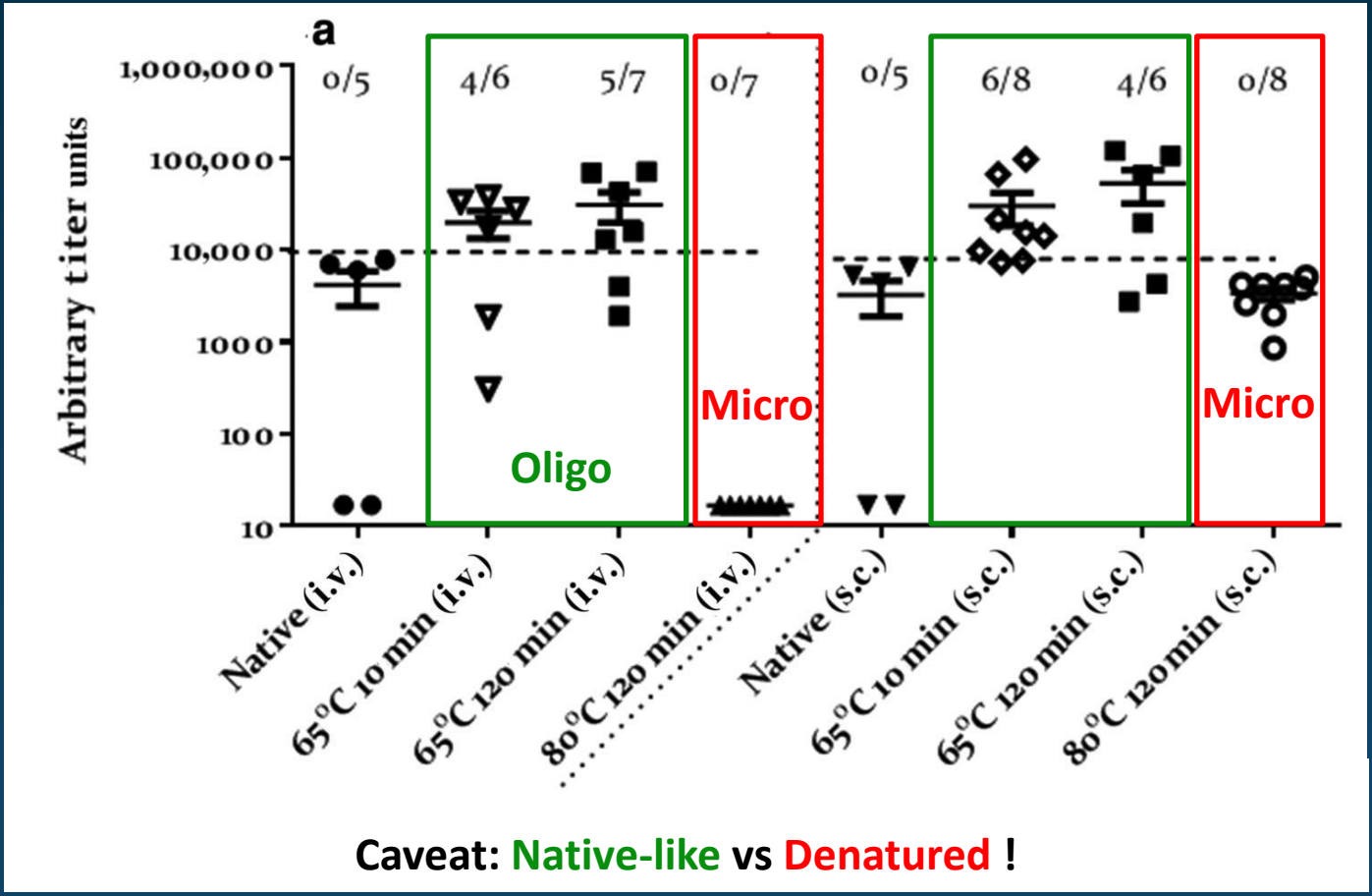
Nano and micro?

Immunogenicity

Size

Bessa et al., Pharm Res 2015

Aggregate size and monoclonal IgG immunogenicity



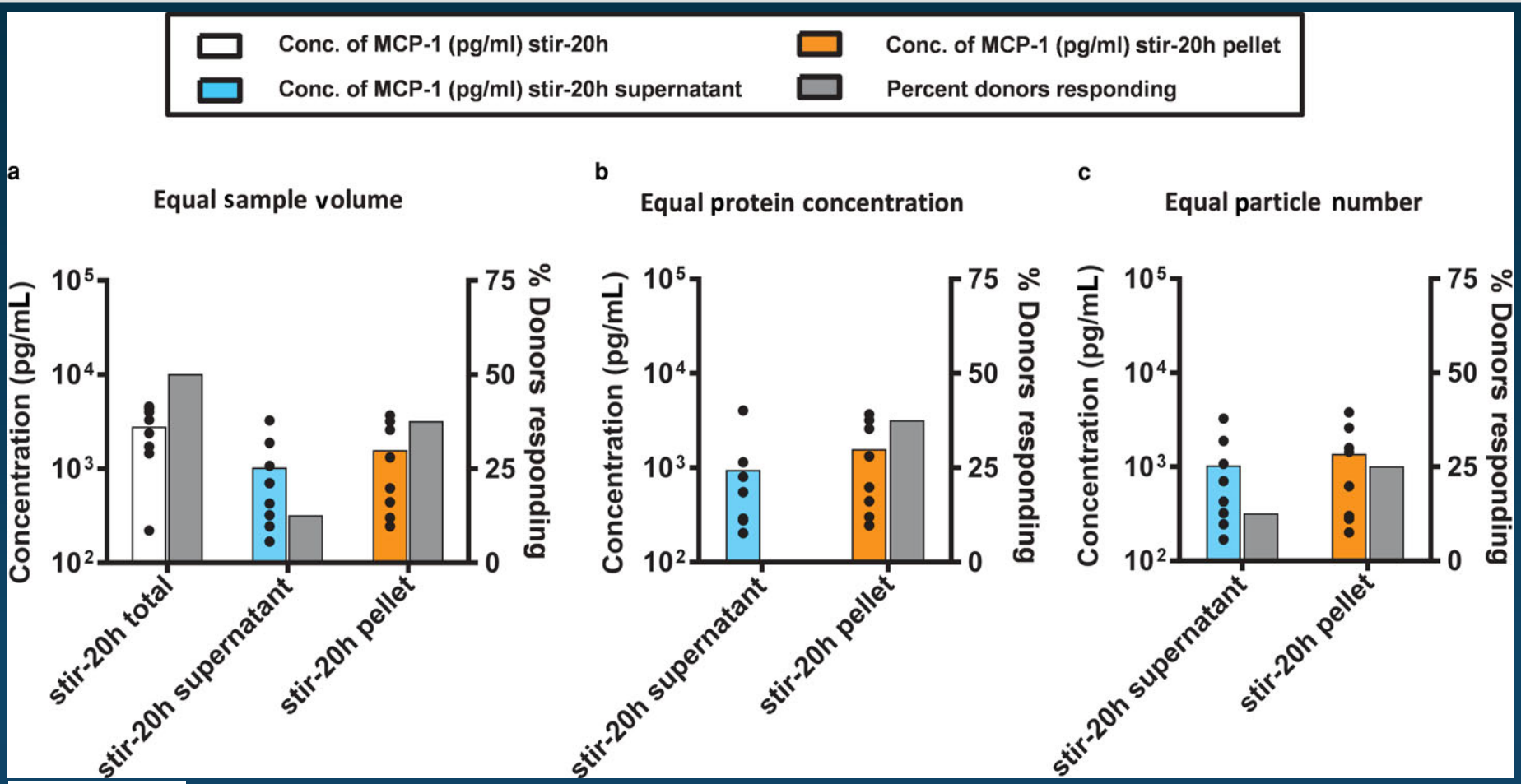
Caveat: Native-like vs Denatured !

