Immunogenicity of protein aggregates:

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Coriolis Pharma
Biopharmaceutical Research and Development Service

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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)

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

Aggregate Size May Affect Immunogenicity

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

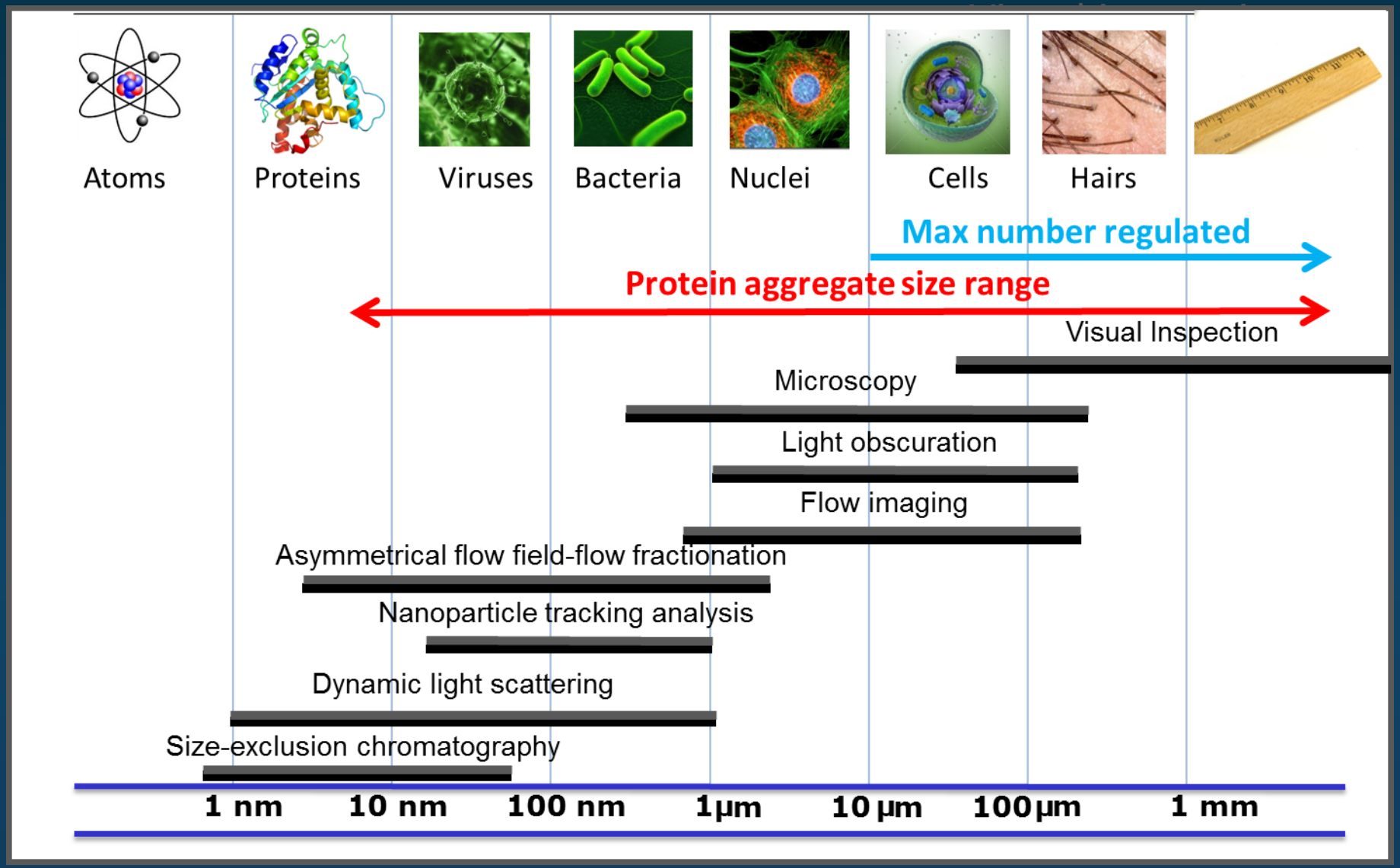
Dose and Dosing Schedule Affect Immunogenicity

Administration Route Affects Immunogenicity

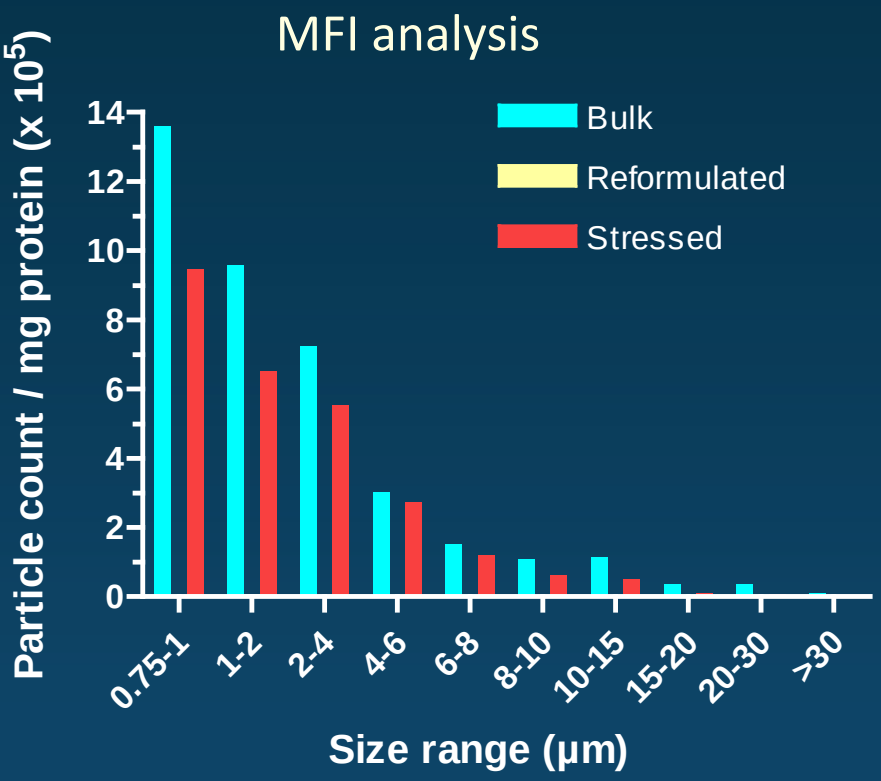
Immune Mechanisms Are Not Yet Fully Understood

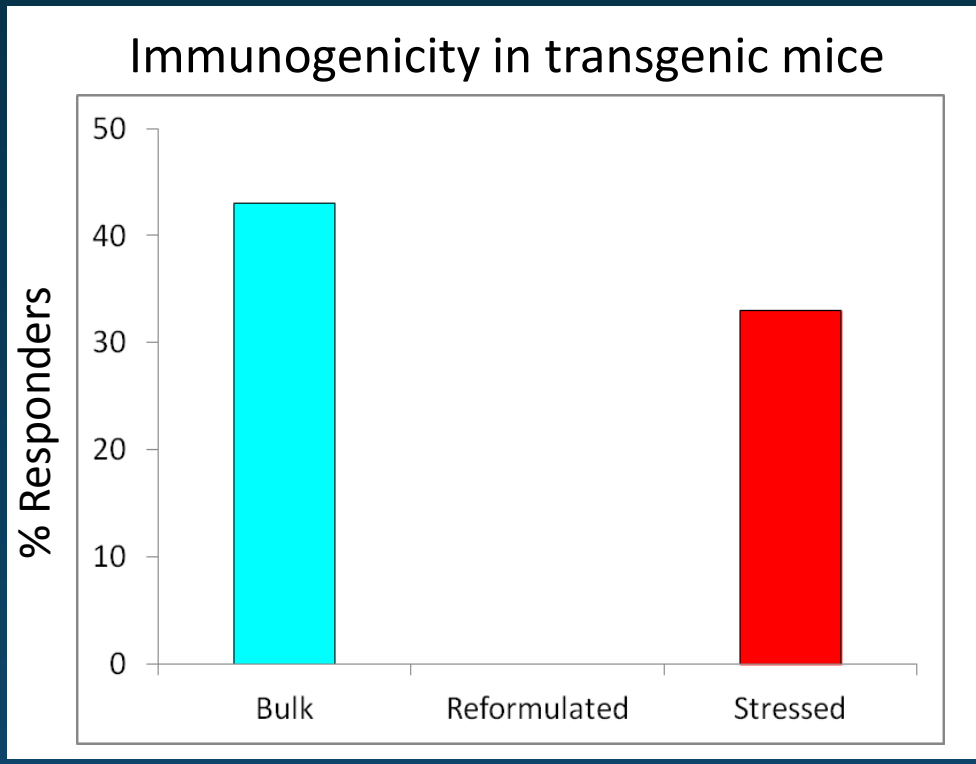
Aggregate size and immunogenicity – is there a link?

