University of Colorado Denver
Leiden Amsterdam Center for Drug Research
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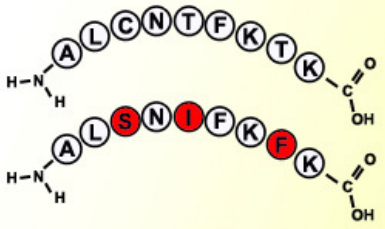
Biological impact of IgG aggregates:
Immunogenicity, Biodistribution, In Vivo Response

Vasco Filipe, 9th May 2014 PEGS - Boston

Sequence variation



Formulation

UNKNOWN

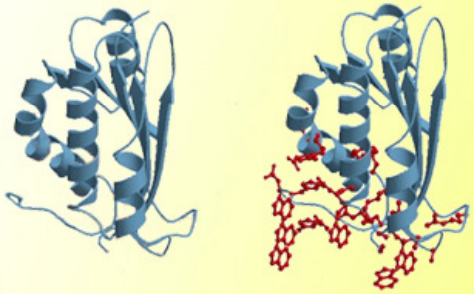
?

Application route

Patient features

Length of treatment

Product modification



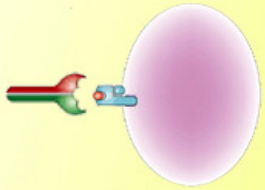
IMMUNOGENICITY

Nature of disease

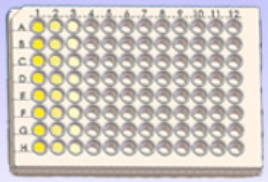
Contaminants and impurities

Dose

Biological activity



Assay technology



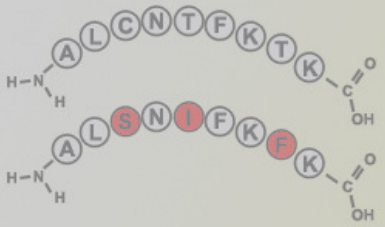
Concomitant medications

PRODUCT RELATED

ANTIBODY ASSAY RELATED

TREATMENT RELATED

Sequence variation



Formulation

UNKNOWN

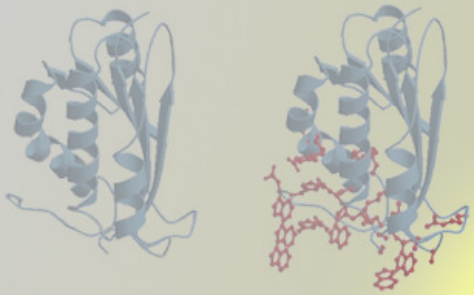
?