Immunogenicity

Size

Fathallah et al., J Pharm Sci 2015

Aggregate size and monoclonal IgG1 immunogenicity



***In vivo*?**

Immunogenicity

Size

Telikepalli et al., J Pharm Sci 2015

Case study

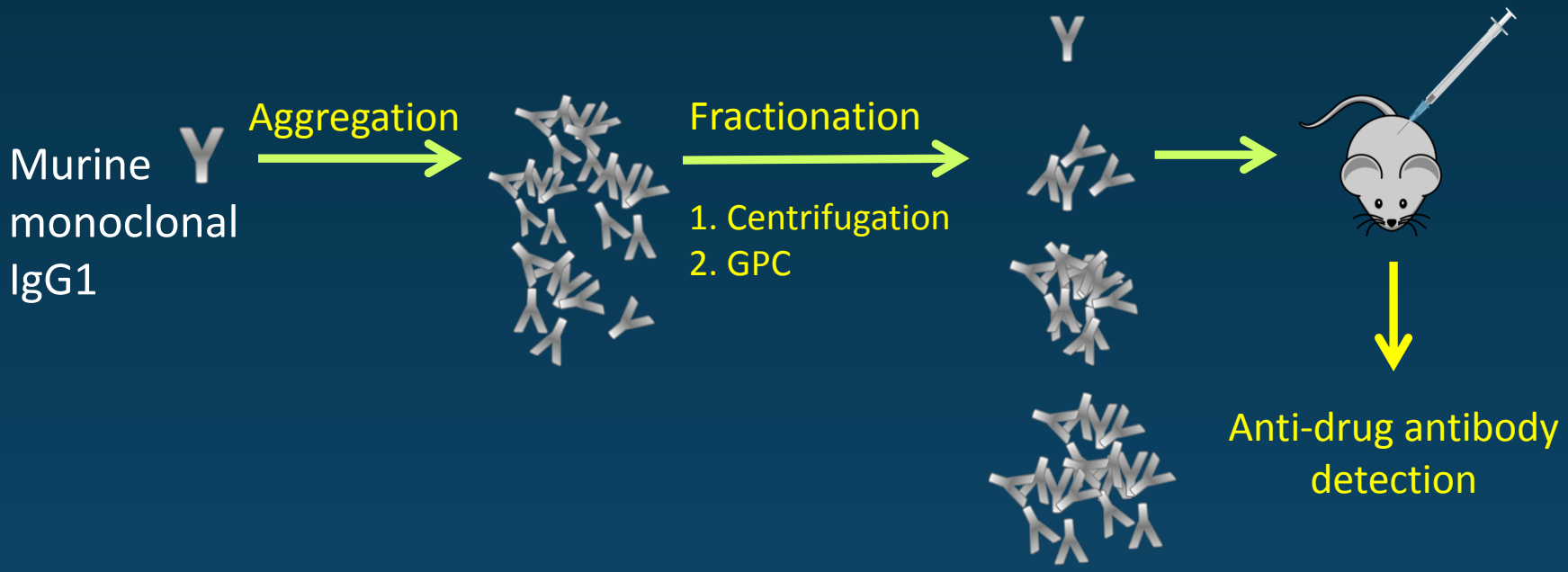
Impact of size of murine monoclonal antibody aggregates on their immunogenicity upon subcutaneous administration in mice

Grzegorz Kijanka, Jared S. Bee, Samuel A. Korman,
Xu Liu, Yuling Wu, Lorin K Roskos,
Mark A. Schenerman, Wim Jiskoot

Kijanka et al., J Pharm Sci 107: 2847-2859 (2018)

Experimental set-up

Stress protocol: pH 4.6, 65˚C, 60 min + stirring (700 rpm, 30 min)