Aggregate size range: 6 orders of magnitude!



Subvisible particle counts and rhIFNβ immunogenicity

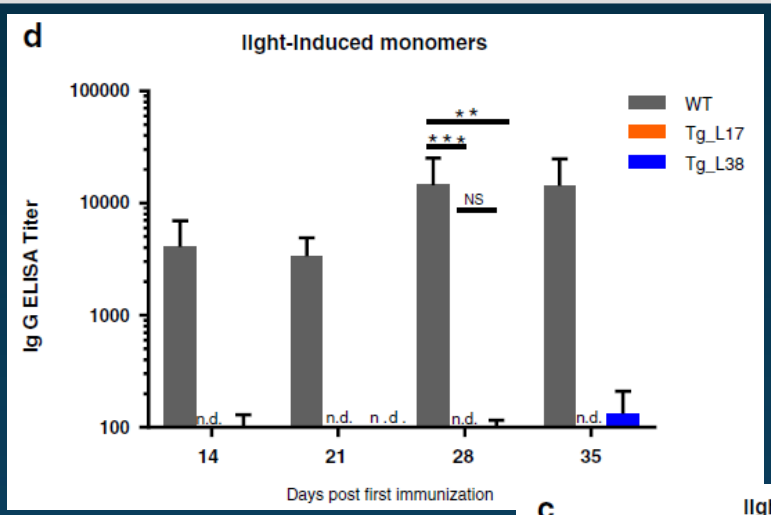


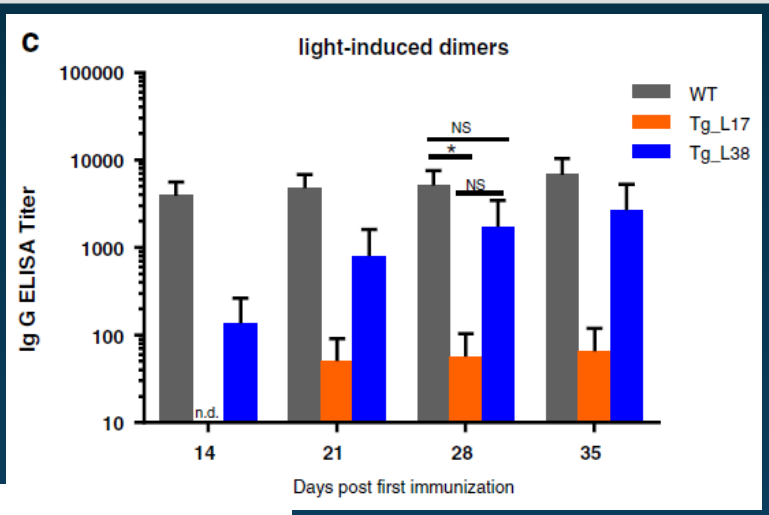


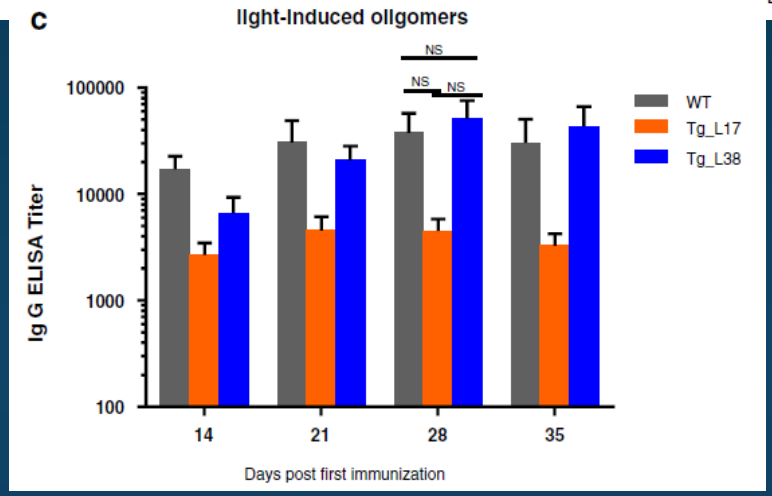
- Virtually particle-free rhIFNβ-1a is non-immunogenic
- Immunogenicity in transgenic immune tolerant mice correlates with subvisible particle counts (rather than total % aggregates)

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

Aggregate size and monoclonal IgG1 immunogenicity







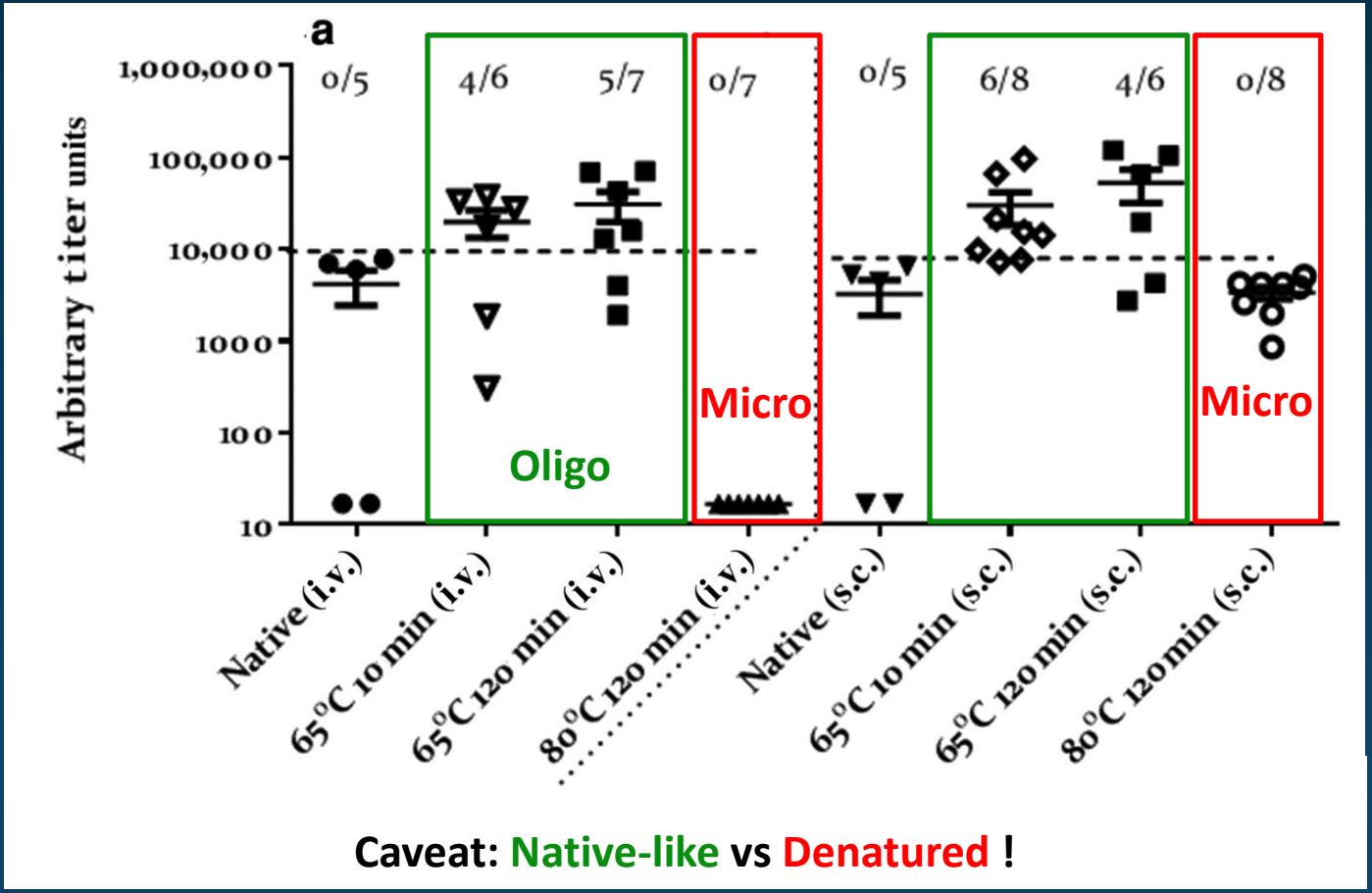
Nano and micro?