Application route

Patient features

Length of treatment

Product modification



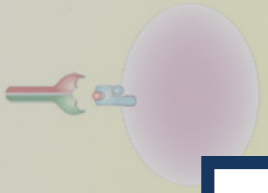
IMMUNOGENICITY

Nature of disease

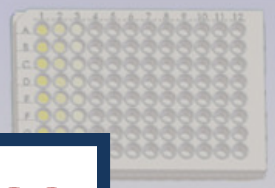
Contaminants and impurities

Dose

Biological activity



Assay technology



Concomitant medications

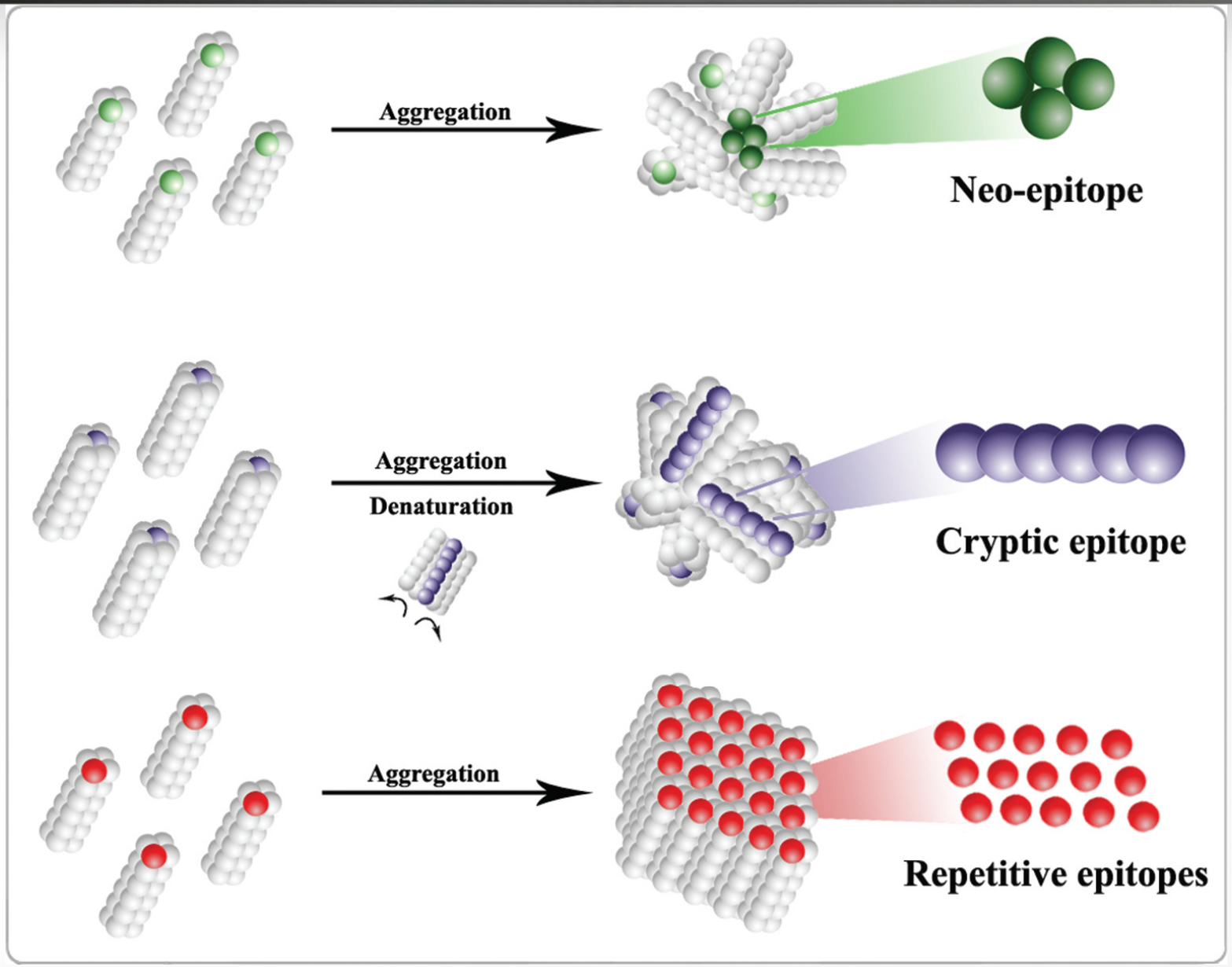
Protein Aggregates

PRODUCT RELATED

ANTIBODY ASSAY RELATED

TREATMENT RELATED

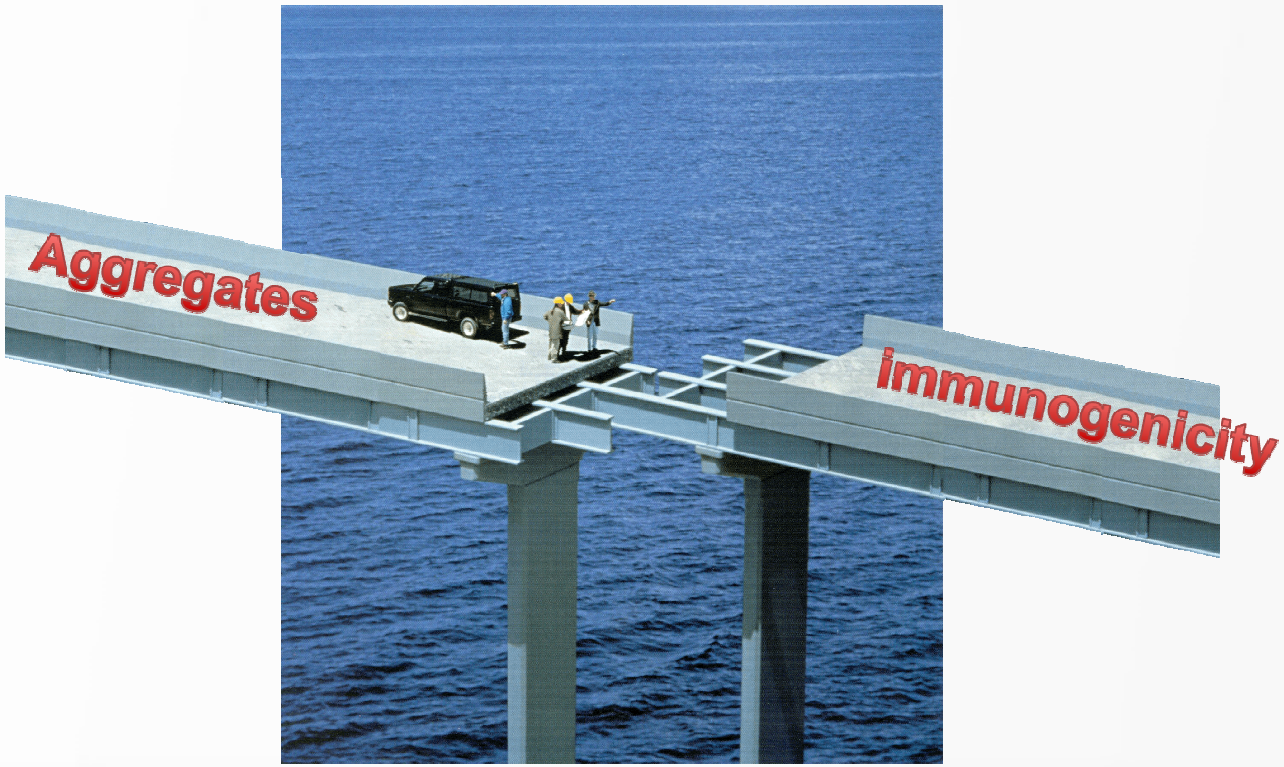
Aggregates and immunogenicity



Filipe et al., Aggregation of therapeutic proteins, John Wiley & Sons, 2010

Key questions

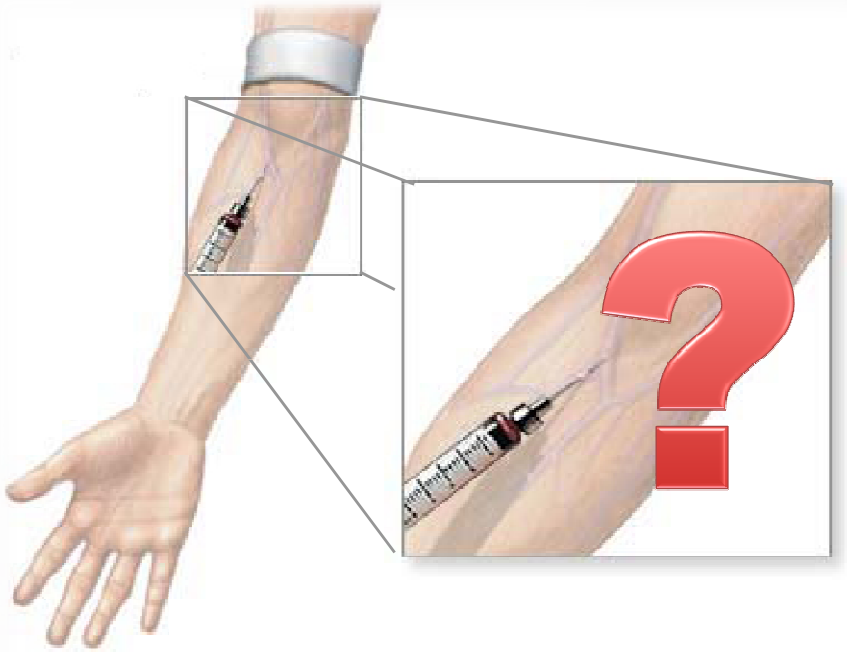
- Why are aggregates immunogenic?
- Which aggregates are the most immunogenic?
- How many aggregates are too much?



Study 1

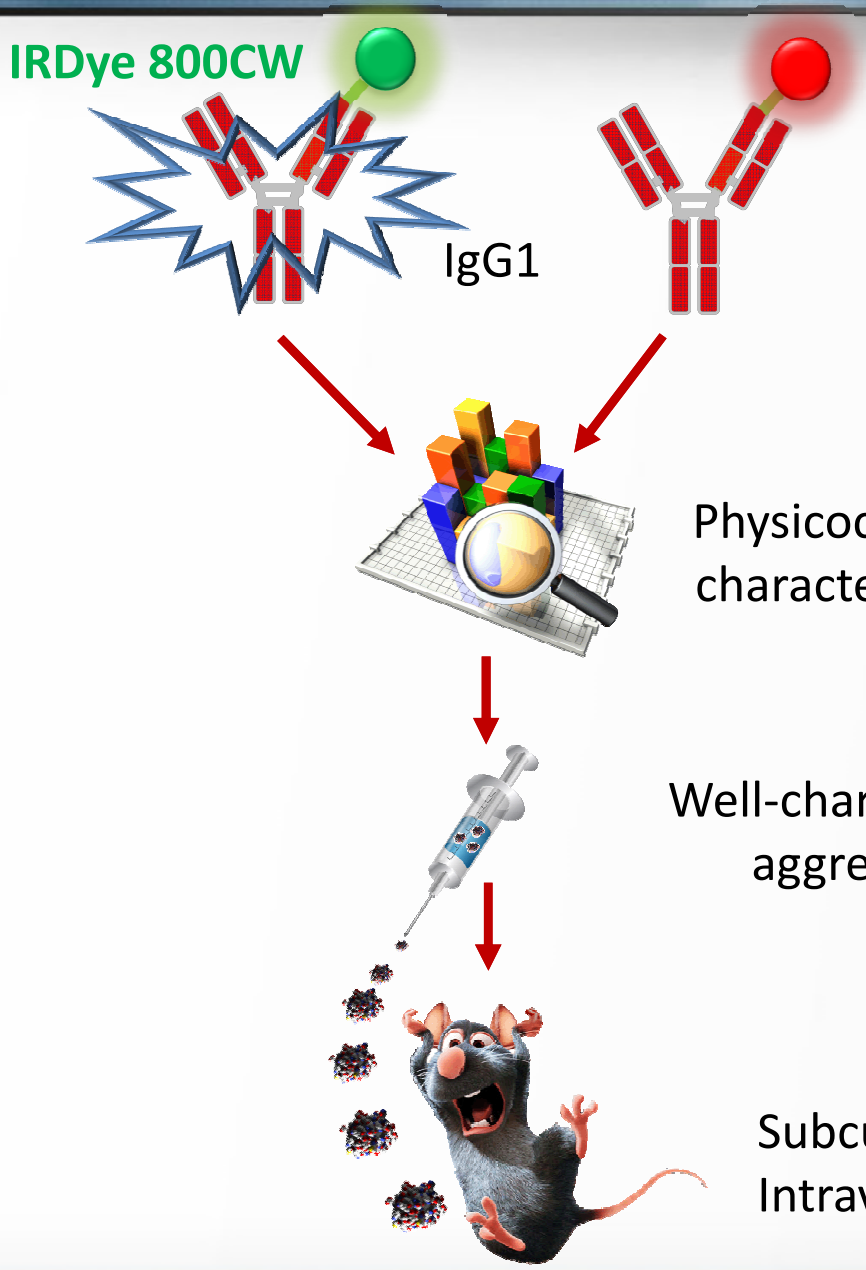
Biodistribution and early immune response to IgG aggregates

What happens upon injection?



- What happens to IgG aggregates at the site of injection?
- Is the biodistribution of aggregates different from monomers?
- Is there a difference in early immune response if IgG is aggregated?

Study 1 – Design



Stress method

- Shake (400 rpm, 3.5 h, RT)

Analytical methods

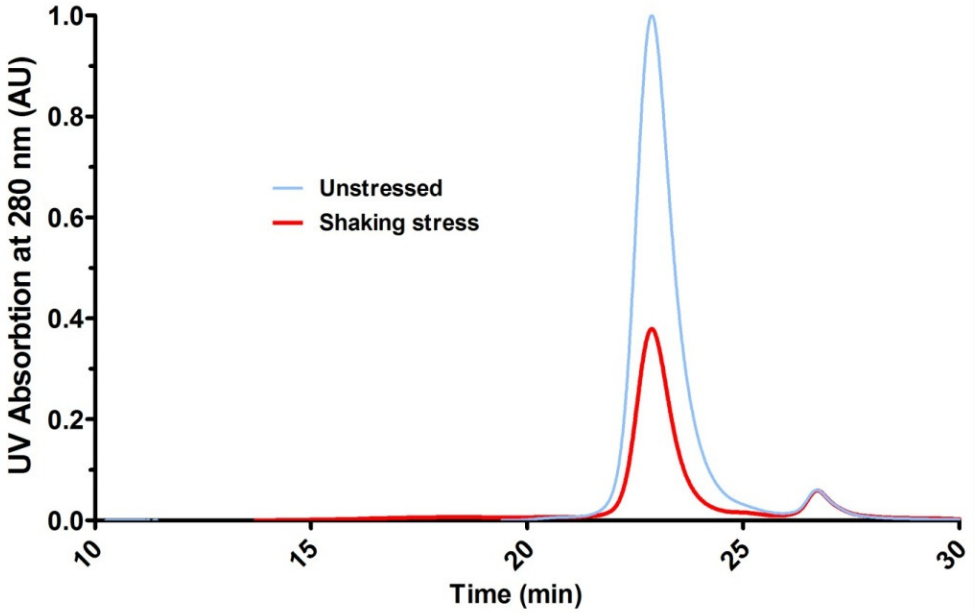
- Size exclusion chromatography (SEC)
- Light obscuration (LO)
- Visual inspection
- Nanoparticle tracking analysis (NTA)
- SDS-PAGE

SKH1 mouse strain

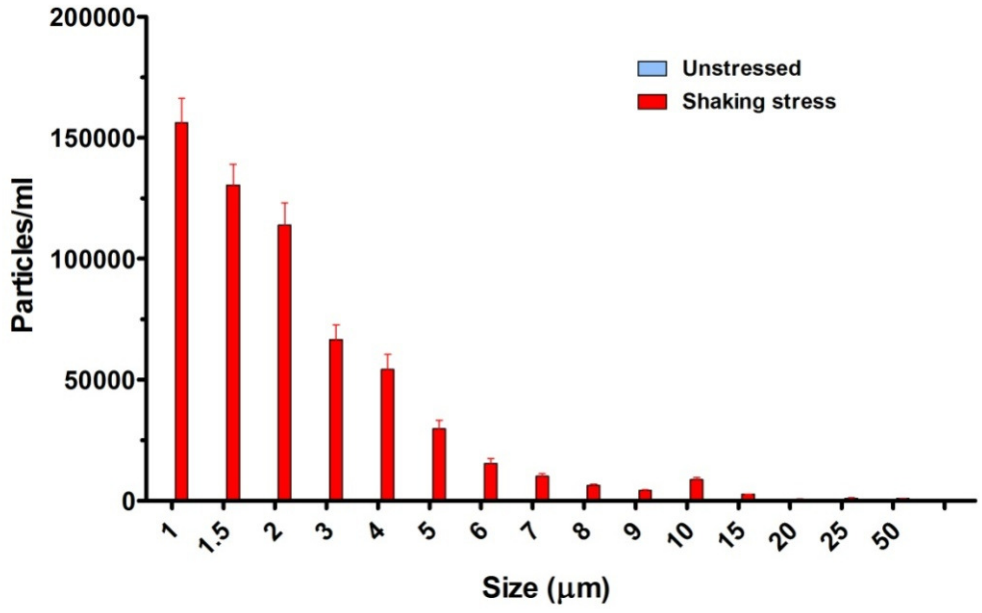
- Hairless (not nude)
- Immune competent
- 8 mice/group