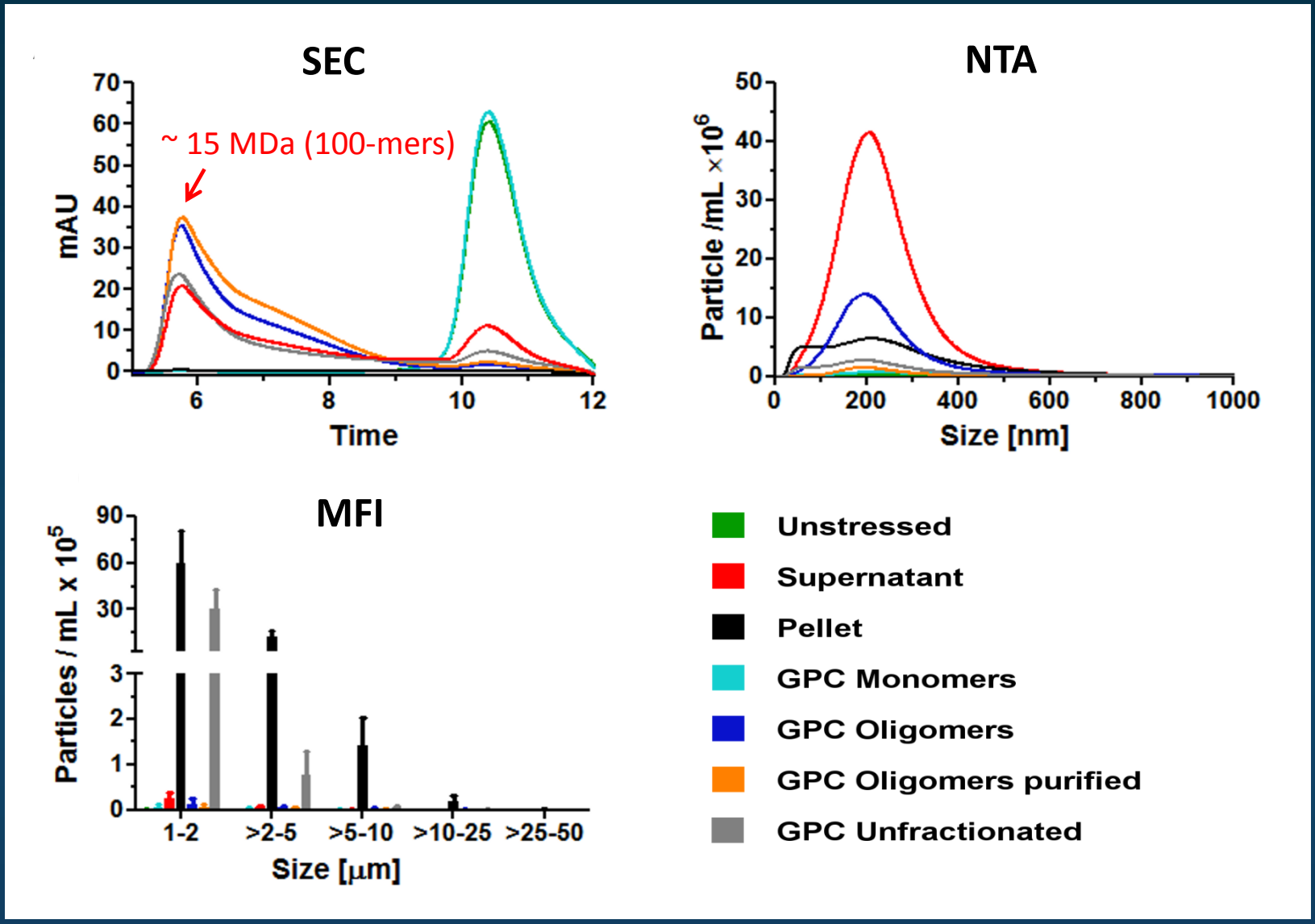


Fractionation: stressed monomers, oligomers, nano-sized aggregates, micron-sized aggregates

Characterization: SEC, SDS-PAGE, Western blotting, DLS, NTA, MFI, fluorescence, CD, MS

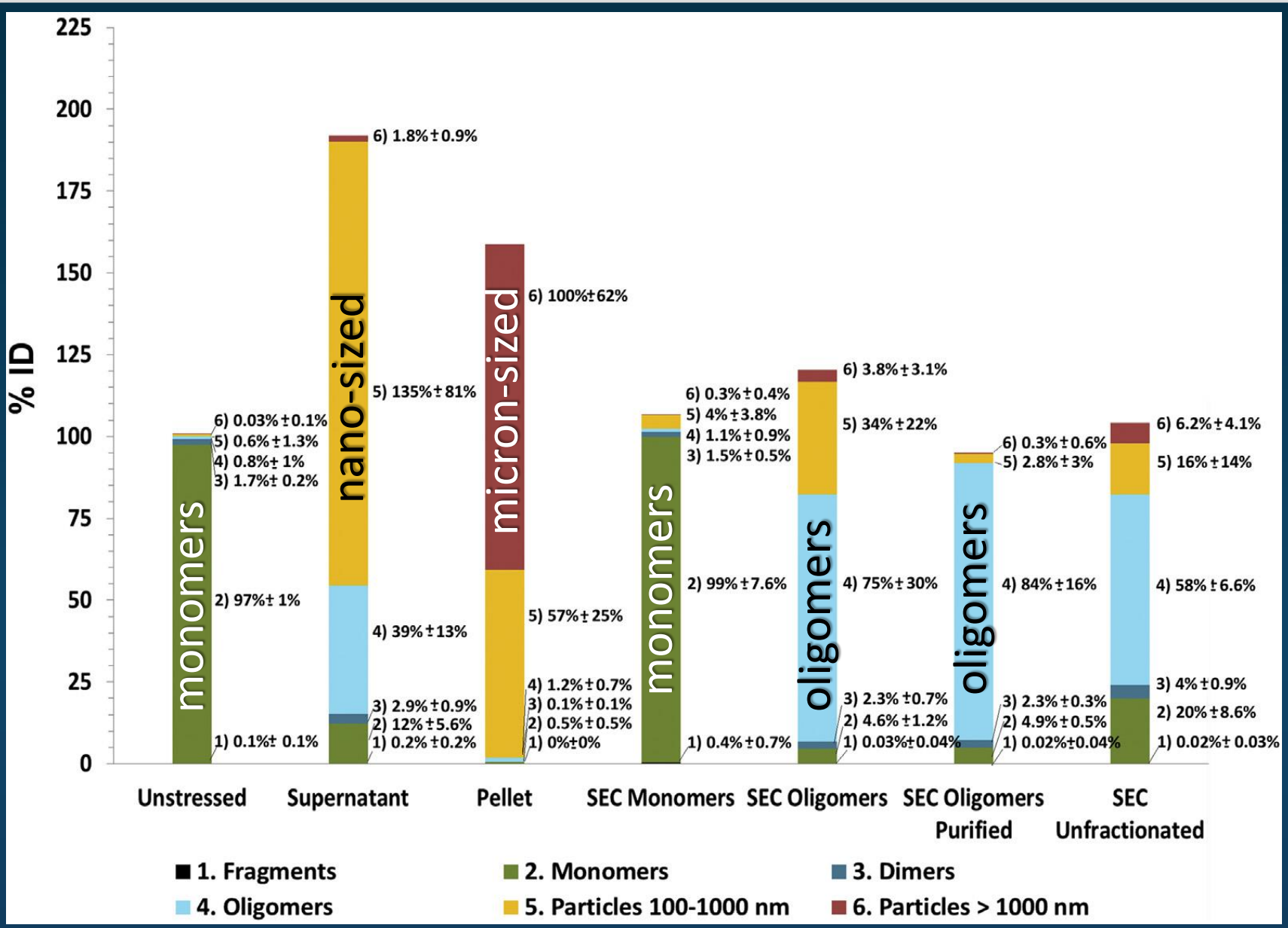
Immunization protocol: 2 subcutaneous injections/week, 8 weeks, 10 µg protein/injection