Immunogenicity

Size

Bessa et al., Pharm Res 2015

Aggregate size and monoclonal IgG immunogenicity



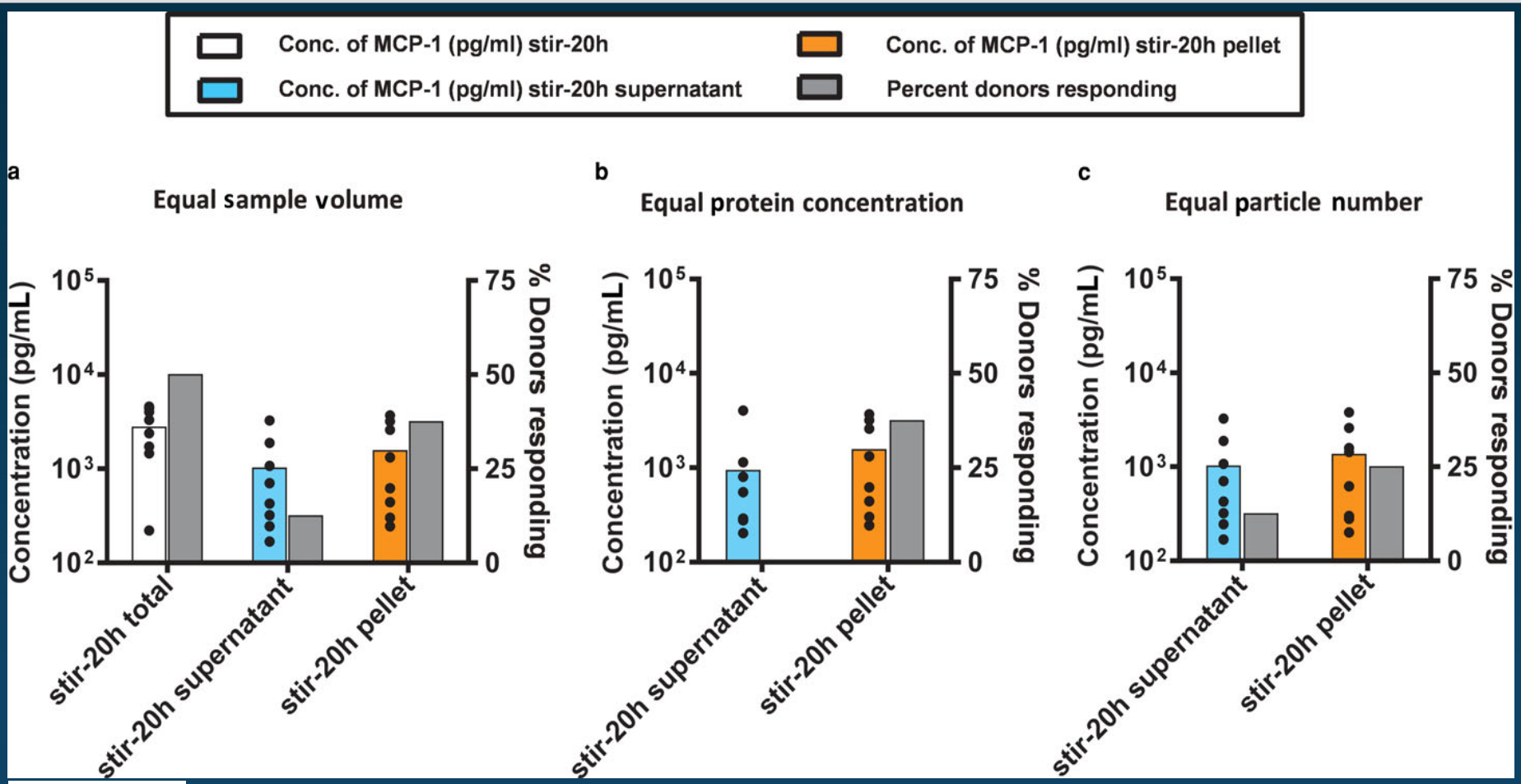
Caveat: Native-like vs Denatured !