Characterization of IgG aggregates

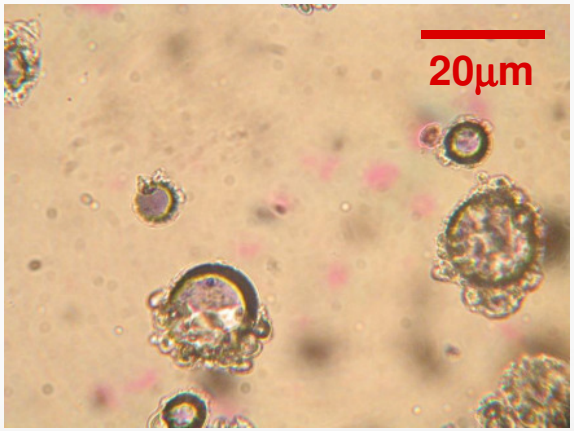
Size Exclusion Chromatography



Light Obscuration



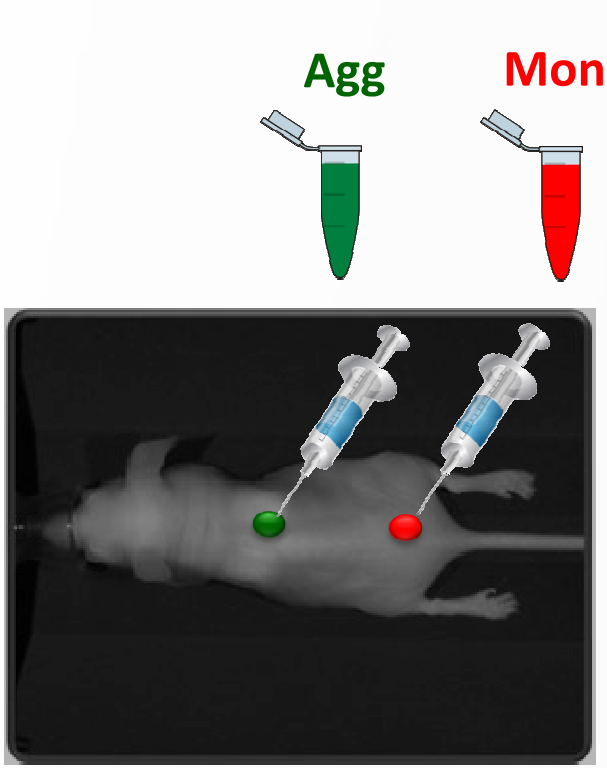
- 70% in aggregated form
- Mostly micron-sized aggregates
- Hydrophobic aggregates →