Aggregate characteristics: size



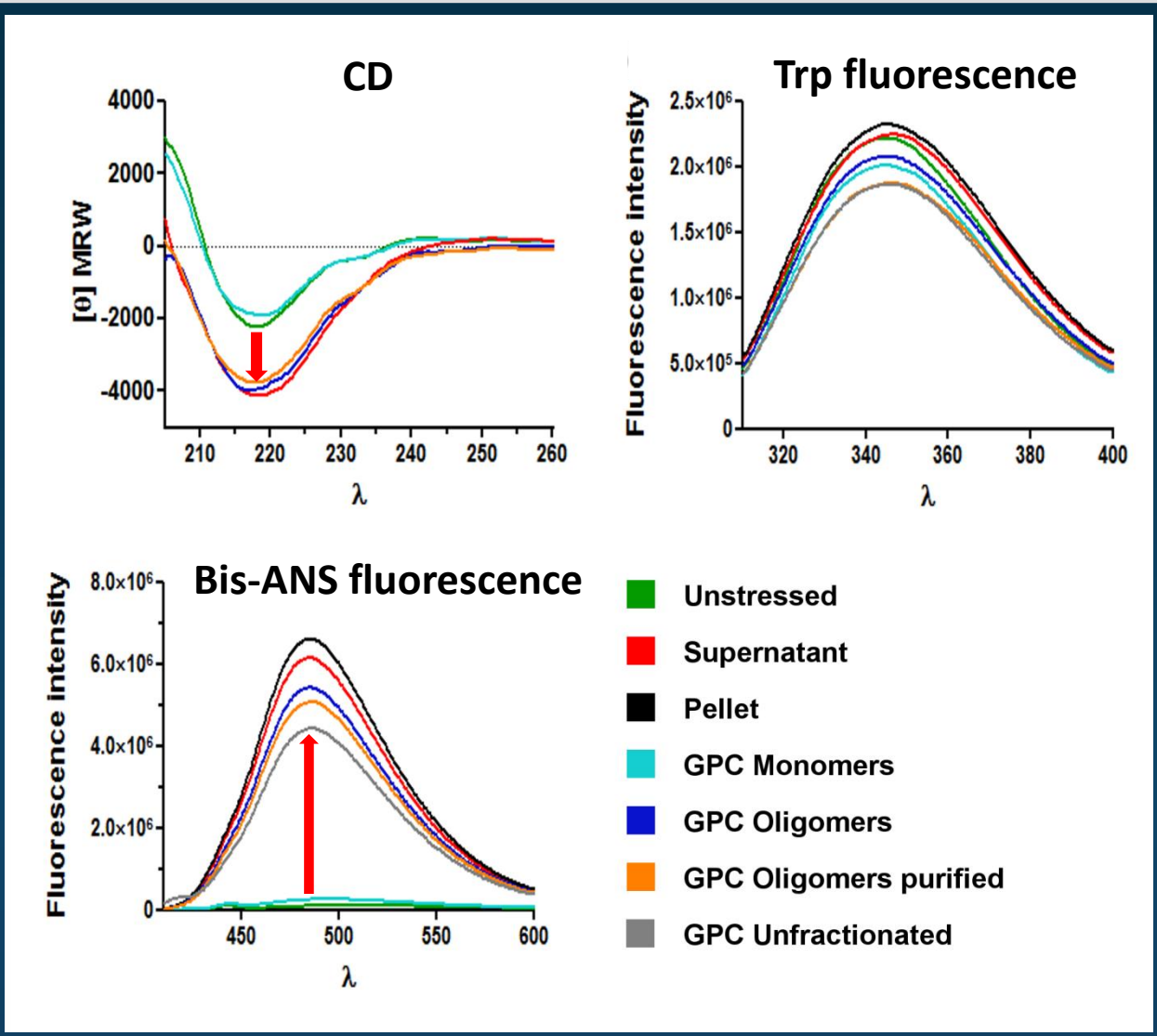
Kijanka et al., J Pharm Sci 107: 2847-2859 (2018)

Aggregate characteristics: estimated mass fractions



Kijanka et al., J Pharm Sci 107: 2847-2859 (2018)

Aggregate characteristics: protein conformation



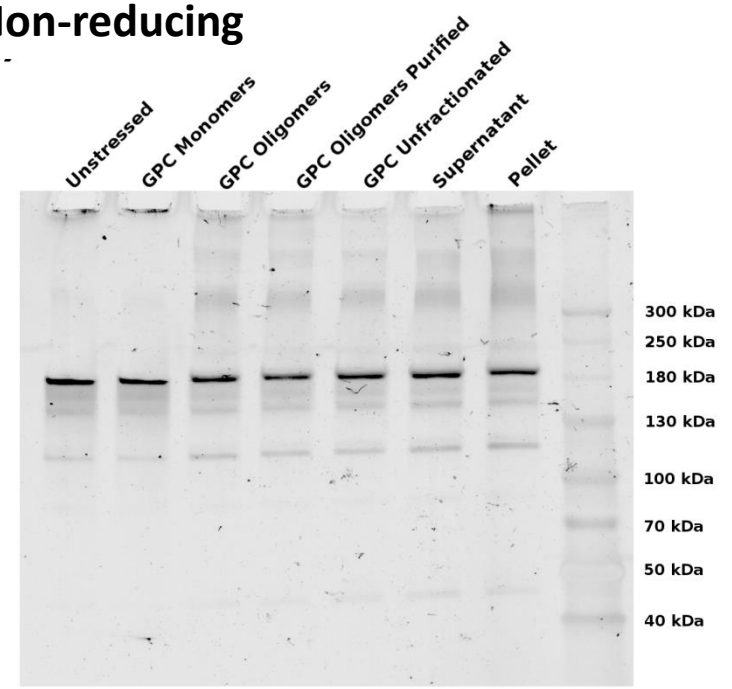
mIgG structure in aggregates altered, not fully denatured

Kijanka et al., J Pharm Sci 107: 2847-2859 (2018)

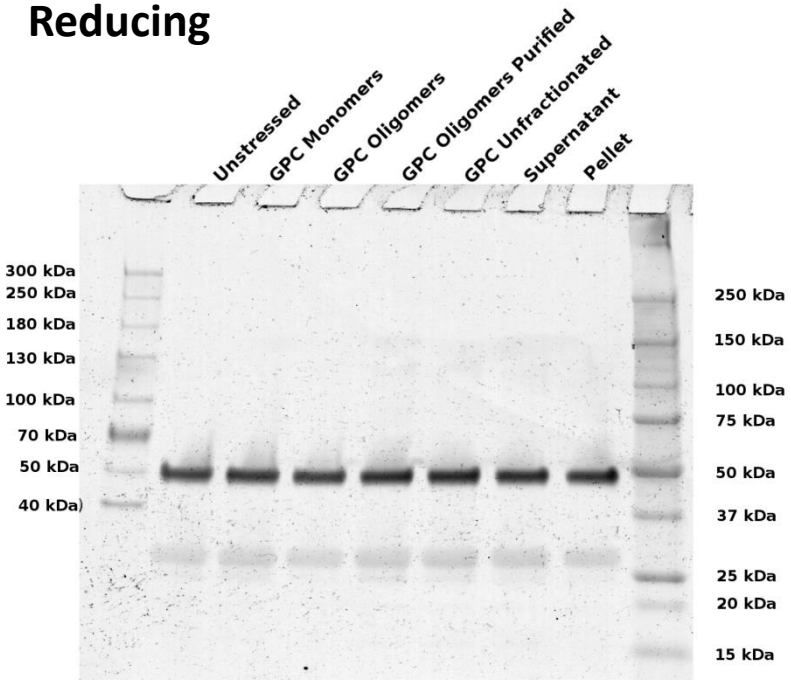
Aggregate characteristics: covalent aggregation