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

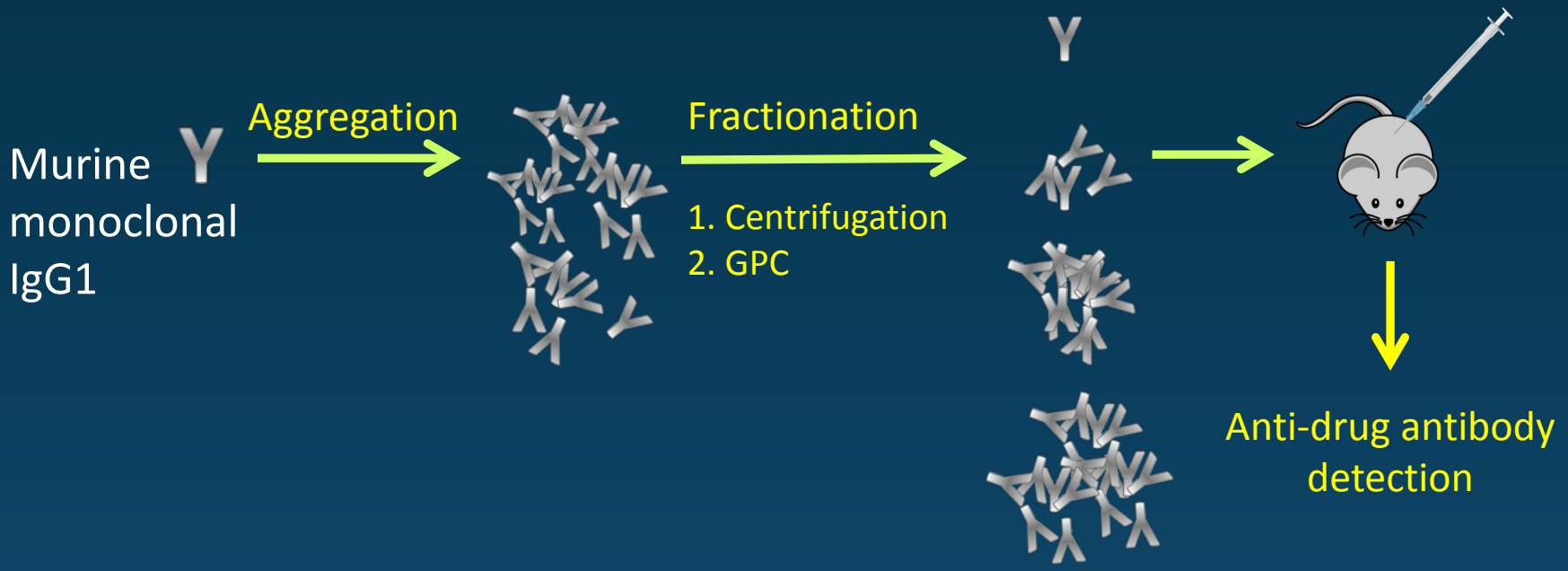
Unpublished study

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

Experimental set-up

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

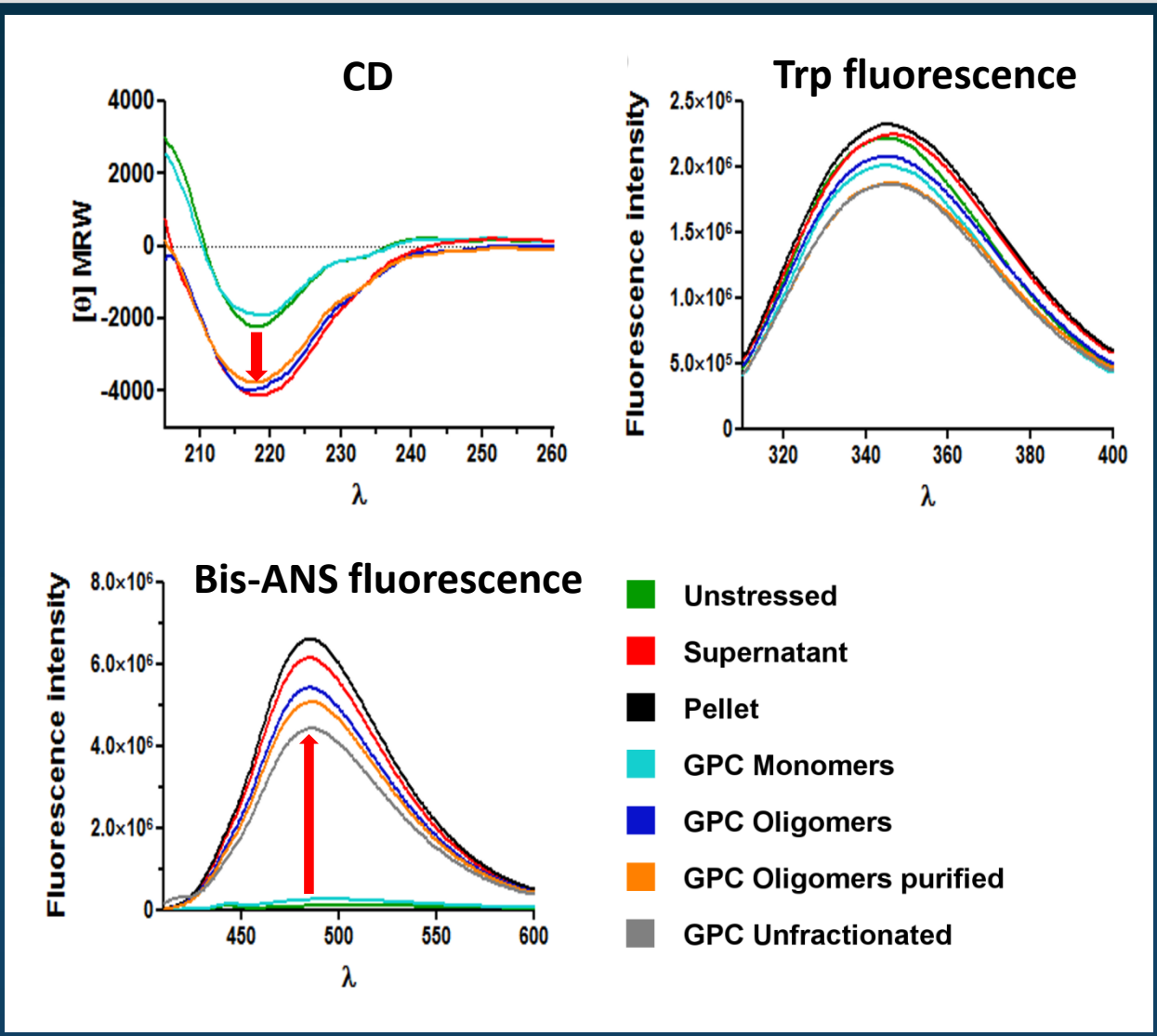


Fractionation: stressed monomers, oligomers, nano-sized aggregates, micron-sized aggregates

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



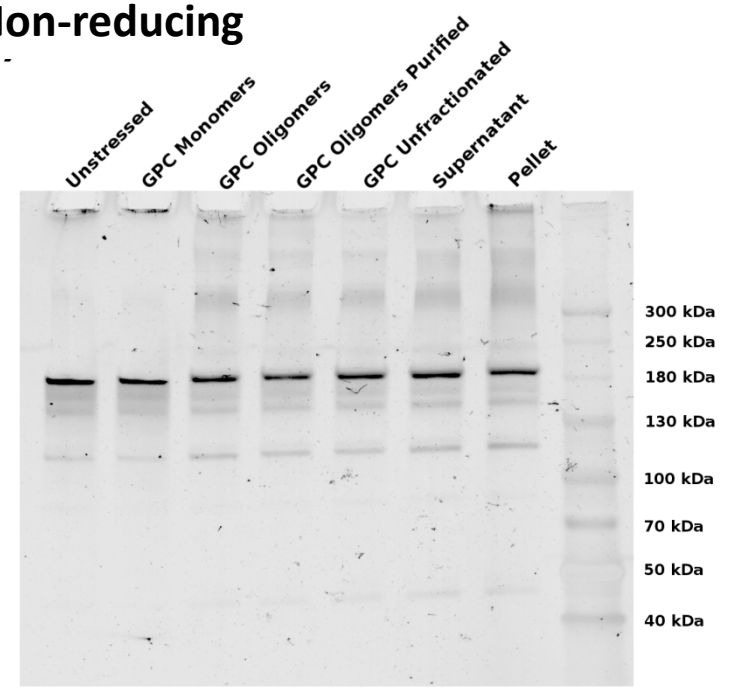
mIgG structure in aggregates altered, not fully denatured