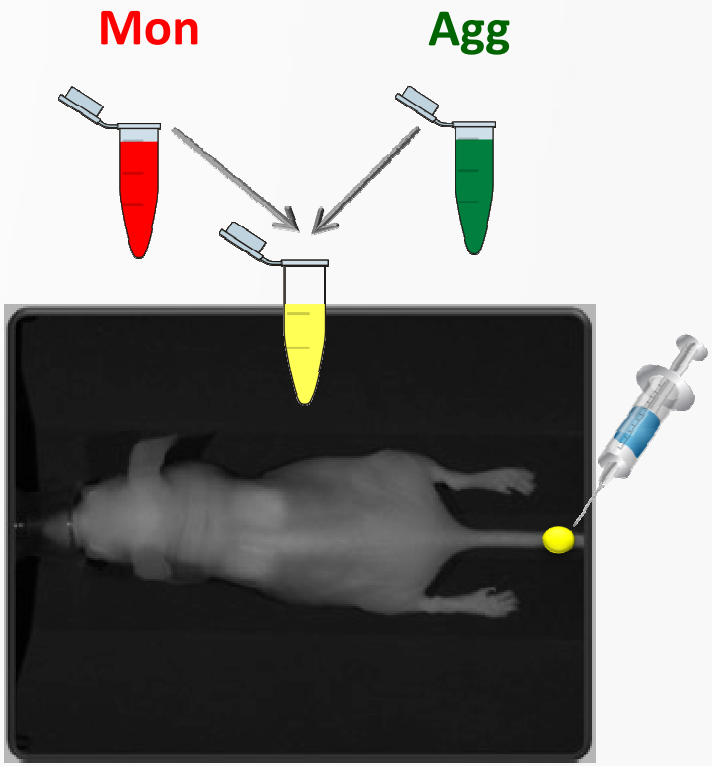
Injection design

Licor Pearl Impulse fluorescence imaging

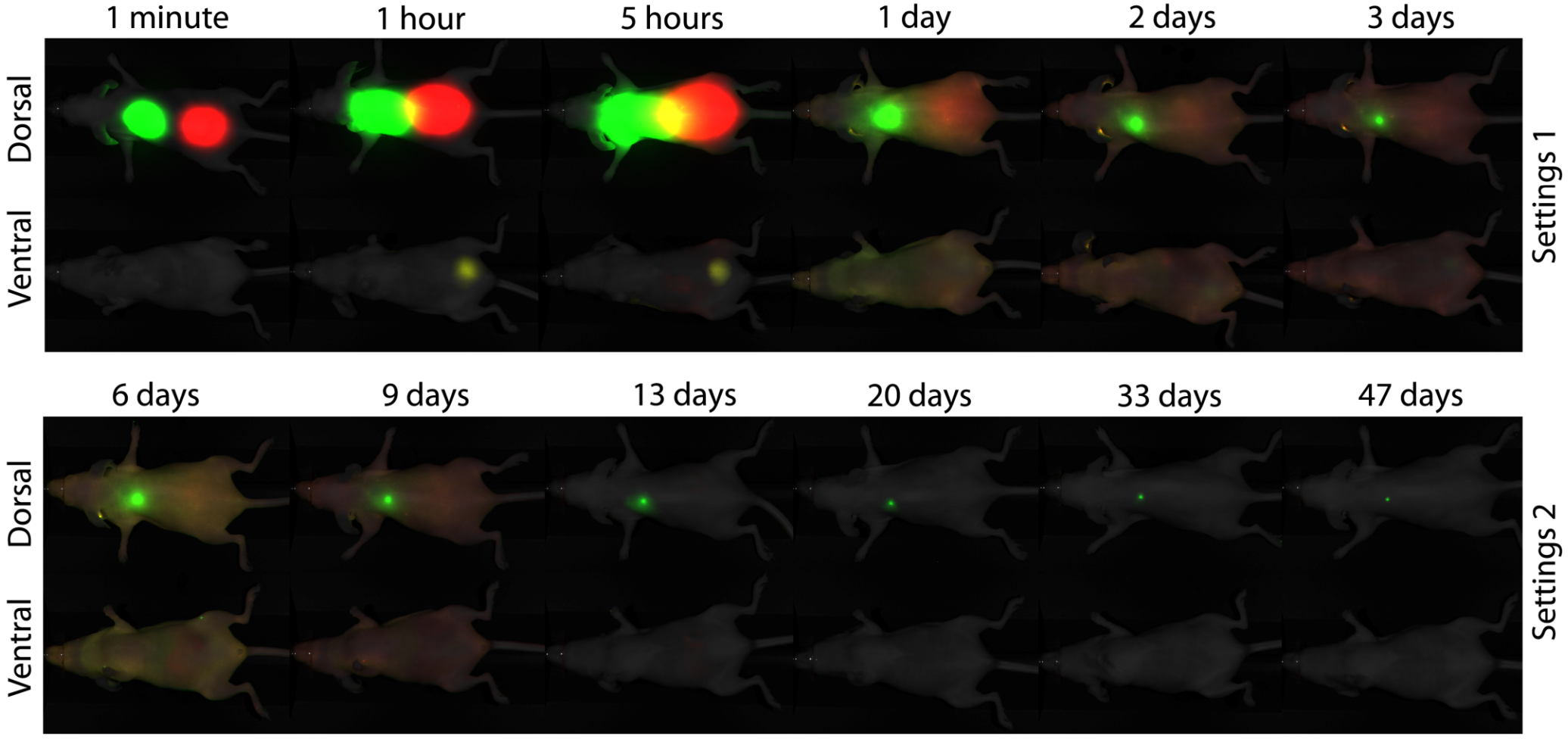
Subcutaneous



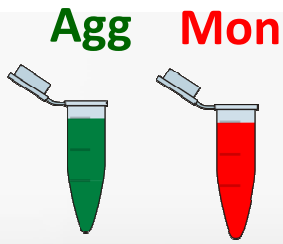
Intravenous



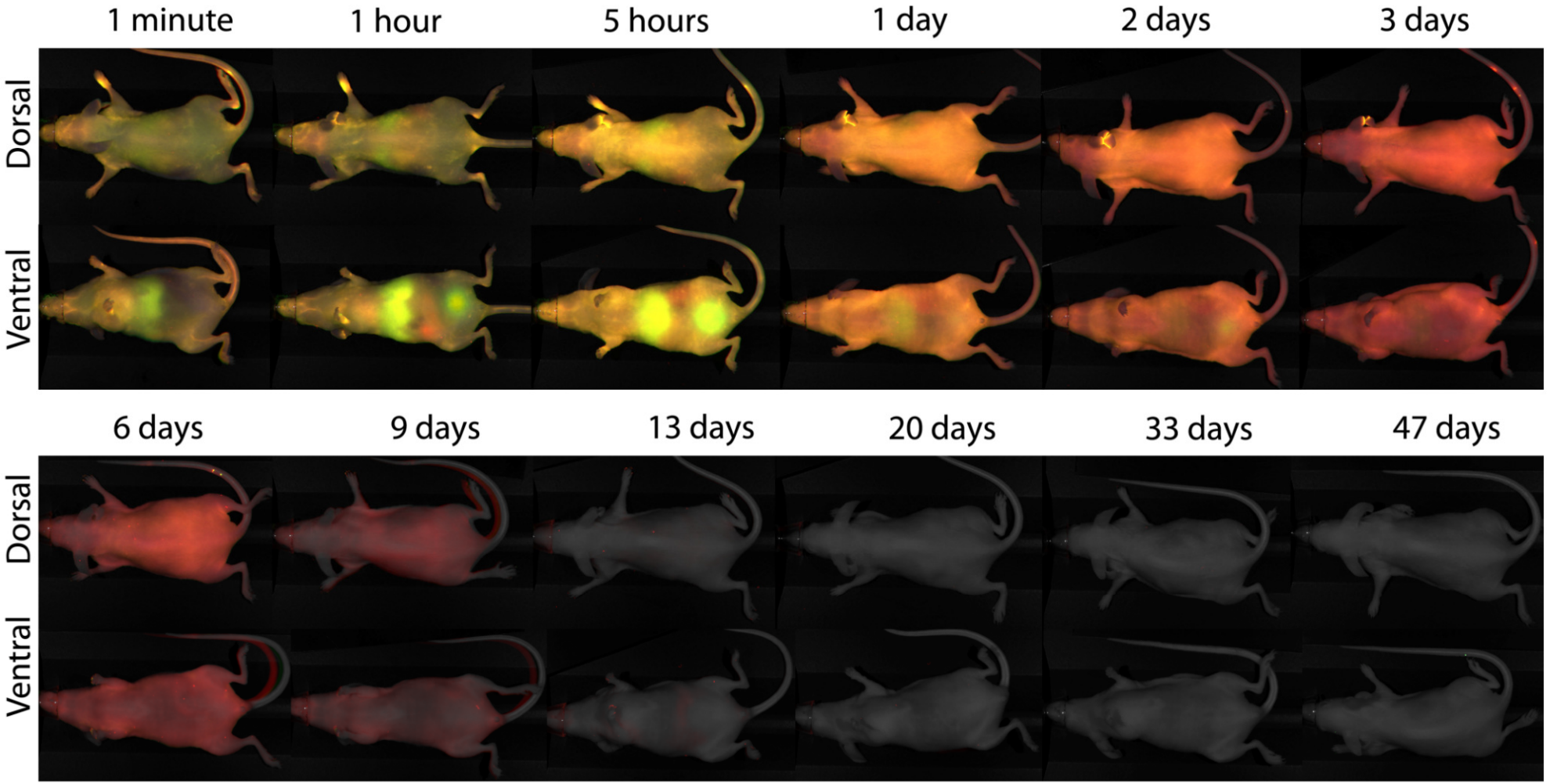
Subcutaneous



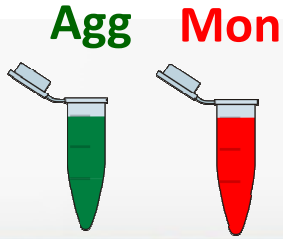
- Monomers spread faster than aggregates
- Aggregates remain at the site of injection for more than a month



Intravenous

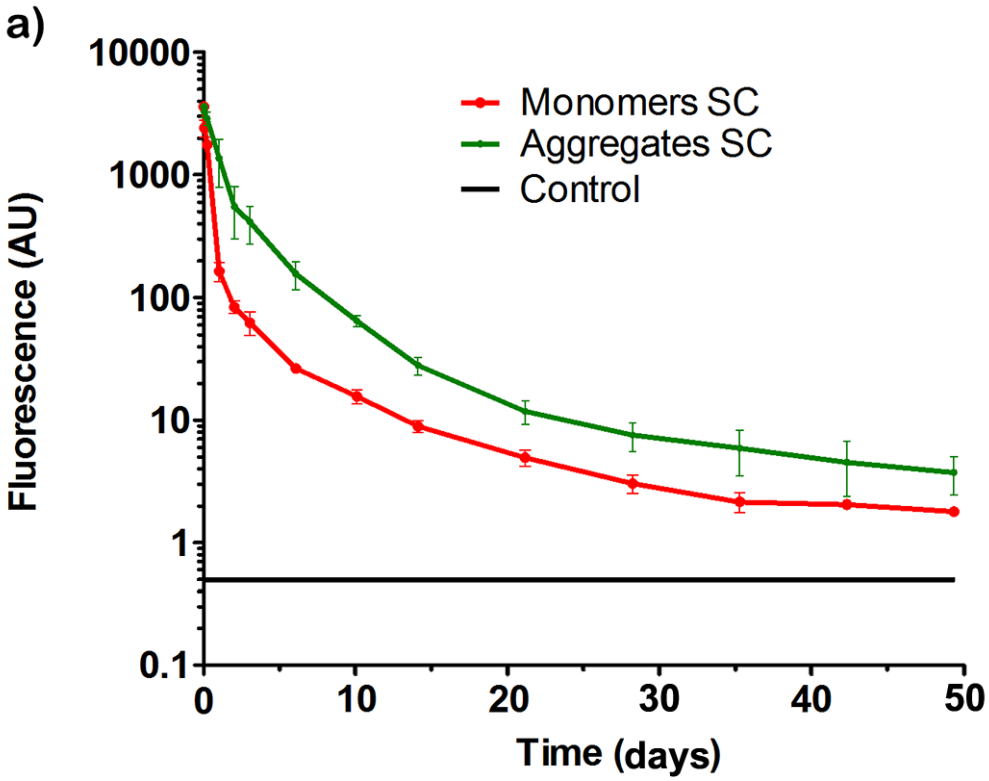


- Accumulation in the liver
- Aggregates disappear from circulation faster than monomers

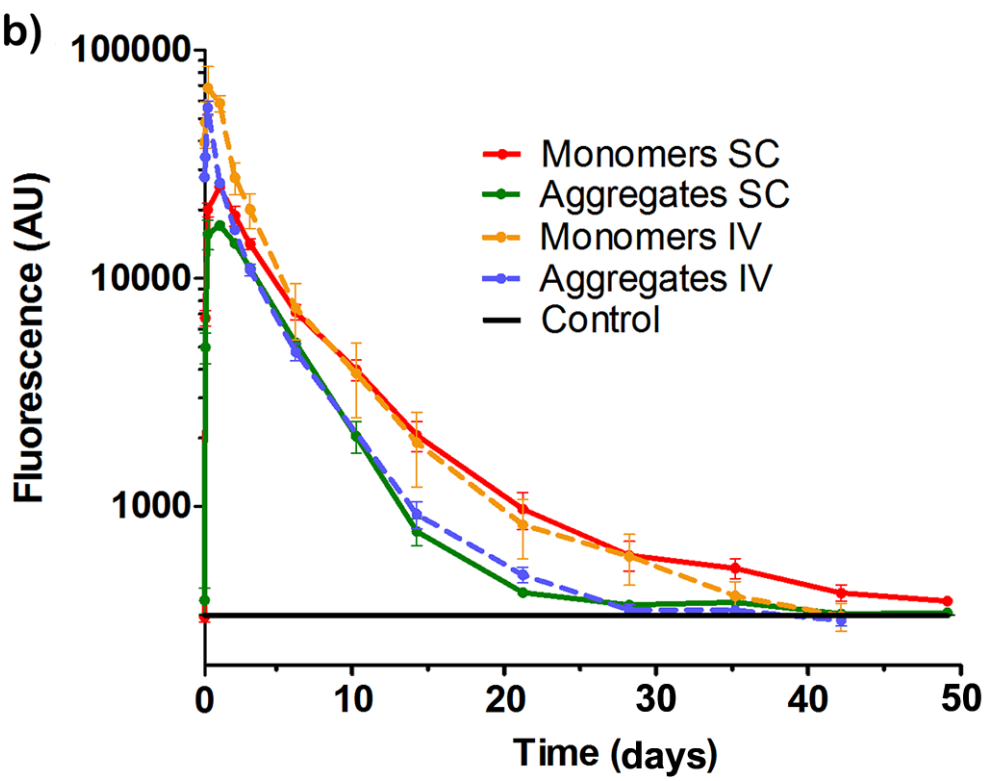


Fluorescence quantification

Injection site



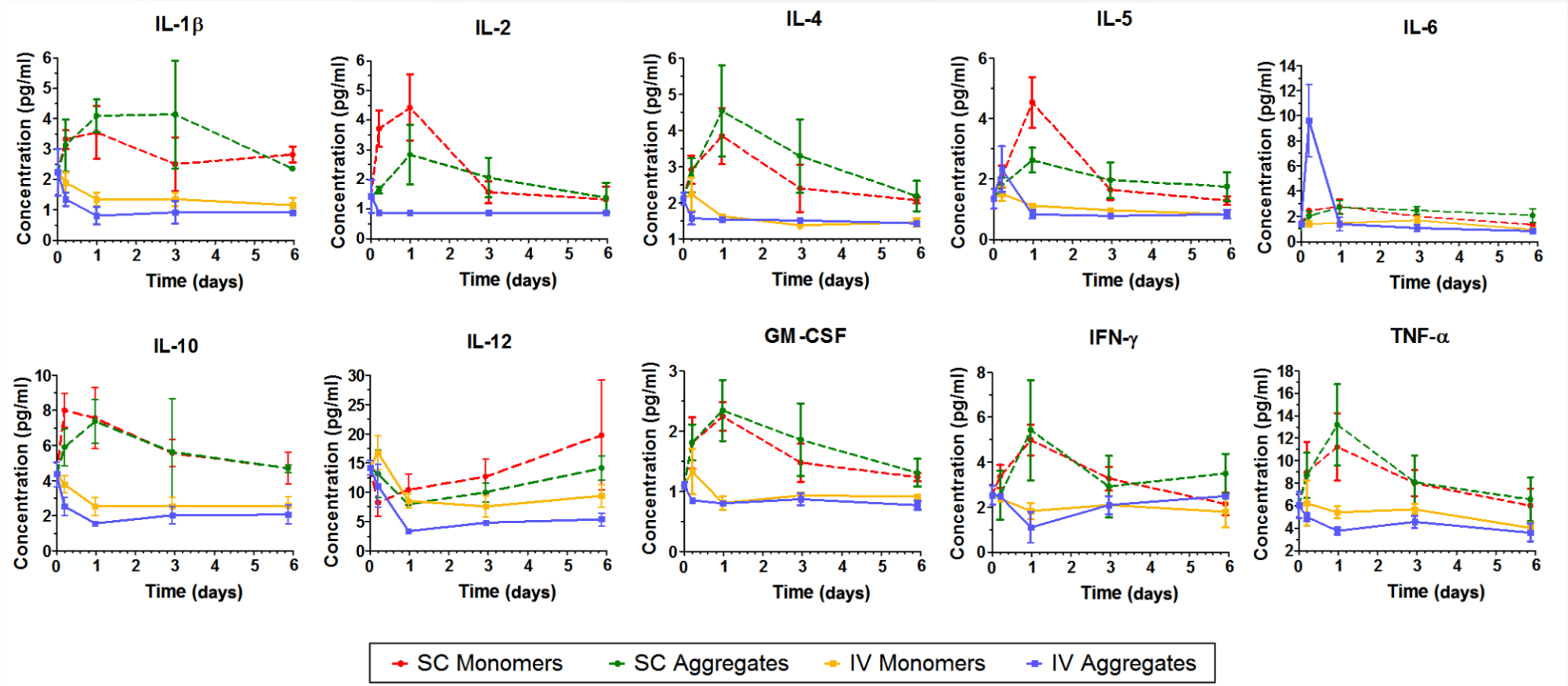
Whole mouse



- Injection site - Aggregates remain at higher concentration for longer time
- Whole mouse – Aggregates disappear faster