SDS-PAGE

Non-reducing



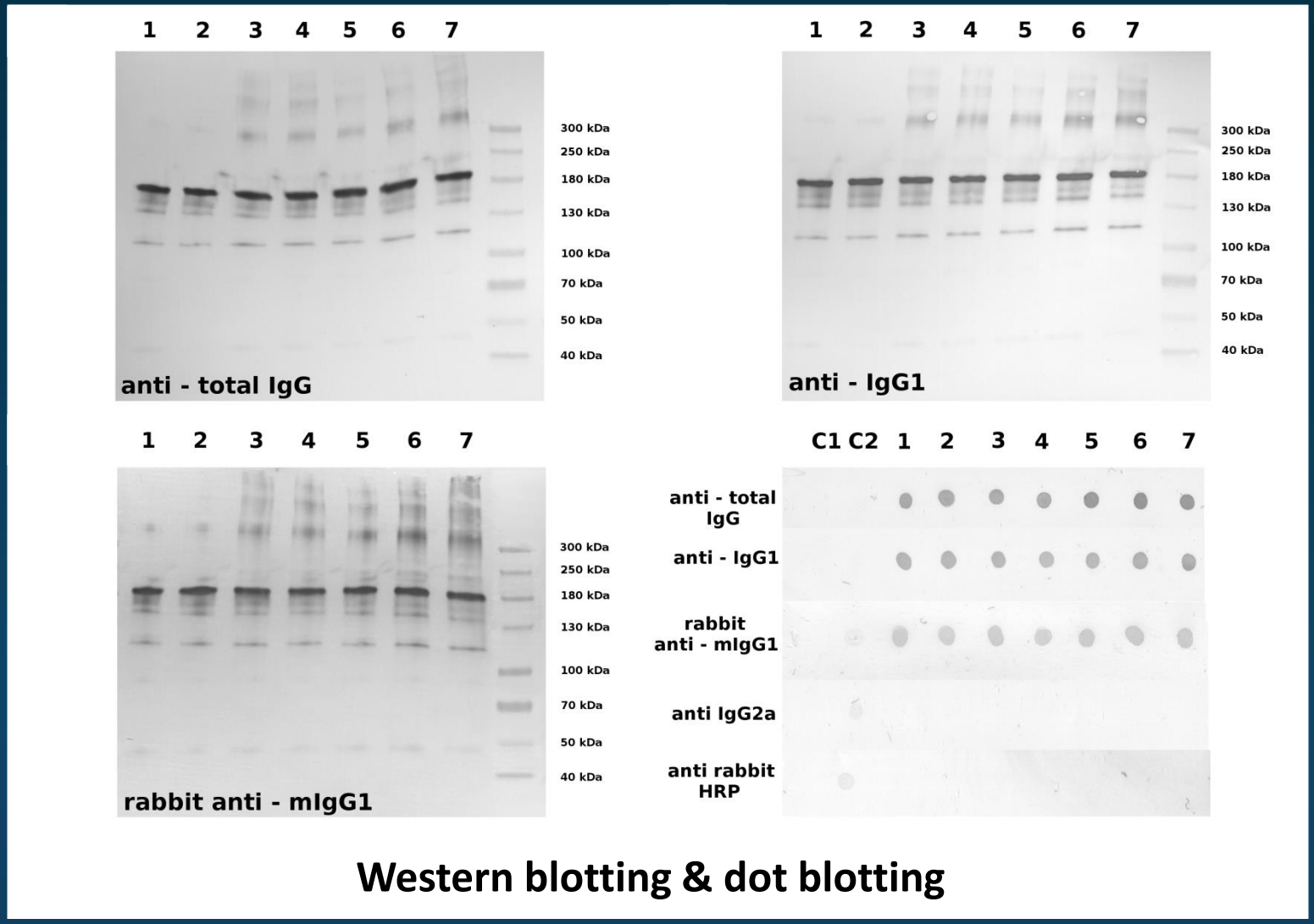
Reducing



Mainly non-covalent, few covalent aggregates

Kijanka et al., J Pharm Sci 107: 2847-2859 (2018)

Aggregate characteristics: antigenicity

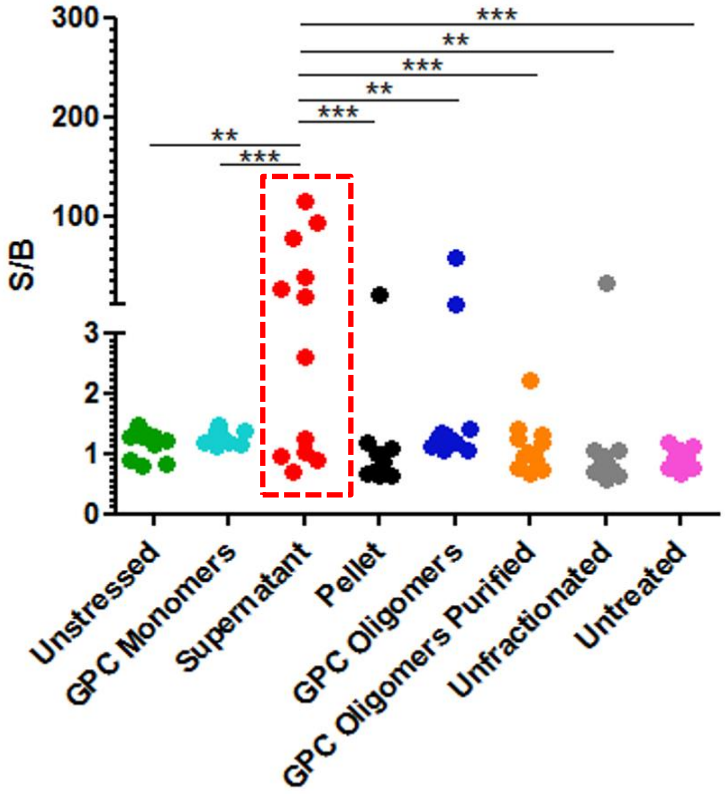


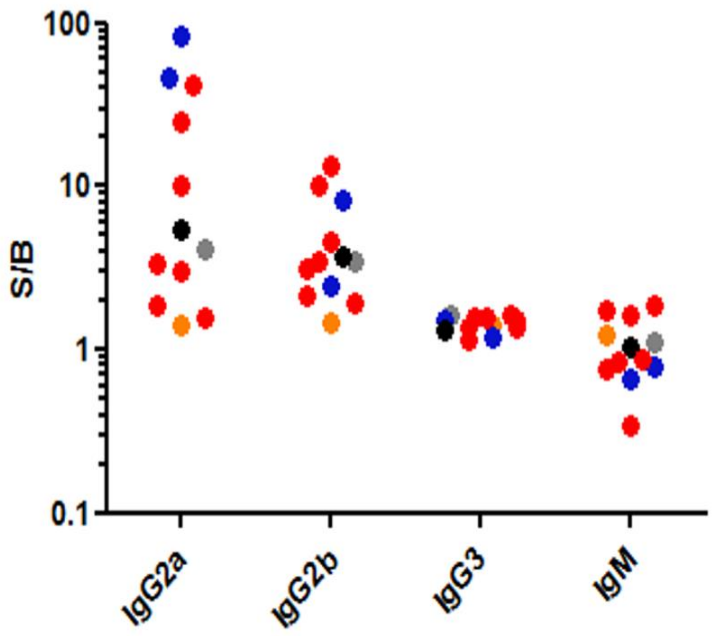
Western blotting & dot blotting

Epitopes preserved in all aggregate fractions

Kijanka et al., J Pharm Sci 107: 2847-2859 (2018)

Immunogenicity





In positive sera, IgG2a and IgG2b were detected (IgG1 was not measured)

Nano-sized aggregates are the most immunogenic

Kijanka et al., J Pharm Sci 107: 2847-2859 (2018)

Follow-up study: are dimers immunogenic?