Kijanka et al., unpublished data

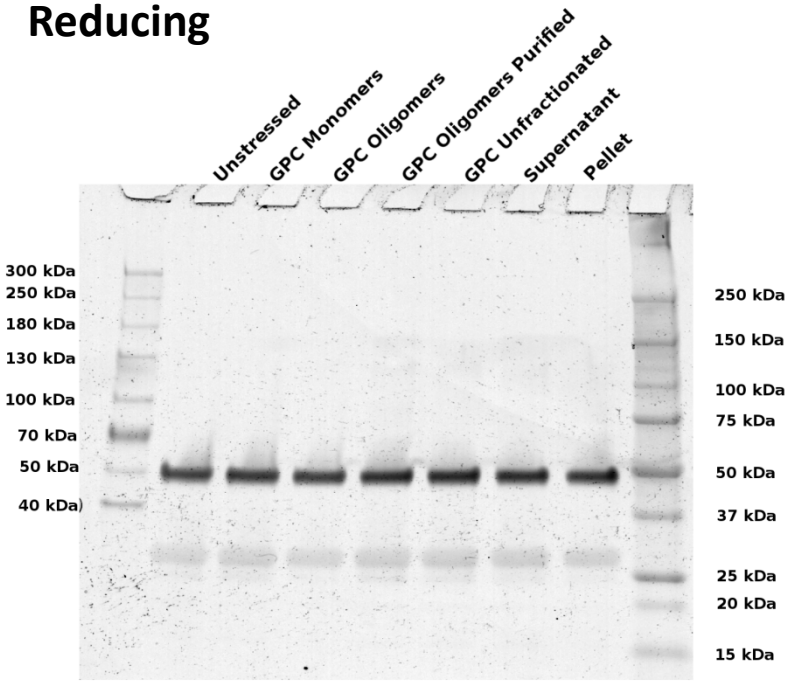
Aggregate characteristics

SDS-PAGE

Non-reducing



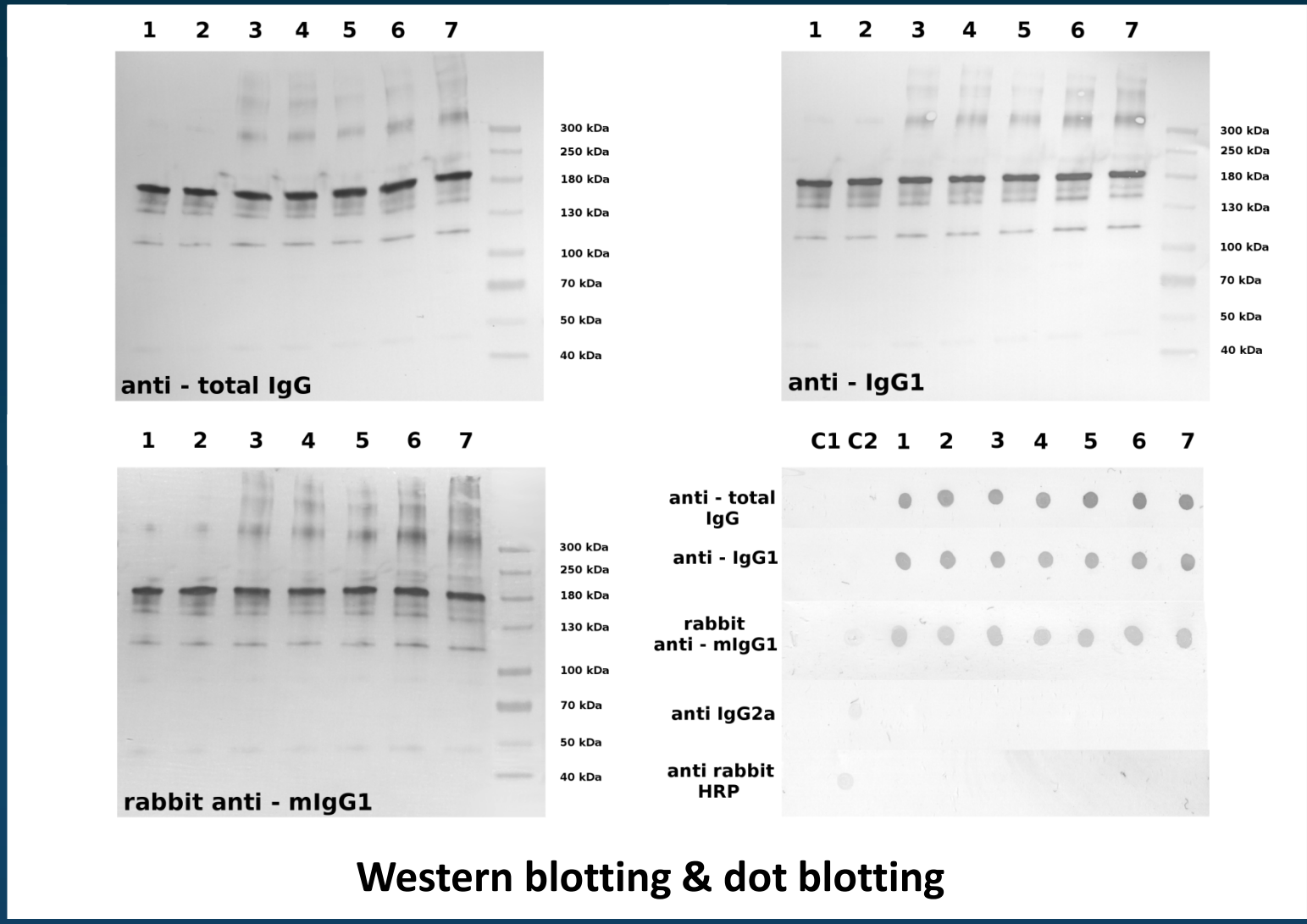
Reducing



Mainly non-covalent, few covalent aggregates

Kijanka et al., unpublished data

Aggregate characteristics

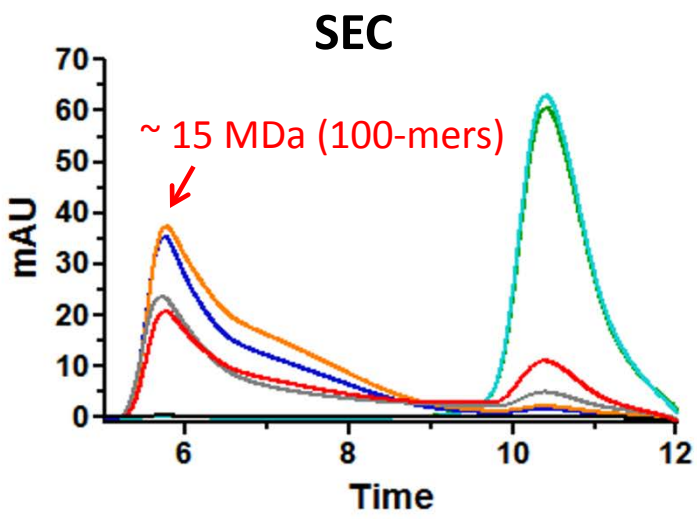


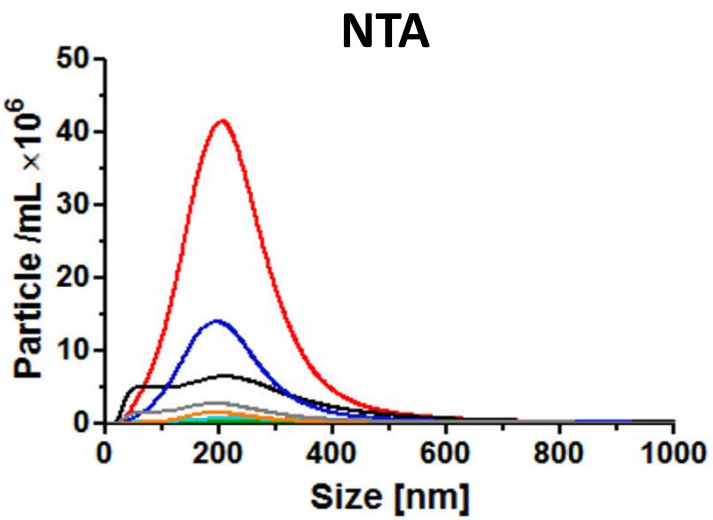
Western blotting & dot blotting

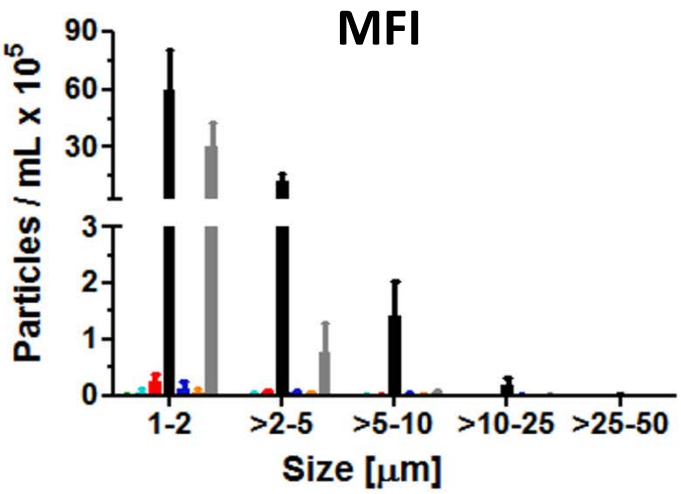
Epitopes preserved on aggregates

Kijanka et al., unpublished data

Aggregate characteristics



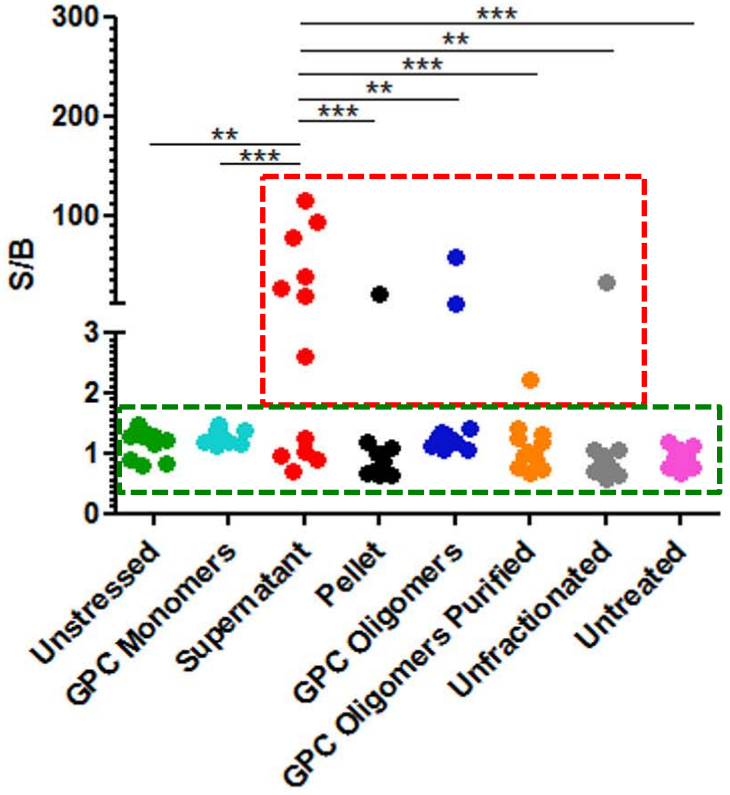




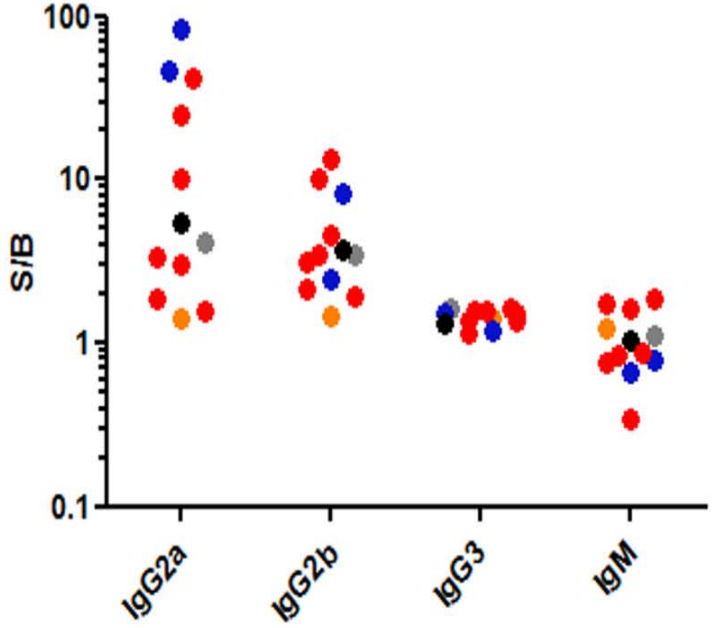