Early immune response cytokines

Luminex assay



- No statistical difference between monomers and aggregates
- Statistical difference between SC and IV routes
- Only IL-6 significantly increased with aggregates (IV)

Conclusions of Study 1

Biodistribution

- IgG aggregates remain more than a month at the site of injection in mice (SC)
- The aggregates spread slower than monomers after (SC)
- IgG aggregates disappear faster than monomers from circulation

Early immune response

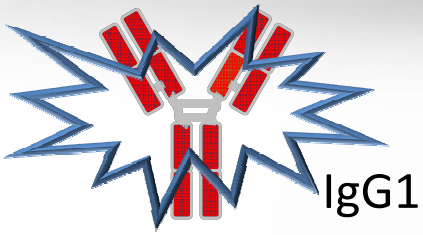
- No statistical difference between monomers and aggregates
- Statistical difference between SC and IV routes

Filipe et al., In vivo fluorescence imaging of IgG1 aggregates after subcutaneous and intravenous injection in mice, Pharm Res, 31(1):216-27, 2014

Study 2

Immunogenicity of different types of IgG aggregates in transgenic mouse model

Study 2 – Design



Apply different stresses

Physicochemical characterization

Well-characterized aggregates

Test immunogenicity in transgenic mice tolerant for human IgG

Stress methods

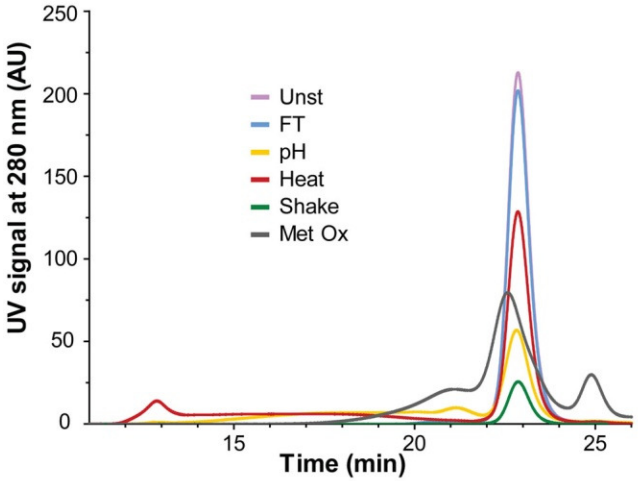
- Freeze-thaw (10 cycles -80°C-RT)
- pH-shift (3 cycles pH 6-1-6)
- Heat (74°C, 15 min)
- Shake (400 rpm, 16 h, RT)
- Metal-catalyzed oxidation (Cu^{2+})

Analytical methods

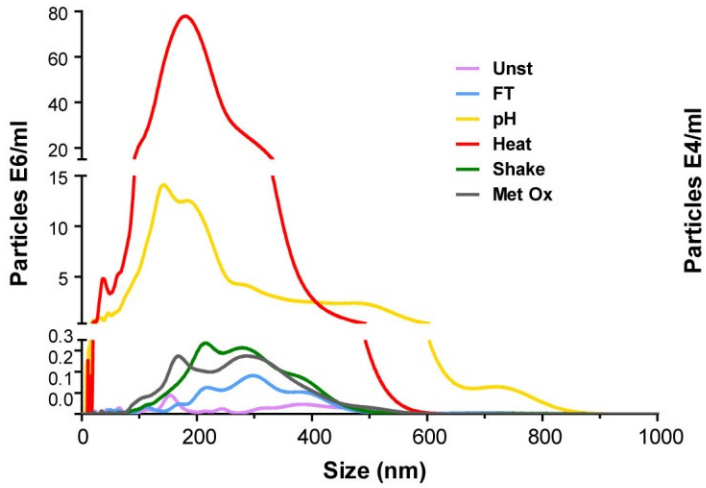
- Size exclusion chromatography (SEC)
- Asymmetric flow field flow fractionation (AF4)
- Nanoparticle tracking analysis (NTA)
- Light obscuration (LO)
- Visual inspection
- Circular Dichroism
- Steady-state fluorescence spectroscopy
- Filtration with Coomassie blue staining
- SDS-PAGE
- Western blotting
- Dot blotting

Size distribution of IgG aggregates

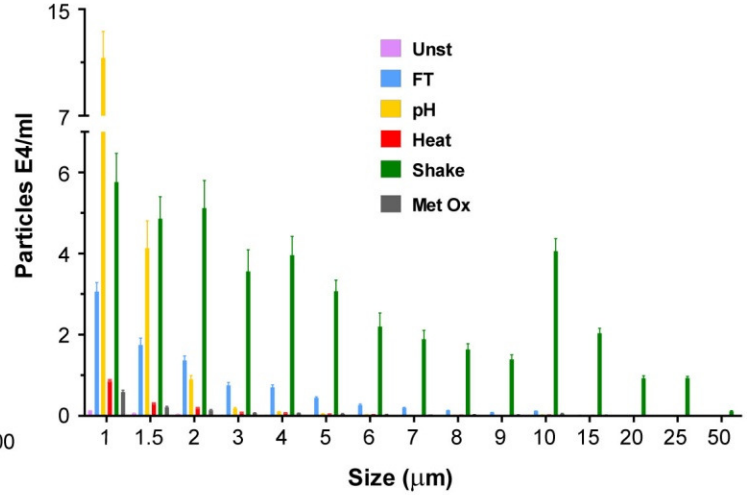
SEC



NTA



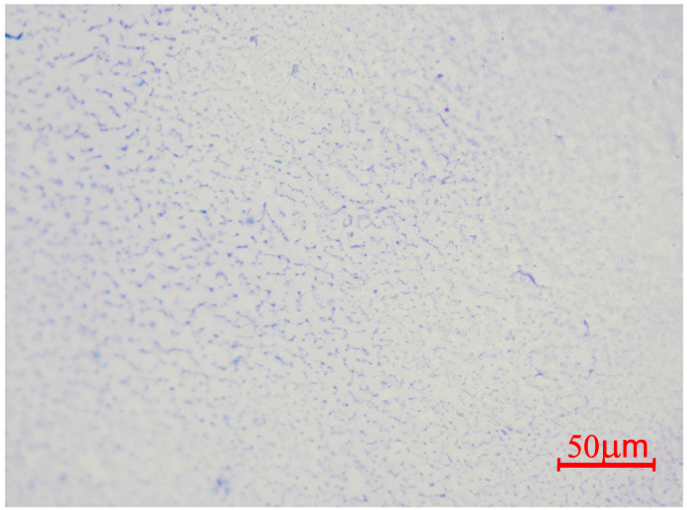
LO



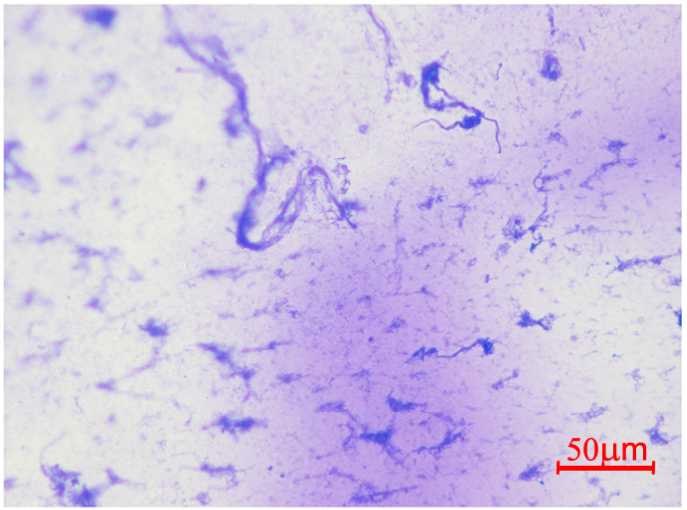
- **Heat** and **pH-shift** induced more **oligomers** and **submicron** aggregates
- **Shake** and **pH-shift** induced more **micron** aggregates
- **Shake** was the only stress inducing **visible** aggregates