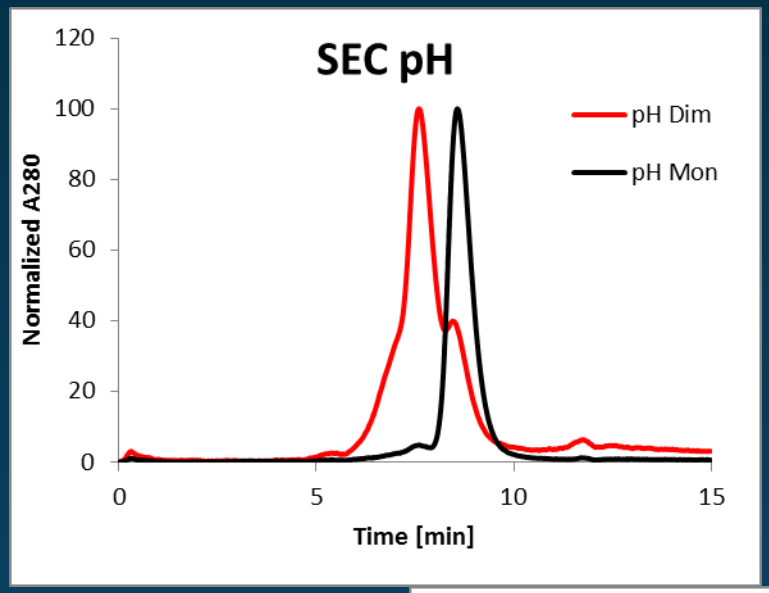
Preparation of dimers by three different stress methods:

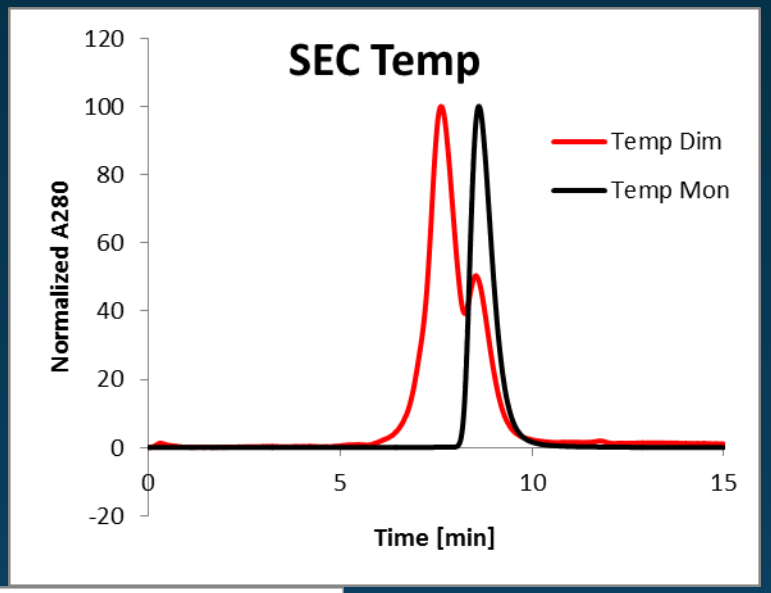
- pH
  - pH 2.5, 1 hour, ambient temperature
- Temperature
  - 65 ˚C, 10 minutes
- Light stress
  - cool white light (13.73 klux) and UV (10.68 W/m^2), 96 h

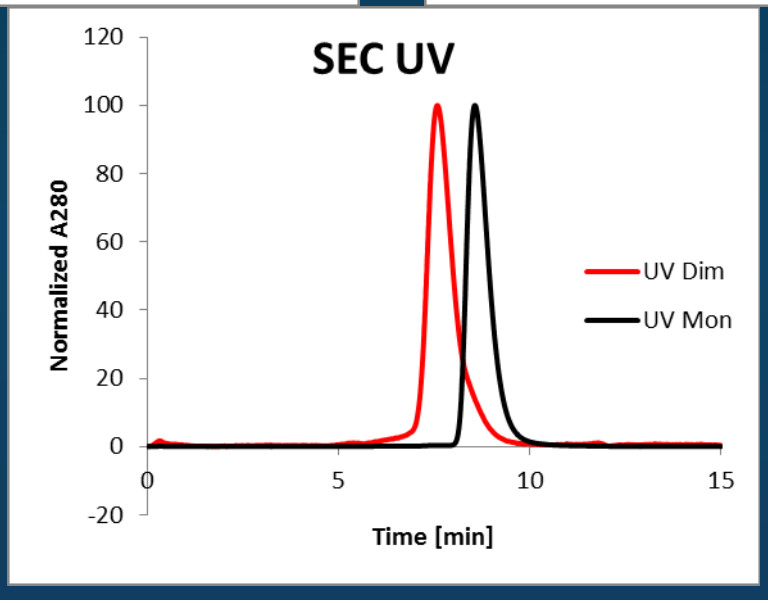
Dimers isolated by preparative SEC (HL Superdex 200 PG)

Kijanka et al., J Pharm Sci , in press (2020)