Kijanka et al., unpublished data

Immunogenicity

Anti-drug antibodies



Antibody isotypes



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

Nano-sized aggregates were the most immunogenic

Kijanka et al., unpublished data

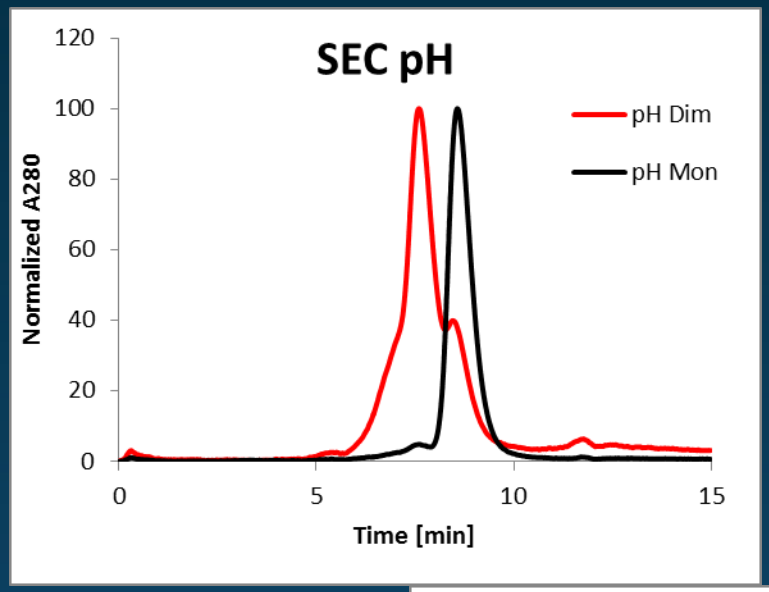
Follow-up study: are dimers immunogenic?

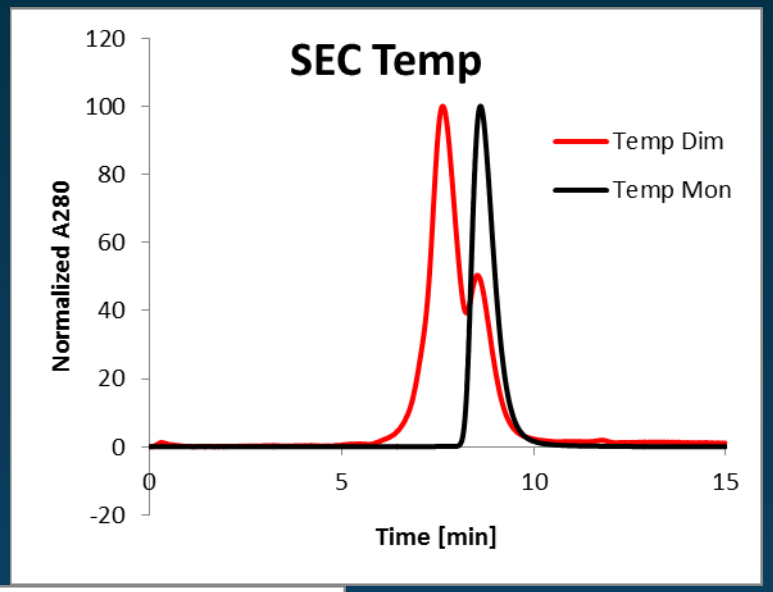
Preparation of dimers by three different stress methods:

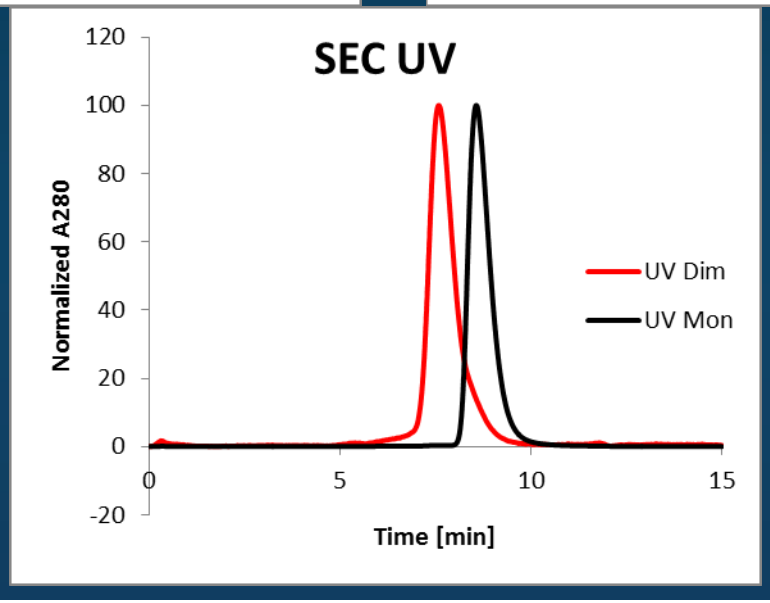
- pH
  - pH 2.5, 1 hour, ambient temperature
- Temperature
  - 65 ˚C, 10 minutes
- Light stress
  - cool white light (13.73 klux) and UV (10.68 W/m^2), 96 h