Morphology of aggregates

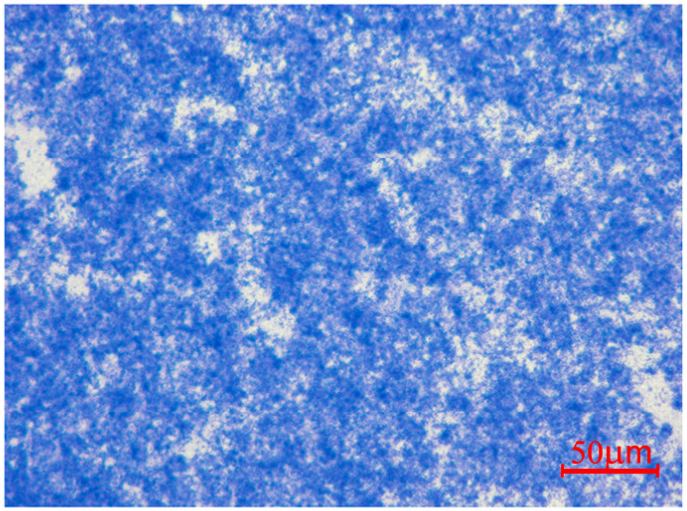
Unst



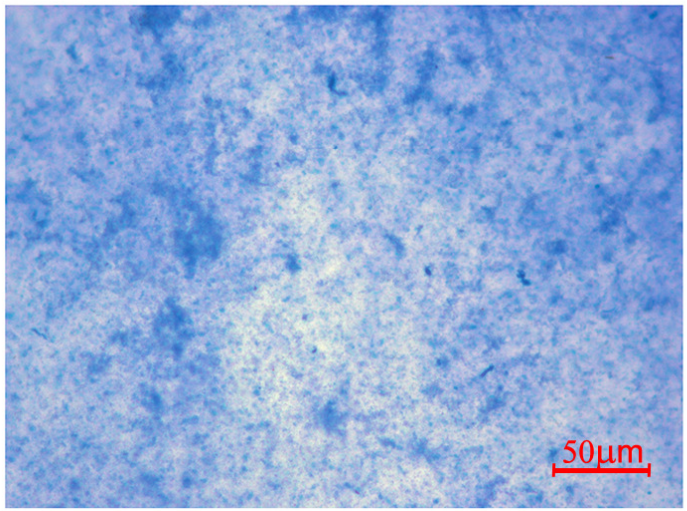
FT



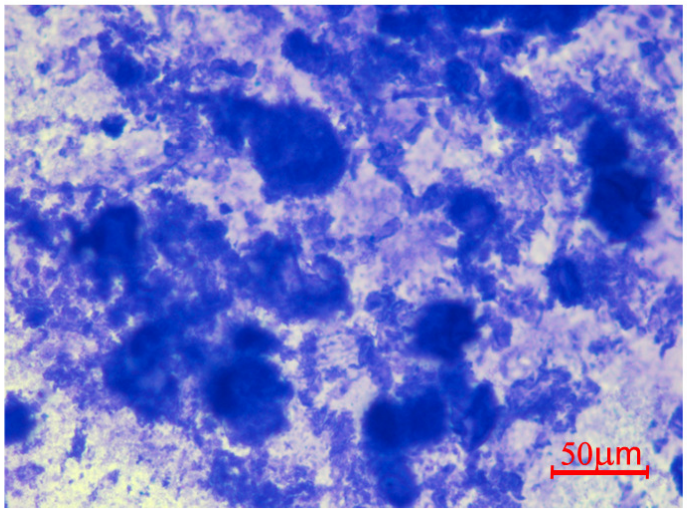
pH



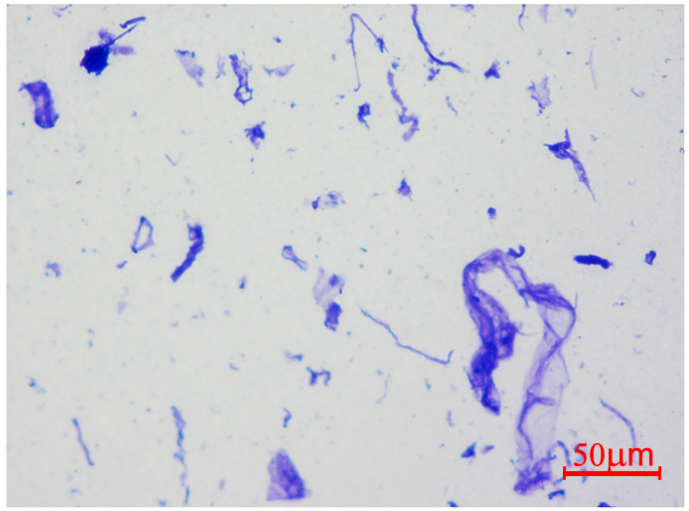
Heat



Shake*



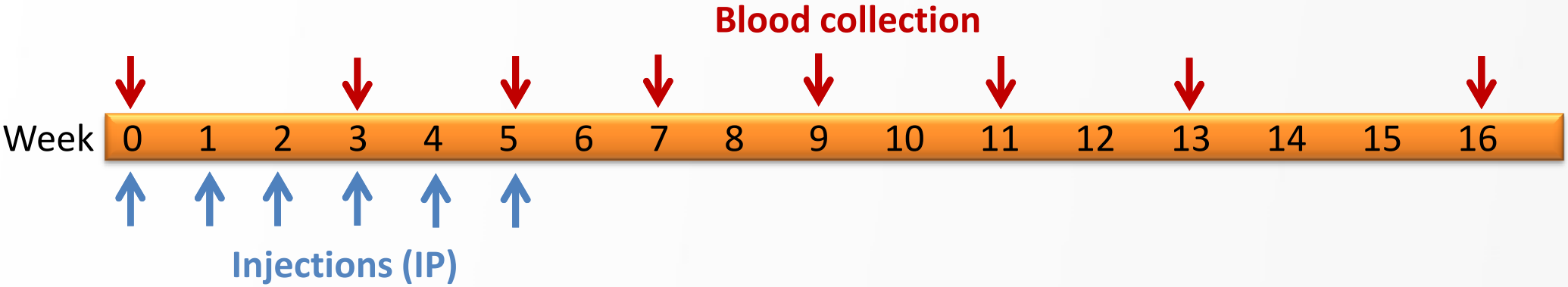
Met ox



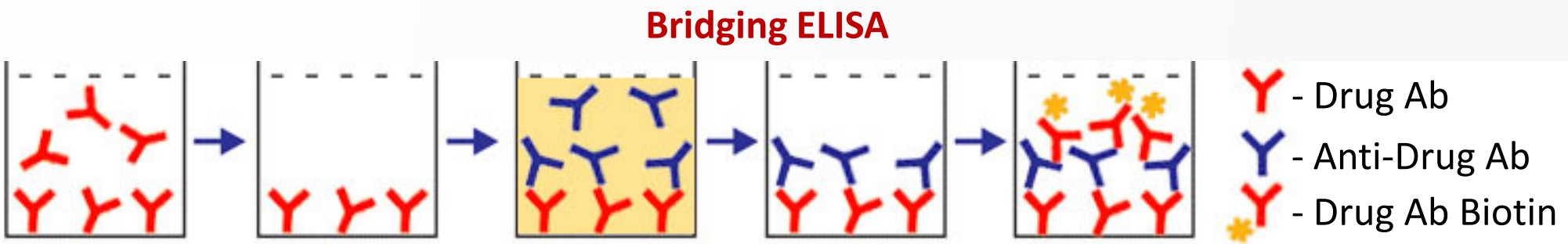
Study 2 – Design

Animal experiment settings

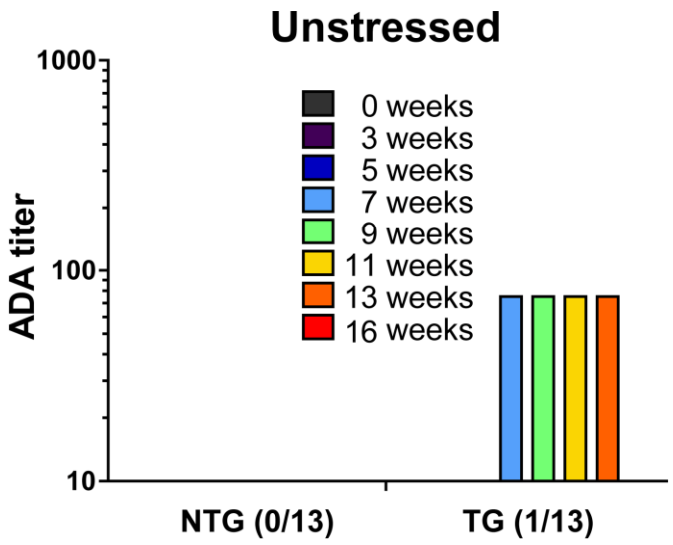
- 13 transgenic mice/group (tolerant for human IgG)
- 13 wild-type mice/group