Characterization of dimers: HP-SEC

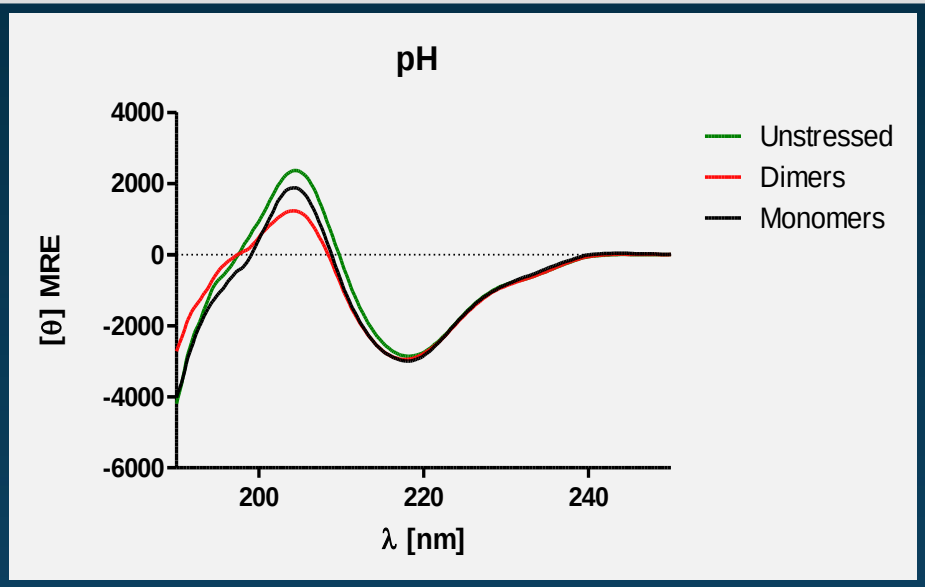


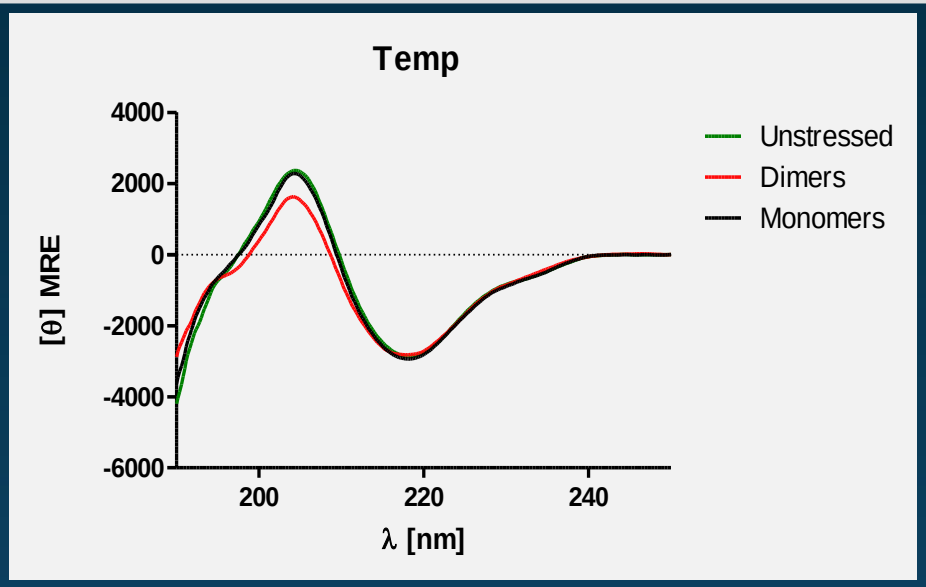


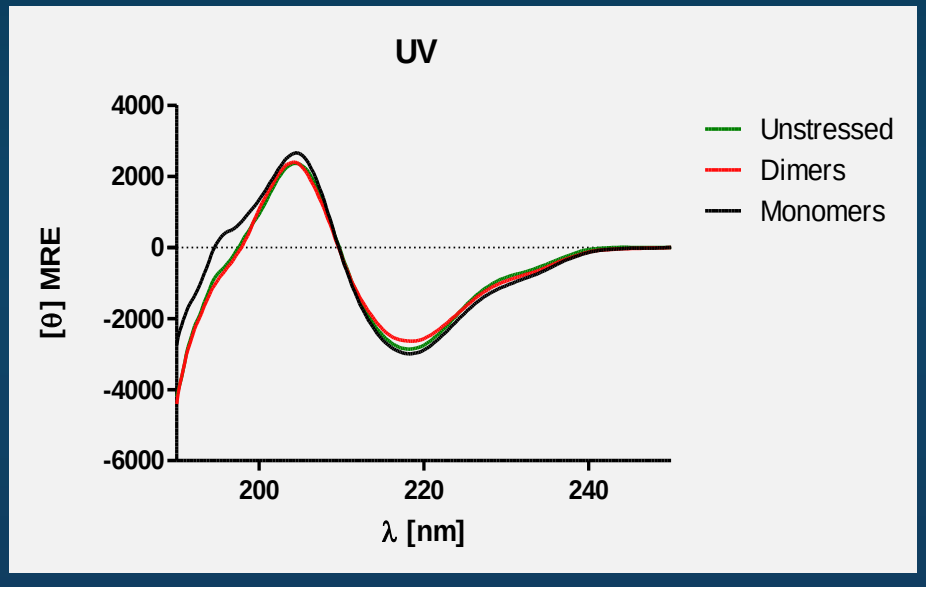


Fractions successfully enriched in stable dimers

Characterization of dimers: far-UV CD

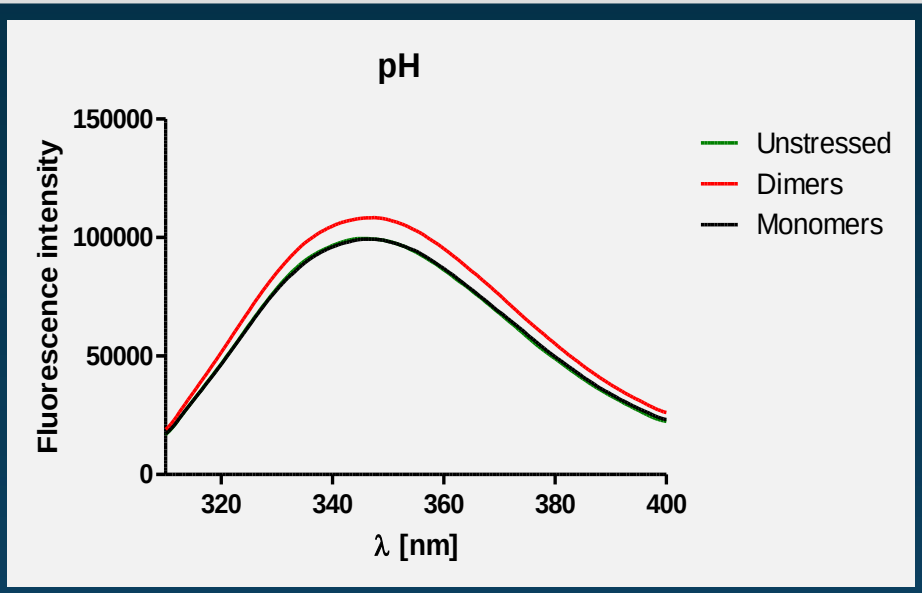


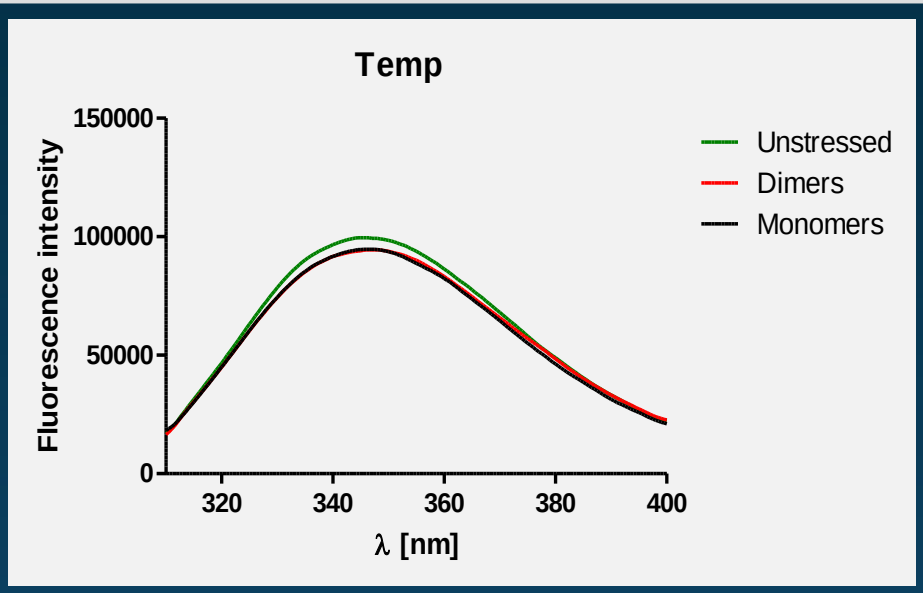


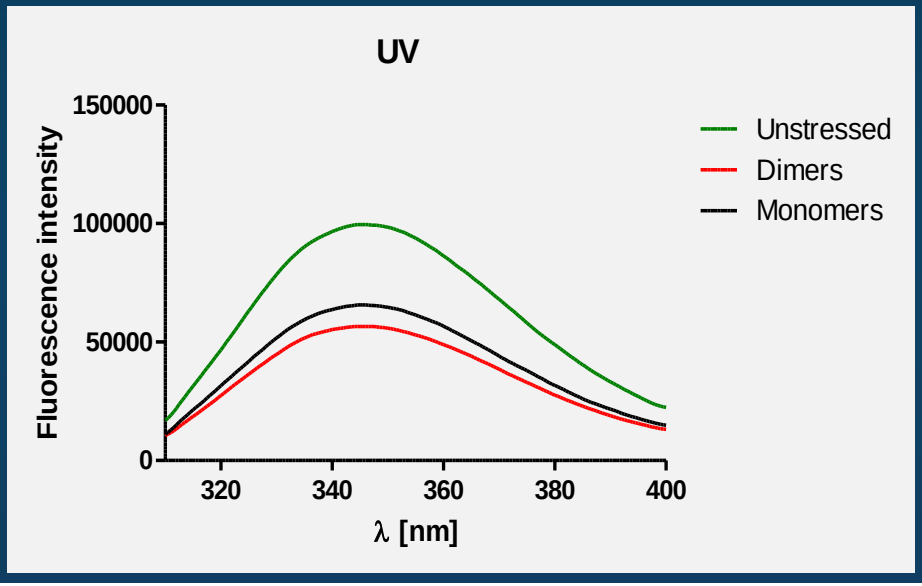


Secondary structure (almost) unaltered

Characterization of dimers: Trp fluorescence

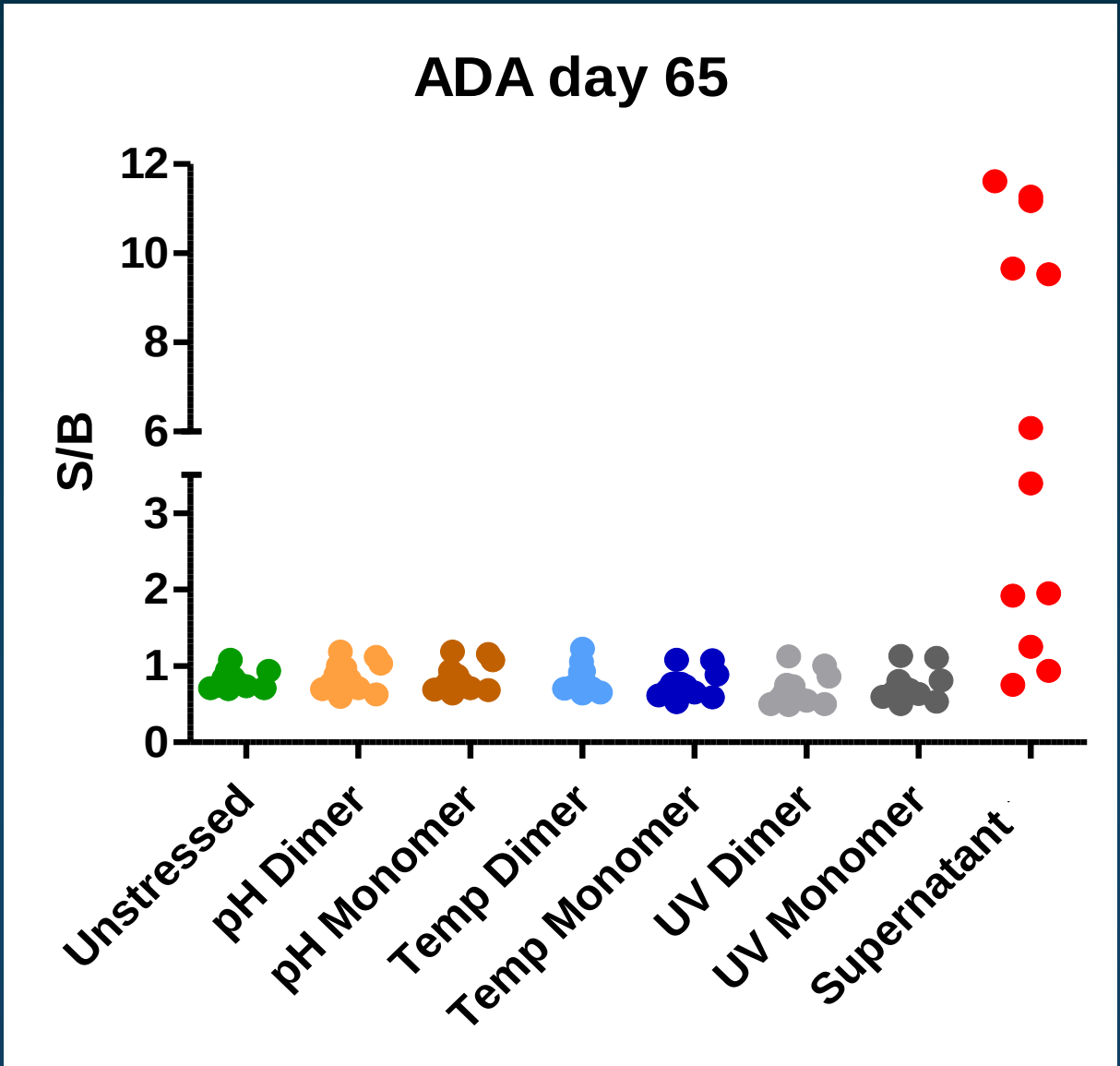






No λ_{max} shifts; intensity reduced in UV stressed mAb

Immunogenicity



Dimers are not immunogenic in our mouse model

Conclusion: size matters!

- In our mouse model, nano-sized aggregates are more immunogenic than micron-sized aggregates or oligomers
- Dimers are not immunogenic in the same mouse model
- But, there is more than size alone…. other aggregate attributes may be equally (or more) important

For comparison:

- Collective studies from the vaccine delivery literature suggest nanoparticles between ca. 20 nm and a few hundred nm (up to a few µm) to be the most effective particulate adjuvants
- But, different types of nanoparticles have widely different levels of adjuvant activity