Dimers isolated by SEC (High Load Superdex 200 PG)

Characterization of dimers: HP-SEC

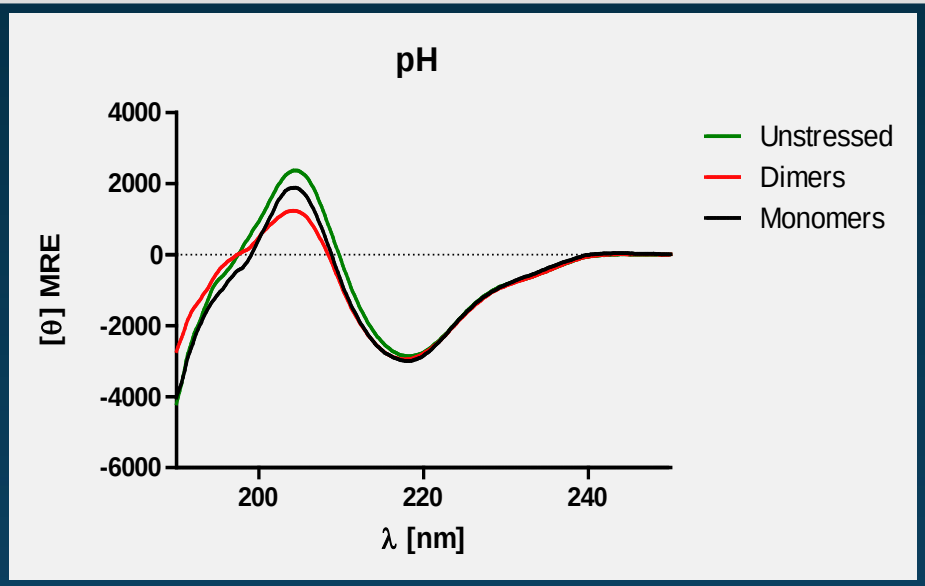


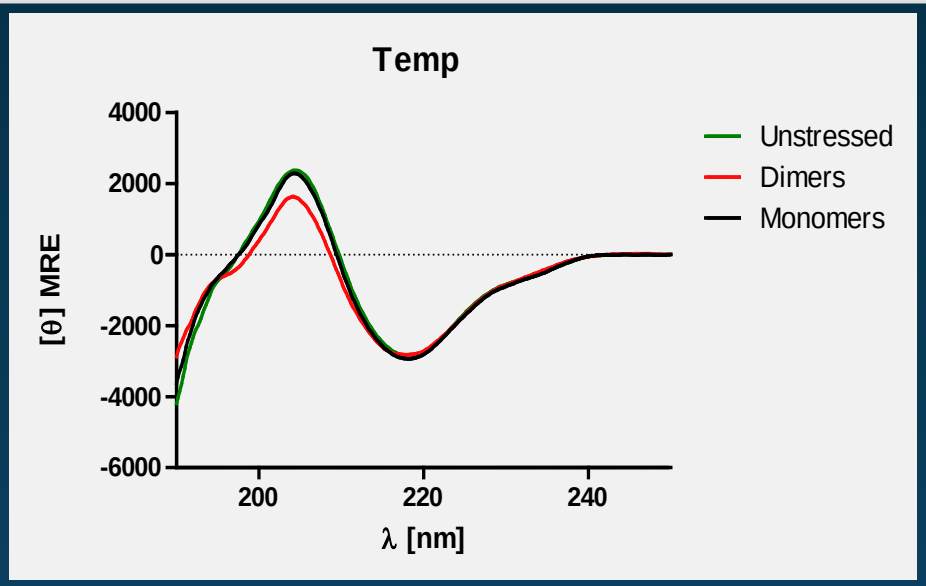


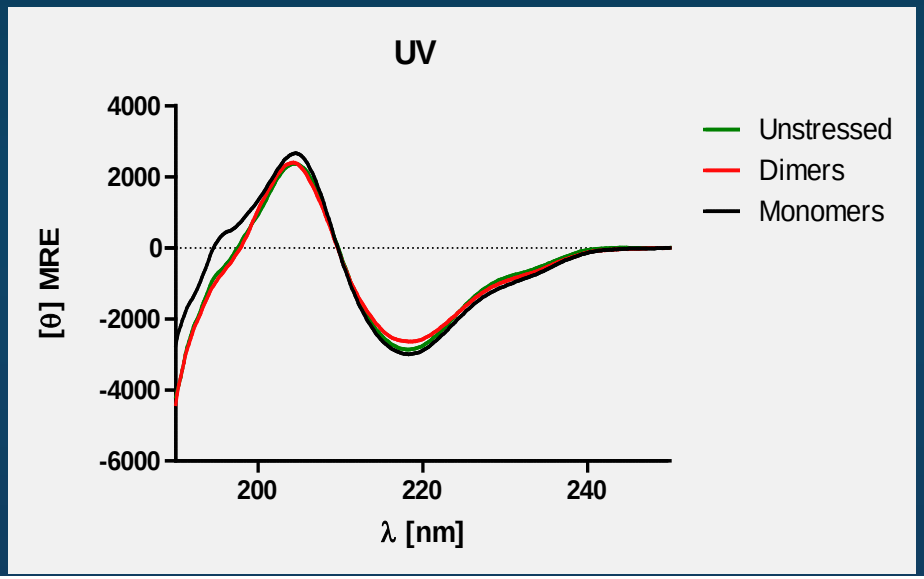


Fractions successfully enriched in dimers

Characterization of dimers: far-UV CD

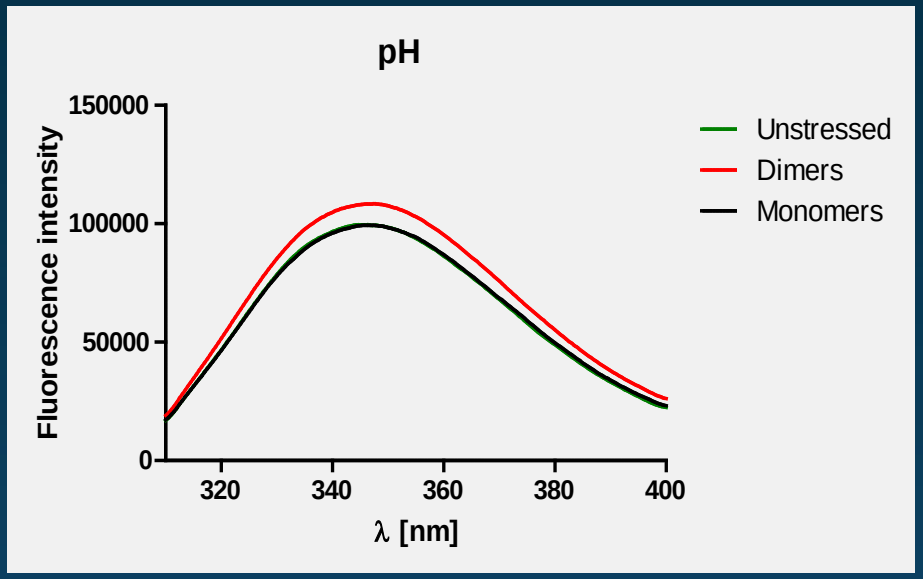


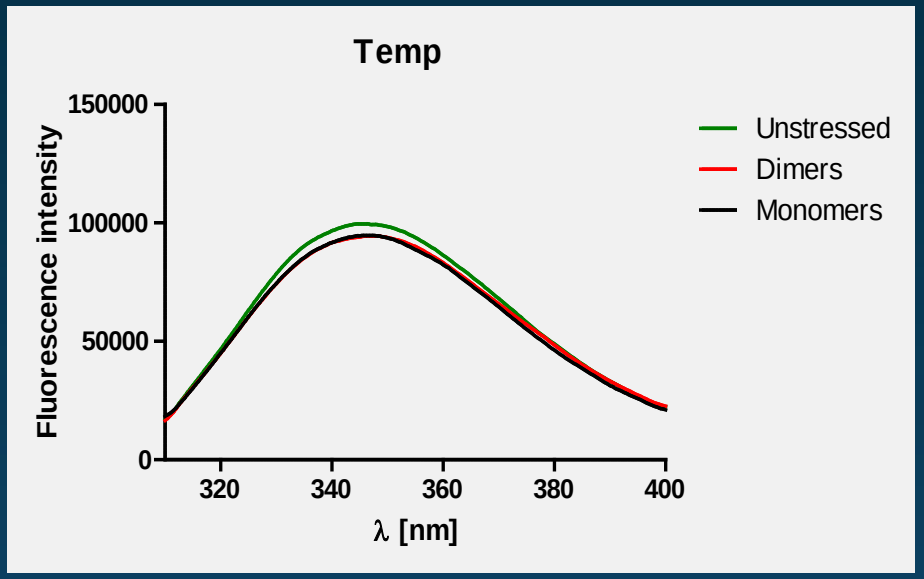


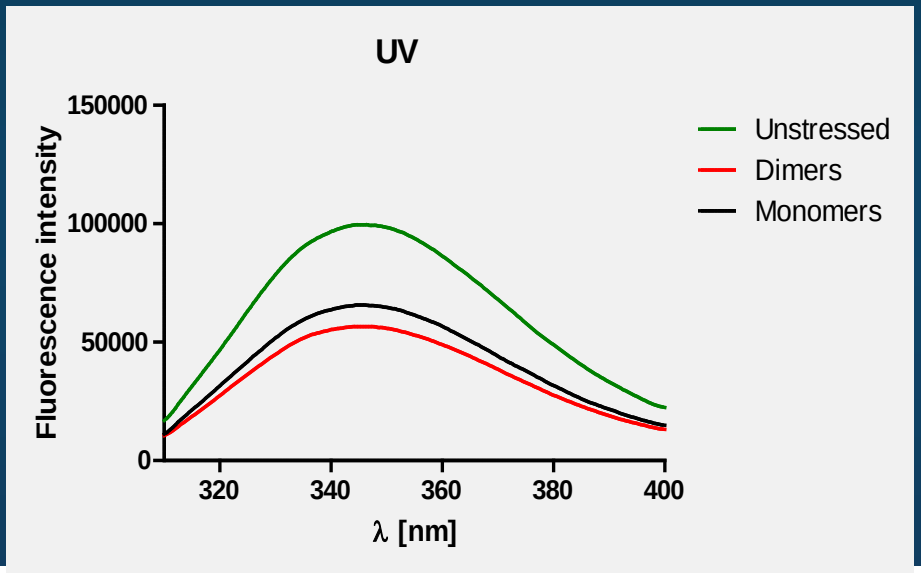


Secondary structure (almost) unaltered

Characterization of dimers: Trp fluorescence