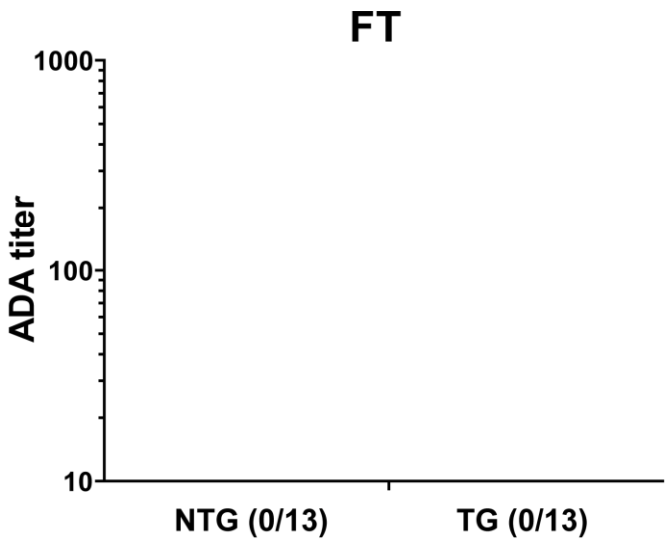


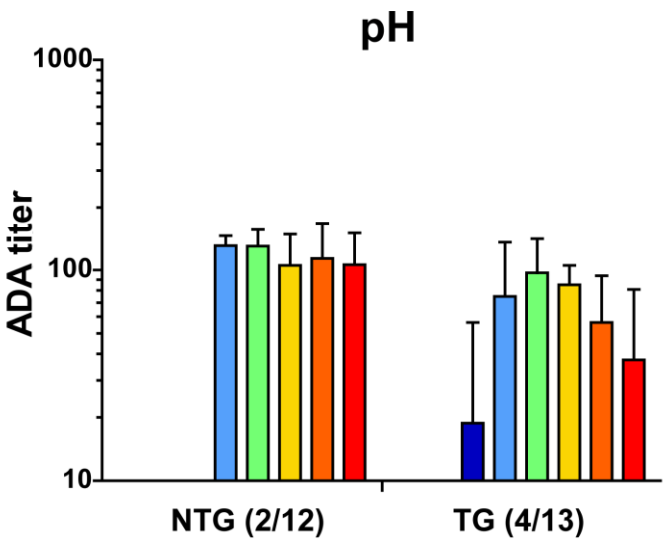
Immunogenicity testing

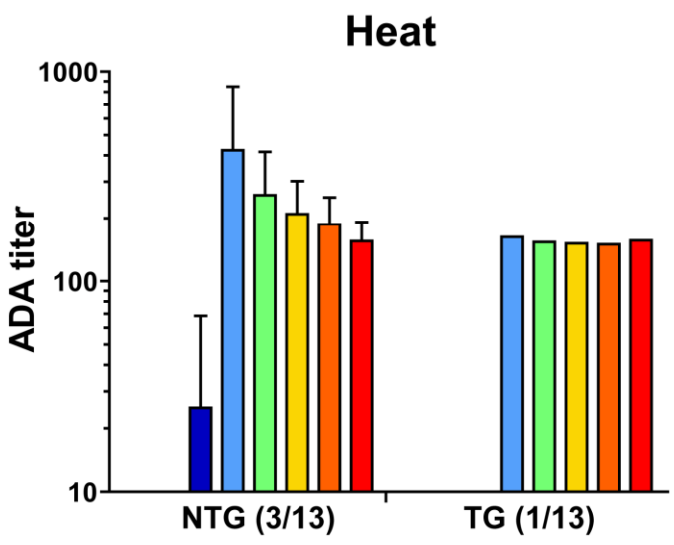


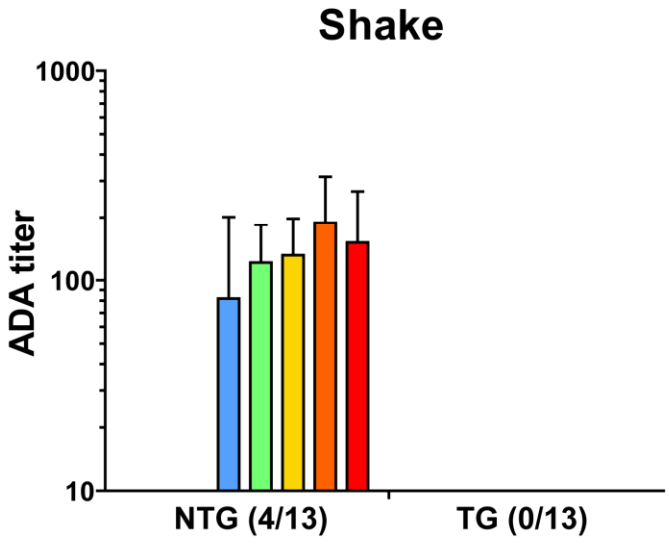
ADA titers

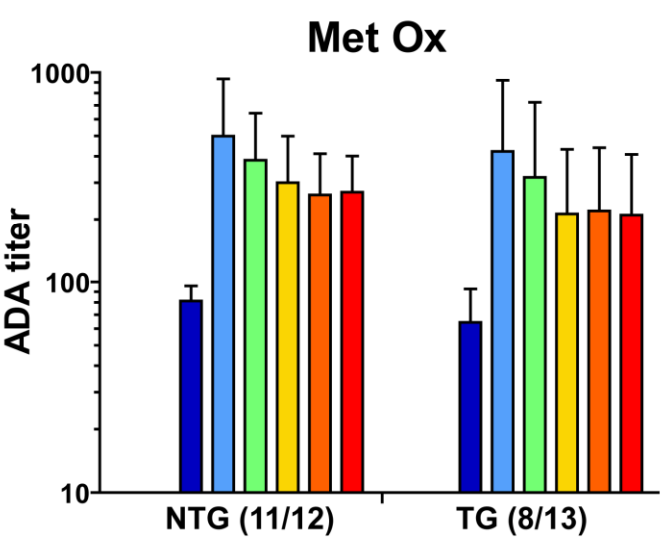

Unstressed
1000
100
10
ADA titer
0 weeks
3 weeks
5 weeks
7 weeks
9 weeks
11 weeks
13 weeks
16 weeks
NTG (0/13)
TG (1/13)


FT
1000
100
10
ADA titer
NTG (0/13)
TG (0/13)


pH
1000
100
10
ADA titer
NTG (2/12)
TG (4/13)

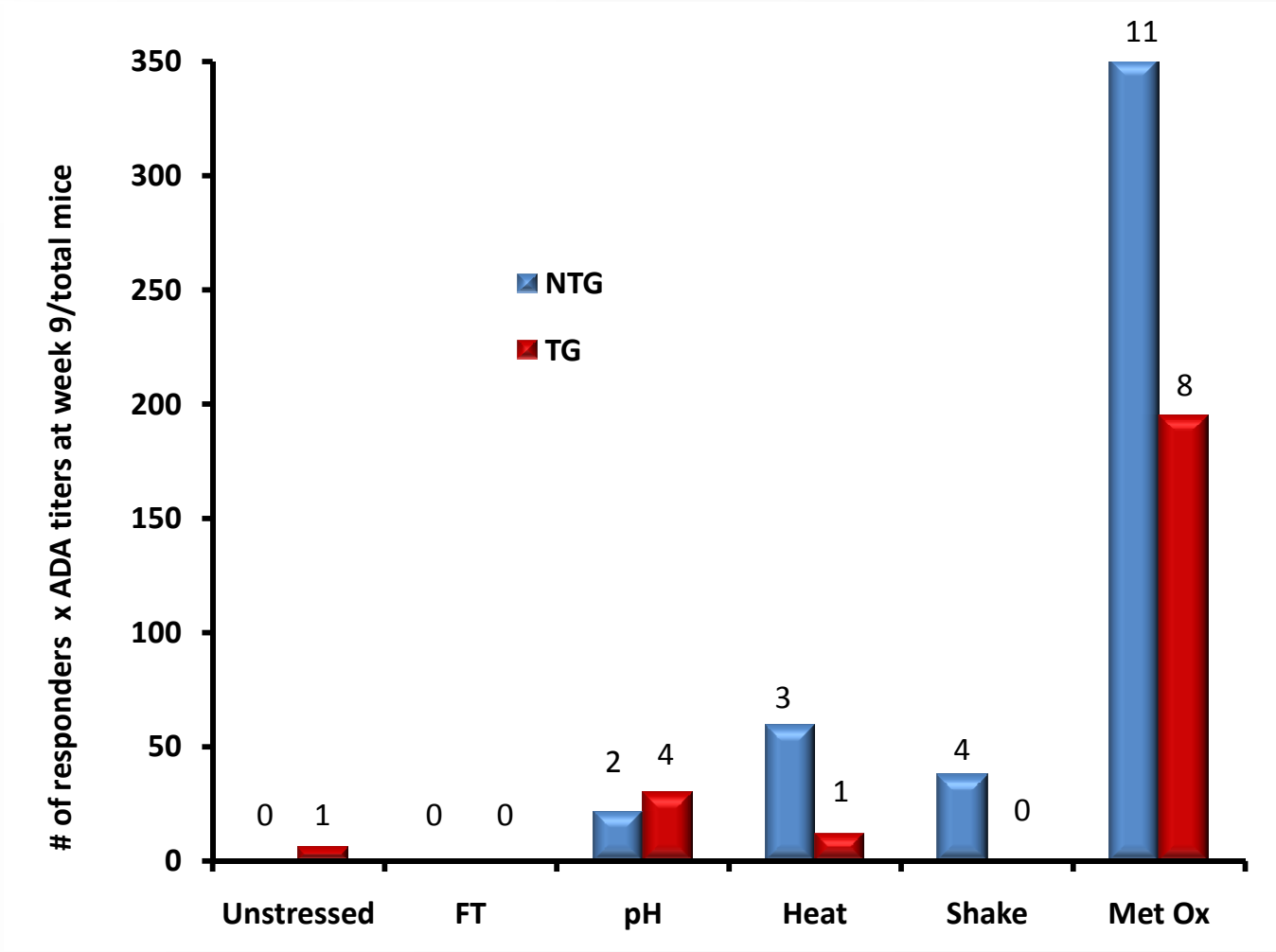

Heat
1000
100
10
ADA titer
NTG (3/13)
TG (1/13)


Shake
1000
100
10
ADA titer
NTG (4/13)
TG (0/13)


Met Ox
1000
100
10
ADA titer
NTG (11/12)
TG (8/13)

Immunogenicity outcome

Weighted immunogenicity



Metal-catalyzed oxidized IgG formulation was able to induce high ADA titers in the majority of the mice

Conclusions of Study 2

- Aggregates increase the risk of immunogenicity
- Not all aggregates pose the same immunogenic risk
- The amount and size of aggregates is not directly related with immunogenicity
- Oxidated aggregates seem to be the most dangerous aggregated species
  - Coherent with by IFN-α studies (Hermeling et al., J Pharm Sci, 2006)
  - Coherent with IFN-β studies (van Beers et al., Pharm Res, 2011)
  - Confirmed by insulin studies (Torosantucci et al., J Pharm Sci, 2014)
  - Contradictory results with IgG2 study by Amgen (Bi et al., JBC, 2013)
  - Confirmed by a recent unpublished IgG study (Antonio Iglesias, Roche, Switzerland)
- Immunogenicity ranking of formulations used the this particular study:

  metal-catalyzed oxidized > heated ≈ pH-shifted ≈ shaken > freeze-thawed
- Case-by-case approach is still necessary

Filipe et al., Immunogenicity of different stressed IgG monoclonal antibody formulations in immune tolerant transgenic mice, MAbs, 4(6):740-52, 2014

Acknowledgements

- Wim Jiskoot
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- Clemens Löwik
- Ivo Que