Conclusion: size matters!

Journal of Controlled Release 234 (2016) 124–134

Review article

Orchestrating immune responses: How size, shape and rigidity affect the immunogenicity of particulate vaccines

Naomi Benne [a,c,1], Janine van Duijn [b,c,1], Johan Kuiper [b,c], Wim Jiskoot [a,c], Bram Slütter [a,b,c,*]

Micron-sized particles can be taken up through receptor-mediated endocytosis and phagocytosis, but their size may restrict macropinocytosis. Dendritic cells (DC) have an exceptional capacity for macropinocytosis [4] and therefore may favor the uptake of nanoparticles over microparticles.[5] Although microparticles are taken up by DC as well, macrophages effectively take up microparticles, especially in the 2 – 3 μm range,[6] the curvature of which corresponds with that of the macrophage's membrane ruffles.[7]


Andhyk Halim
Andrea Hawe
Daan Crommelin
Daniel Weinbuch
Grzegorz Kijanka
Huub Schellekens
Jared Bee & colleagues
John Carpenter
Linda Narhi
Mark Fogg
Matthew Baker
Melody Sauerborn
Miranda Van Beers
Riccardo Torosantucci
Suzanne Hermeling
Ted Randolph
Vasco Filipe
Vera Brinks
LACDR
Coriolis Pharma
Biopharmaceutical Research and Development Service

Thank you!