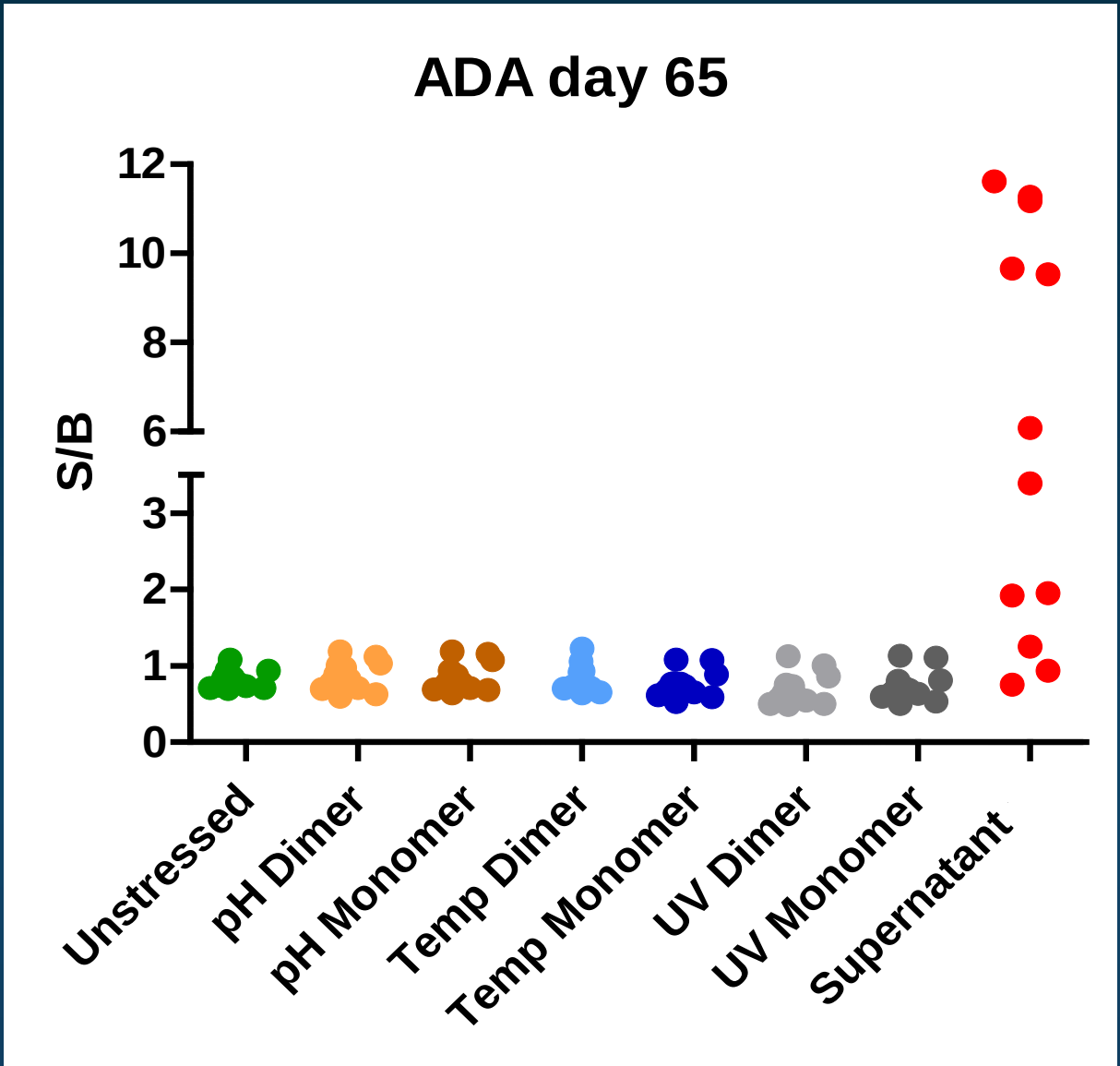






Fluorescence intensity reduced in UV stressed mAb

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 20 nm and a few hundred nm to be the most effective particulate adjuvants
- But, different types of nanoparticles have widely different levels of adjuvant activity


Andhyk Halim
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Daan Crommelin
Daniel Weinbuch
Grzegorz Kijanka
Huub Schellekens
Jared Bee & collegues
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!