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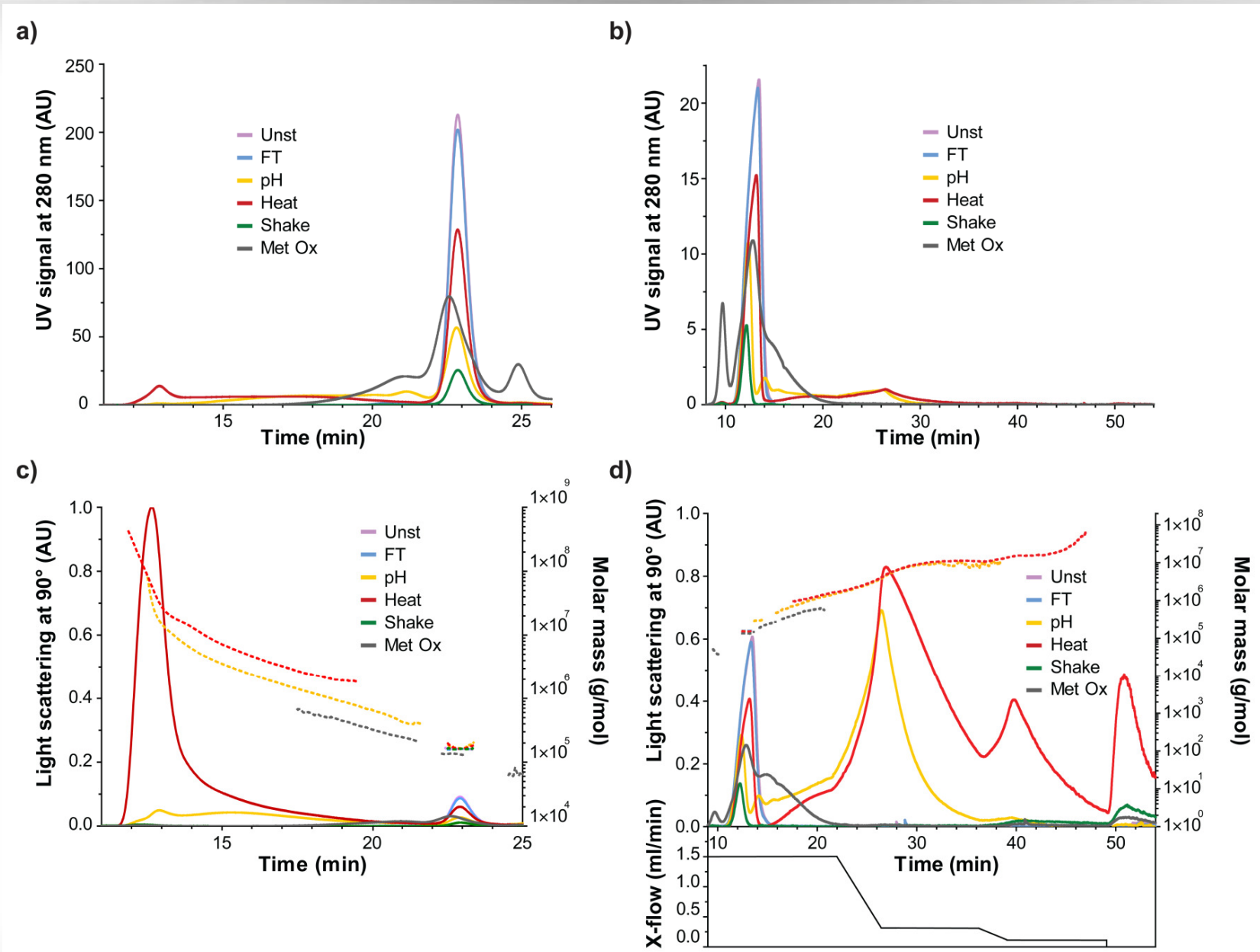
Leiden Amsterdam Center for Drug Research

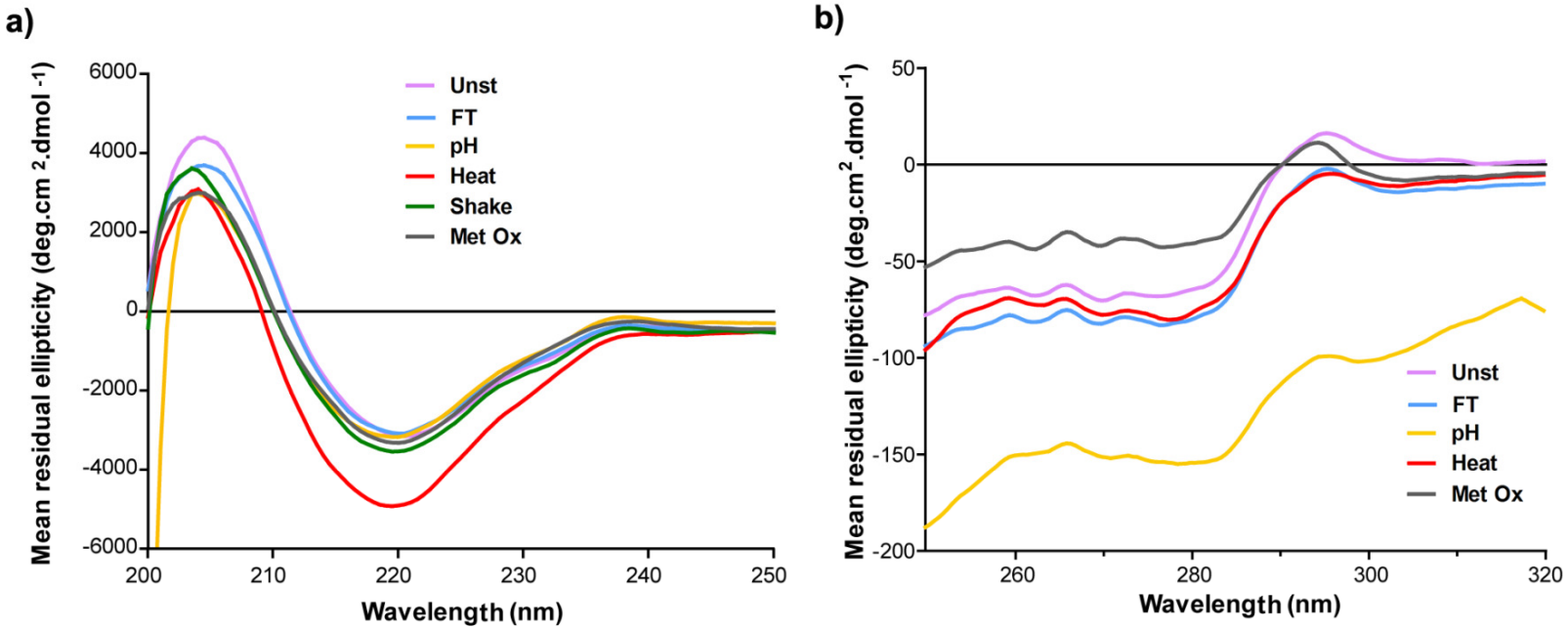
Leiden University Medical Center

Utrecht University

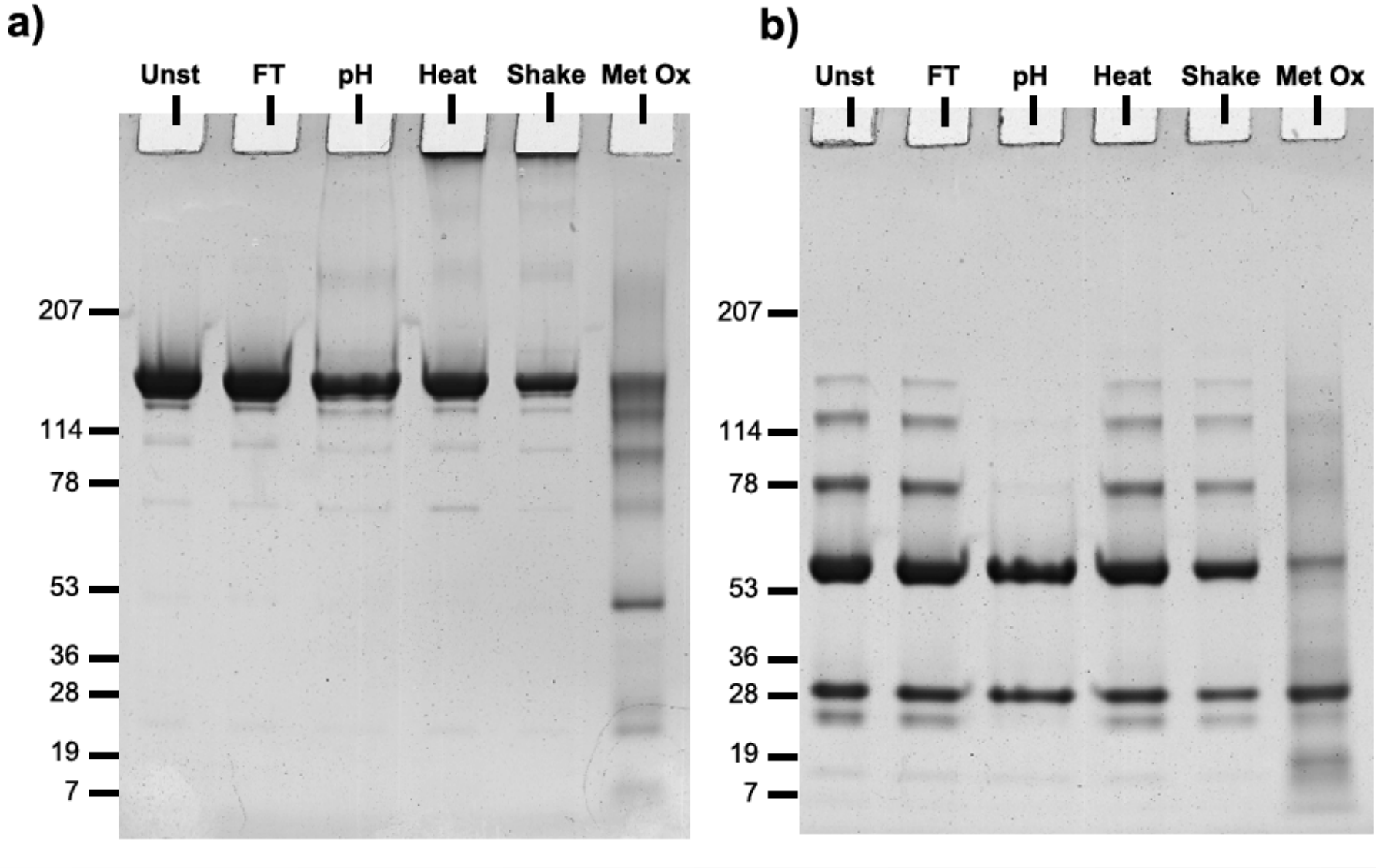
SEC + AF4


a)
UV signal at 280 nm (AU)
Time (min)
Unst
FT
pH
Heat
Shake
Met Ox
b)
UV signal at 280 nm (AU)
Time (min)
Unst
FT
pH
Heat
Shake
Met Ox
c)
Light scattering at 90° (AU)
Molar mass (g/mol)
Time (min)
Unst
FT
pH
Heat
Shake
Met Ox
d)
Light scattering at 90° (AU)
Molar mass (g/mol)
X-flow (ml/min)
Time (min)
Unst
FT
pH
Heat
Shake
Met Ox

Far UV CD + Near UV CD + bis-ANS Fluorescence



SDS-PAGE


a)
Unst
FT
pH
Heat
Shake
Met Ox
207
114
78
53
36
28
19
7
b)
Unst
FT
pH
Heat
Shake
Met Ox
207
114
78
53
36
28
19
7

Western Blot + Dot Blot